Woodhead Publishing Series in Food Science, Technology and Nutrition

Handbook of herbs and spices

Second edition

Volume 2

Edited by K. V. Peter

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Handbook of herbs and spices

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Handbook of herbs and spices
Second edition, Volume 1
(ISBN 978-0-85709-039-3)
Herbs and spices are among the most versatile ingredients in food processing, and alongside their sustained popularity as flavourants and colourants, they are increasingly being used for their natural preservative and potential health-promoting properties. This authoritative new edition, in two volumes, of *Handbook of herbs and spices* provides a comprehensive guide to the properties, production and application of a wide variety of commercially-significant herbs and spices. Volume 1 begins with an introduction to herbs and spices, discussing their definition, trade and applications. Both the quality specifications for herbs and spices, and the quality indices for spice essential oils are reviewed in detail, before the book goes on to look in depth at individual herbs and spices, ranging from basil to vanilla.

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Volume 3 (ISBN 978-1-84569-735-8)
Volume 4 (ISBN 978-0-85709-090-4)
While products such as bananas, pineapples, kiwifruit and citrus have long been available to consumers in temperate zones, new fruits such as lychee, longan, carambola, and mangosteen are now also entering the market. Confirmation of the health benefits of tropical and subtropical fruit may also promote consumption further. Tropical and subtropical fruits are particularly vulnerable to postharvest losses, and are also transported long distances for sale. Therefore maximising their quality postharvest is essential and there have been many recent advances in this area. Many tropical fruits are processed further into purees, juices and other value-added products, so quality optimization of processed products is also important. These books cover current state-of-the-art and emerging post-harvest and processing technologies. Volume 1 contains chapters on particular production stages and issues, whereas Volumes 2, 3 and 4 contain chapters focused on particular fruit.

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1

Introduction to herbs and spices: medicinal uses and sustainable production

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Abstract: This introductory chapter contains a brief history of herbs and spices, including cultivation, trade and uses. The cultivation requirements of important herbal spices are discussed, as well as uses of herbs and spices in food and beverages, perfumes and cosmetics, and medicinal and nutraceutical uses. The important flavour compounds in major culinary and herbal spices are considered. Other topics discussed in this chapter are antioxidants isolated from herbs and spices, active plant constituents and the molecular phytopharmacology of a few herbs and spices. It also deals with biosafety and efficacy issues from a phytochemical perspective.

Key words: acids, alcoholic beverages, alkaloids, anthraquinones, antioxidant and antimicrobial properties, biosafety, bitters, colouring agents, cosmetics, coumarines, flavones, glycosides, gums, herbal remedies, herbs, medicinal and nutraceutical uses, perfumery, pharmaceuticals, resins, saponins, spices, tannins, volatile oils.

1.1 Introduction

The history of herbs and spices is as long as the history of mankind. People have used these plants for medicinal purposes since the earliest times, and the knowledge of herbs has been handed down from generation to generation for thousands of years (Brown, 1995). The terms 'herb' and 'spice' have more than one definition. According to a common one, herbs are plants, some parts of which contain essential oils useful in food, medicine and/or cosmetics and which usually grow in temperate regions, both in the wild and under cultivation. They do not develop persistent woody tissue. Spices are generally derived from woody plants that grow in tropical areas. They have to be imported to other parts of the world, making them expensive. The earliest gardens were herb gardens and the present-day concept of a herb garden has developed largely from ancient Egyptian, Christian and Islamic traditions. Herbs gardens were planted about 4000 years ago in Egypt. Herb growing was often associated with temples, which required herbs and sacred flowers for daily worship and rituals. Both horticulture and botany began with the study of herbs. In most parts of the world, herbs are grown mainly as field crops or on a small

scale as a catch-crop among vegetables and ornamentals as they were thousands of years ago.

Ancient cultures of the Middle East, Greece, China and India revered the power of nature and developed herbal remedies based on the plants found in their home environments. The first known herbal compilation of herbal remedies was ordered by the King of Sumeria around 2000 BC and included 250 medicinal substances including garlic (Fetrow and Avila, 1999). In earlier times, medicinal plants were chosen for their colour or shape of leaves, for example, heart-shaped leaves were used for heart problems, while plants with red flowers were used for treating bleeding disorders. This primitive approach is called the Doctrine of Signatures. The lives of people and plants are more entwined than is often realized. About 80 % of the world population is dependent upon medicinal plants for primary healthcare, particularly in the developing economies where local communities are offered immediate access to safe and effective products so as to treat ill health through self-medication (Akerele, 1992). Currently there is great commercial interest in reinvestigating and developing new pharmaceuticals from natural sources, including herbs and spices. Lastly, it should also not be forgotten that many medicinal herbs are also food, oil and fibre plants and have always been grown for a range of purposes (Parry, 1969; Rosengarten, 1973; Andi *et al*., 1997). The popularization of traditional healthcare in most parts of the world has led to a tremendous demand for medicinal plants, which are often still collected from their natural habitats. This practice has the potential to lead to their depletion and, ultimately, their extinction. This chapter reviews the uses of herbs and spices, with a focus on medicinal uses, as well as the requirement for sustainable production methods.

1.1.1 Sustainable production of herbs and spices

People all over the world have picked and uprooted herbs from the wild since ancient times.

Medicinal herbs, in particular, have always been mainly collected from the wild, and the knowledge of where they grow and the best time to gather them has formed an important oral tradition among healers from many different countries and cultures. These ancient traditions aimed at successfully balancing supply and demand, allowing plant stock to regenerate seasonally. However, some of the most commonly used culinary herbs, such as chilli peppers (*Capsicum annuum* var. *annuum*) and basil (*Ocimum basilicum*), have such a long history of use and cultivation that truly wild plants have never been recorded. They presumably became extinct because of over-collection. Owing to the strong commercial pressures of food and pharmaceutical industries of today, unregulated gathering has led to severe genetic erosion of a range of herbs and spices. Wild populations frequently contain genes of value in plant-breeding – for instance to increase the levels of active principles, or to confer resistance to disease, so their conservation is important.

Out of about 2000 medicinal and aromatic plants traded in Europe, 1200–1300 are native to the continent with only 130–140 species predominantly derived from cultivated stock. Large-scale cultivation is one practice that can take the pressure off wild stocks. Countries with large-scale cultivation include Argentina, Chile, China, India and Poland (Kuipers, 1997). By enhancing cultivation, food and

pharmaceutical companies can have greater control over quality and supply while reducing pressure on populations of wild plants. Increased exchange of information and self-regulation between stakeholders and by manufacturers of herbal products is also desirable. *In situ* conservation measures and demonstration gardens can be incorporated into management systems for wild populations, and can raise awareness of conservation issues. Substitution by a different species with the same constituents can in addition take pressure off a vulnerable species (e.g. *Calendula officinalis* or *Sambucus nigra* as a substitute for Goldenseal, *Hydrastis canadensis*). Lastly, changing the method of harvest may make practices more sustainable. A Swiss-based herbal remedies and cosmetics company has found that arnica (*Arnica montana*) can be harvested sustainably if only sections of the above-ground parts of the plants are harvested. Sustainable cultivation can be an effective means of providing income for the poorest sectors of the society and can contribute social stability while supporting conservation. Exhaustive details of general herb and spice cultivation practices in India have been compiled by Parthasarathy and Rajeev (2010). Cultivation requirements for some herbs are summarized in Table 1.1.

1.2 Main uses of herbs and spices

1.2.1 Uses in the foods and beverage industry, perfume and cosmetics industries

Herbs and spices have tremendous importance in the way we live, as ingredients in food, alcoholic beverages, medicine, perfumery, cosmetics, colouring and also as garden plants. Spices and herbs are used in foods to impart flavour, pungency and colour. They also have antioxidant, antimicrobial, pharmaceutical and nutritional properties. In the USA, as well as other western countries, there is a trend towards the use of culinary herbs and spices to produce more appealing foods with reduced levels of fat, sugar and salt, and a more general increase in the popularity of ethnic foods from Asia and Latin America. Between 1970 and 2005, the overall per capita consumption of spices doubled, increasing from 1.6 pounds per year to 3.3 pounds per year; however, in the case of garlic, usage has increased more than six-fold (USDA ERS, 2011)

The basic effects of spices when used in cooking and confectionery can be for flavouring, deodorizing/masking, pungency and colouring (Table 1.2). They are also used to make food and confectionery more appetizing and palatable. Some spices, such as turmeric and paprika, are used more for imparting an attractive colour than for enhancing taste. Because of their antioxidant and antimicrobial properties, spices have dual function – in addition to imparting flavour and taste, they play a major role in food preservation by delaying the spoilage of food. Spices also form an important component in quite a few alcoholic beverages and beers (Table 1.3).

Many herbs and spices have been used in cosmetics, perfumery and beauty and body care since ancient times. The toiletries and allied industries use spices and herbs and their fragrant oils for the manufacture of soaps, toothpastes, face packs, lotions, freshness sachets, toilet waters and hair oils. They are essential ingredients in beauty care as cleansing agents, infusions, skin toners, moisturizers, eye lotions, bathing oils, shampoos and hair conditioners, cosmetic creams, antiseptic

Table 1.1 Cultivation requirements of some herbal spices and their uses

Plant	Propagation	Common uses
Anise	Annual. Seeds are sown in a dry, light soil in early summer. Seedlings should be thinned to 15–18 inches apart. Anise needs 120 frost-free days to produce fully ripened seed heads.	The aromatic seeds are used in cooking, in pot-pourris and in some simple home remedies.
Basil	Perennial. Grows easily from seed. It is frost sensitive. Basil needs medium-well-drained soil and full sun. Pinch tips and flower buds to promote bushiness.	The leaves are a classic complement to enrich the flavour of tomatoes; they are also used to enhance the flavour of salads, sauces and vegetables.
Chervil	Annual and resembles parsley. Seeds are sown in spring. Thin to 15 cm (6 inches) apart. Likes moist, well-drained soil and partial shade. Will self-sow.	The leaves, with their delicate anise-like flavour, are often used in soups and salads.
Lavender	Perennial, with many varieties. English lavender is the hardiest. Mulch it over the winter. Propagation is easiest by root division. Likes full sun and alkaline, gravelly soil.	Grown for its fragrance in the garden and to be used in pot-pourris and sachets.
Oregano	Perennial. Prefers well-drained slightly alkaline soil and full sun. Propagate by seed, root division or cuttings.	The leaves are a favourite seasoning for pizza and other Italian dishes.
Parsley	Biennial, usually grown as an annual. Both the curly and the flat-leaved types like a rich, well-drained soil and full sun or partial shade. Parsley seeds germinate slowly. Be patient; keep the soil moist. Thin to 20 cm (8 inches) apart.	Curly leaved parsley is popular as garnish, but flat leaved (Italian) parsley is more flavourful and is used as addition to salads and sauces. Parsley tea makes a healthful tonic.
Rosemary	Perennial, grown indoors in cold climates. Propagate by layering or cuttings. Rosemary needs full sun, and a sandy well-limed soil. Cut it back after flowering to prevent it from becoming leggy.	This is an aromatic flavouring for meat and poultry dishes. Also used for making wreaths.
Savory	Winter savory, a perennial, has a peppery, pungent flavour. Summer savory, an annual, is similar but more delicate. Plant seeds of summer savory in a rich, light, moist soil; thin to 20 cm (8 inches) apart. Winter savory thrives in poorer soil and with less water. It can be propagated by seed, division or cuttings.	Savory is used to flavour sausages and other meats and is sometimes included in a bouquet garni.
Thyme	Perennial. There are many species and varieties including lemon, English, golden and garden. The garden variety is the most popular for cooking. Thyme grows well on dry slopes; pruning after flowering will keep it from getting woody. Propagated by cuttings.	The leaves add pungent taste to meats and vegetables; thyme sprigs are a main ingredient in bouquet garni for soups and stews.

Source: Reader's Digest (1990).

Table 1.2 Basic uses of herbs and spices

Basic function	Major function	Subfunction
Flavouring	Parsley, cinnamon, allspice, dill, mint, tarragon, cumin, marjoram, star anise, basil, anise, mace, nutmeg, fennel, sesame, vanilla, fenugreek, cardamom, celery	Garlic, onion, bay leaves, clove, thyme, rosemary, caraway, sage, horseradish, Japanese pepper, saffron,
Deodorizing/ masking	Garlic, savory, bay leaves, clove, leek, thyme, rosemary, caraway, sage, oregano, onion, coriander	
Pungency	Garlic, savory, bay leaves, clove, leek, thyme, rosemary, caraway, sage, oregano, onion, coriander, Japanese pepper, mustard, ginger, horseradish, red pepper, pepper	Parsley, pepper, allspice, mint, tarragon, cumin, star anise, mace, fennel, sesame, cardamom, mustard, cinnamon, vanilla, horseradish, Japanese pepper, nutmeg, ginger
Colouring	Paprika, turmeric, saffron	

Source: Ravindran *et al*. (2002).

Table 1.3 Spices and herbs used in alcoholic beverages

Alcoholic beverages	Spices and herbs used
Vermouth	Marjoram, sage, coriander, ginger, cardamom, clove, mace, peppermint, thyme, anise, juniper berry
Gin	Coriander, juniper berry
Aquavit	Anise, fennel, dill, caraway
Curaçao	Cinnamon, clove, nutmeg, coriander
Kummel	Caraway, fennel, coriander
Anisette	Anise, fennel, nutmeg
Ganica	Cinnamon, cardamom, coriander, mint, fennel, clove, pepper
Geme de cumin	Cumin
Geme de cacao	Clove, mace, vanilla
Geme de menthe	Peppermint
Peppermint schnapps	Peppermint

Source: Ravindran *et al*. (2002).

and antitanning lotions and creams, and for improvement of complexion and purifying blood (Pamela, 1987; Ravindran *et al*., 2002). A few important chemical flavour constituents in herbs and spices are summarized in Table 1.4.

1.2.2 Medicinal and nutraceutical uses

Herbs and spices have been an essential factor in health care through the ages in all cultures. There are a number of different systems of herbal medicine, the most important of which are Chinese and Indian (*Ayurvedic*) systems of medicine. The use of major medicinal spices in Ayurveda has been reviewed by Mahindru (1982). Herbs and spices are prepared in a number of ways to extract their active

Table 1.4 Antioxidants isolated from herbs and spices

Spice and herbs	Systematic names	Substances and type of substances
Rosemary	*Rosemarinus officinalis*	Carnosic acid, carnosol, rosemarinic acid, rosmanol
Sage	*Salvia officinalis*	Carnosol, carnosic acid, rosmanol, rosmarinic acid
Oregano	*Origanum vulgare*	Derivatives of phenolic acids, flavonoids, tocopherols
Thyme	*Thymus vulgaris*	Thymol, carvacrol, *p*-cunene-2, 3-diol, biphehyls, flavonoids
Ginger	*Zingiber officinale*	Gingerol-related compounds, diarylheptanoids
Turmeric	*Curcuma domestica*	Curcumins
Summer savory	*Satureja hortensis*	Rosemarinic acid, carnosol, carvacrol, thymbol
Black pepper	*Piper nigrum*	Phenolic amides, flavonides
Red pepper	*Capsicum annum*	Capsaicin
Chilli pepper	*Capsicum frutescence*	Capsicin, capsaicinol
Clove	*Eugenia caryphyllata*	Eugenol, gallates
Marjoram	*Marjorana hortensis*	Flavonoids
Common balm	*Melissa officinalis*	Flavonoids
Licorice	*Glycyrrhiza glabra*	Flavonoids, licorice phenolics

ingredients for internal and external use, including infusions, decoctions, macerations, tinctures, fluid extracts, teas, juices, syrups, poultices, compresses, oils, ointments and powders. Important spices, their botanical names and their medicinal properties are given in Table 1.5.

Active plant constituents

Herbs and spices are rich in volatile oils, which give pleasurable aromas. In addition, herbs may contain alkaloids and glycosides, which are of greater interest to pharmacologists. Some of the main active constituents in herbs are as follows (Brown, 1995; De Guzman and Sienonsma, 1999):

- **Acids**: These are sour, often antiseptic and cleansing.
- **Alkaloids**: These are bitter, often based on alkaline nitrogenous compounds. They affect the central nervous system and many are very toxic and addictive.
- **Anthraquinones**: These are bitter, irritant and laxative, acting also as dyes.
- **Bitters**: Various compounds, mainly iridoides and sesquiterpenes with a bitter taste that increases and improves digestion.
- **Coumarines**: These are antibacterial, anticoagulant, with a smell of new-mown hay.
- **Flavones**: These are bitter or sweet, often diuretic, antiseptic, antispasmodic and anti-inflammatory. Typically yellow, and present in most plants.
- **Glycosides**: There are four main kinds of glycosides:
  - *cardiac*: affecting heart contractions;
  - *synogenic*: bitter, antispasmodic sedative, affecting heart rate and respiration;
  - *mustard oil*: acrid, extremely irritant;
  - *sulphur*: acrid, stimulant, antibiotic.
- **Gums and mucilages**: These are bland, sticky or slimy, soothing and softening.

Table 1.5 Important spices and their medicinal properties

Serial number	Common name	Botanical name	Medicinal uses
1	Ajowan	*Trachyspermum ammi* L. Sprague ex. Tussil	Antispasmodic, stimulant, tonic and carminative. Administered in flatulence, dyspepsia, diarrhoea and cholera. Effective in relaxing sore throat and in bronchitis. External application of fruit paste recommended in asthma. Used in preparation of ointment for checking chronic discharge. It is diuretic and carminative.
2	Allspice	*Pimenta dioica* (L.) Merr	Used to treat flatulence, dyspepsia and diarrhoea, rheumatism and arthritis. Remedy for depression, nervous exhaustion and stress. Has antioxidant properties.
3	Angelica	*Angelica archangelica* L.	Has antispasmodic, aphrodisiac, anticoagulant, bactericidal, carminative, diuretic, expectorant, nervine, stimulant and tonic properties. Used in stomach complaints, vomiting and leucoderma. Reduces accumulation of toxins.
4	Anise	*Pimpinella anisum* L	Carminative with good flavour and fragrance.
5	Asafoetida	*Ferula asafetida* L.	Stimulant of mucous membrane, carminative, antispasmodic, expectorant, laxative and digestive. Also used in asthma, bronchitis and whooping cough.
6	Basil	*Ocimum basilicum* L	Carminative and antimicrobial used against gas, nausea and dysentery.
7	Black caraway	*Bunium persicum* (Bosis) B Fedtsh	Stimulant and carminative. Used in treating diarrhoea, dyspepsia, fever, flatulence, stomachic, haemorrhoids and hiccups.
8	Black cumin	*Nigella sativa* L.	Seeds are carminative, stimulant, diuretic, emenagogue and galactogogue. They are used in mild cases of puerperal fever and skin eruptions. Alcoholic extract shows antibacterial activity. Also used as preservative.
9	Black mustard	*Brassica nigra* (L)Koch	Used against lung congestion, bronchial problems and inflammation.
10	Black Pepper	*Piper nigrum* L.	Used as an aromatic stimulant in cholera, vertigo and coma and as a stomachic in dyspepsia and flatulence. Externally valued for its rubefacient properties. Used to protect against filariasis.
11	Capers	*Capparis spinosa* L.	Reduce flatulence and have antirheumatic and antioxidant properties. Used as hepatic stimulants, diuretics, kidney disinfectants, vermifuges and tonics.
12	Caraway	*Carum carvi* L.	Antispasmodic and used against gas pains.

Table 1.5 *Continued*

Serial number	Common name	Botanical name	Medicinal uses
13	Cardamom	*Elettaria cardamomum* Maton	Used as adjuvant to carminative drugs, as stomachic and in dyspepsia. Home remedy for indigestion, nausea, halitosis, bronchial infections, skin diseases, inflammations, itching and poisons.
14	Capsicum	*Capsicum annum* L	Source of capsasin, capsorubin and vitamins C, A and E, it has health-enhancing effects in clearing lungs, increasing flow of digestive juices, triggering brain to release endorphins (pain killers) and anti-oxidant and as a muscle relaxant. Used in flavouring and colouring food products.
15	Celery	*Apium graveolens* L	Carminative and sedative used against gas pains and as a tonic.
16	Cinnamon	*Cinnamomum verum*	Carminative, antispasmodic, aromatic stimulant, diuretic, haemostatic, astringent, stomachic and germicide. Used in pain balms, cold, cough and gastric troubles. It also has antimicrobial and anti-oxidant properties.
17	Clove	*Syzygium aromaticum* Merr & Perry	Aromatic, stimulant and carminative, used in gastric irritation and dyspepsia. Administered in powdered form to relieve nausea and vomiting, to correct flatulence. Oil used as local analgesic for hypersensitive dentine and carious cavities. Has antiseptic and pain-relieving qualities.
18	Coriander	*Coriandrum sativum* L.	Fruits carminative, diuretic, tonic, stomachic, antibilious, laxative, refrigerant and aphrodisiac. Fruits and leaves used against colic, dizziness, kidney stones, indigestion and sore throat.
19	Cumin	*Cuminum cyminum* L.	Seeds are stimulant, carminative, stomachic, astringent and useful in diarrhoea and dyspepsia and in veterinary medicine. Is an appetite stimulant and good digestive. Used for common gastrointestinal upsets.
20	Dill	*Anethum graveolens* L	Folk remedy for infant cholic and digestive disorders.
21	Fennel	*Foeniculam vulgare* Mill	Antispasmodic and used in indigestion and stomach cramps.
22	Fenugreek	*Trigonella foenum-graceum* L.	Seeds are carminative, tonic, anti-arthritic and galactogogue. Used externally in poultices as emollient for intestinal inflammations. Aqueous extract shows antibiotic activity. Used in treatment of chronic bronchitis, diabetis, hepato- and splenomegaly.

Table 1.5 *Continued*

Serial number	Common name	Botanical name	Medicinal uses
23	Galangal	*Kaempferia galanga*	Stimulant, expectorant, carminative and diuretic. Also used for dyspepsia, headache and malaria.
24	Garlic	*Allium sativum* L.	Has a significant carminative effect with a release or nausea. It brings about a decrease in triglycerides and cholesterol. Oil drops used in earache. Preparations given in pulmonary phthisis, bronchiectasis, gangrene of the lung and whooping cough. Used in laryngeal tuberculosis, lupus and duodenal ulcers and pulmonary tuberculosis. Used in dyspepsia, flatulence and colic. Antiseptic, antispasmodic and used in lowering cholostrol and reducing hypertension.
25	Ginger	*Zingiber officinale* Rosc	Carminative, stimulant, remedy for flatulence and colic, adjunct to stimulant remedies. Contains antihistaminic factor, remedy for diarrhoea and constipation, anorexia and indigestion. Ginger tea is used for colds, coughs, flu and hangovers. Ginger compresses are used to relieve sinus congestion, kidney problems, menustral cramps and various aches and pains. Its is also rubefacient.
26	Greater galangal	*Alpinia galangal* L. Willd.	Rhizomes are bitter, acrid, thermogenic, nervine tonic, stimulant, carminative, stomachic, disinfectant, aphrodisiac, bronchodilator and have tonic properties. Also known for antimicrobial, antifungal, antiprotozoal and expectorant activities. Used in skin diseases, indigestion, colic, dysentery, enlarged spleen, respiratory diseases, cholera, mouth and stomach cancer.
27	Horse radish	*Armoracia rusticana* Gart.	Antimicrobial, diuretic, stimulant and diaphoretic. Used in treatment of arthritis, respiratory and urinary infections and fevers.
28	Juniper	*Juniperous communis* L	Diuretic and carminative.
29	Kokkam and cambodje	*Garcinia indica* Chiocy *Garcinia cambogia* Desr.	Source of natural red pigment and hydroxycitric acid which reduces cholesterol and used as anti-obesity agent. It is used against bilious infections, dysentery, mucous diarrhoea, etc.
30	Lavender	*Lavendula officinalis* Chaix	Carminative, spasmolytic, tonic and antidepressant. Used in headache, neuralagia, rheumatism, depression, etc.

Table 1.5 *Continued*

Serial number	Common name	Botanical name	Medicinal uses
31	Long pepper	*Piper longum* L.	As a stimulant, anticolic, antitussive and inducing resistance to infections. Fruits and roots used in respiratory tract diseases, as counter-irritant and analgesic, as a snuff in coma and drowsiness, sedative in insomnia and epilepsy, as a cholagogue, emmenogogue and abortifacient and as ingredient in rejuvenating medicine.
32	Lovage	*Levisticum officinale* W.D.J.Koch	Used against gas pains and breath deodorizer.
33	Marjoram	*Marjorana hortensis* M	Source of sweet marjoram oil. Anti-oxidant antispasmodic, antimicrobial, carminative, stimulant and nerve tonic. Used in asthma, coughs, indigestion, rheumatism, tooth ache and heart conditions.
34	Mints	*Mentha piperita* L (pepper mint), *M. spicata* (spear mint)	Menthol from peppermint is added in many medicines for its therapeutic effects. They have carminative and emmenagogue effects. They make refreshing herbal teas.
35	Nutmeg	*Myristica fragrans* Houtt.	Used as stimulant, carminative, astringent, aphrodisiac, tonic, electuaries and forms constituent of preparations prescribed for dysentery, stomach ache, flatulence, nausea, vomiting, malaria, rheumatism, sciatica and early stages of leprosy. Mace has been recommended for treatment of inflammations of bladder and urinary tract. Butter is a mild external stimulant in ointments and hair lotions. Used in helminthiasis, cough, asthma, amenorrhoea, dysmenorrhoea, etc.
36	Onion	*Allium cepa* L	External antiseptic has many medicinal properties. Also used in reducing intestinal disorders, hypertension, diabetes, cholesterol, fat in the blood and inflammation.
37	Oregano	*Origanum vulgare*	Rich in vitamins E and B6, riboflavin, niacin, pantothenate and biotin. Is an antioxidant, carminative, stomachic, diaphoretic and expectorant. Used in colic, coughs, headaches and irregular menstrual cycles.
38	Parsley	*Petroselenium crispum* Mill	Used as liver tonic, laxative, carminative and against kidney stones. Relieves flatulence and colic. Rich in minerals and vitamins A, C.
39	Pomegranate	*Prunica granata* L.	Astringent, anthelminthic and used against tapeworm. Cooling and refrigerant and used against dysentery and diarrhoea.

Table 1.5 *Continued*

Serial number	Common name	Botanical name	Medicinal uses
40	Rosemary	*Rosemarinum officinalis* L.	Carminative, antidepressant, anticarcinogenic, antispasmodic, rubefacient, antimicrobial and anti-inflammatory. Used in pulmonary diseases and as an antidiarrhoeic, antidiabetic, antispasmodic and antidepressant.
41	Sage	*Salvia officinalis* L	Used for excessive sweating, fever and nervous disorders. Carminative and antiseptic.
42	Saffron	*Crocus sativus*	
43	Star anise	*Illicium verum* Hooker	Antimicrobial, carminative, diuretic and stomachic. Used in digestive disturbances, cough mixtures and colic pain.
44	Summer savory	*Satureja hortensis* L.	Aromatic, carminative and has expectorant properties.
45	Sweet flag	*Acorus calamus* L	Constituent of tonics. Also has antacid, purgative, anti-oxidant, antimicrobial and anti-insecticidal properties. Used in skin and hair care and also as stimulant.
46	Tarragon	*Artemisia dracunculus* L	Diuretic, stimulant and emmenagogue.
47	Thyme	*Thymus vulgaris* L.	Used in bronchitis and whooping cough. Has antimicrobial, antifungal, anti-oxidant, spasmolytic and anti-inflammatory activities.
48	Turmeric	*Curcuma longa* L.,	As an ingredient of curry powders, improves flavour and functions as antiseptic, antipoison factor. Aromatic stimulant tonic, carminative and anthelmintic. Paste of turmeric and neem leaves is applied to facilitate the process of scabbing. Used in treating eosnophilia. Ingredient of recipes intended for promotion of health and intelligence of children. As stomachic, tonic, blood purifier, antiperiodic, alterative, etc. Anti-oxidant and has anticarcinogenic and anti-AIDS properties.

Sources: Ravindran *et al*. (2002); Peter (2004); Reader's Digest (1990).

- **Resins**: Often found as oleoresins or oleogum resins – they are acrid, astringent, antiseptic, healing.
- **Saponins**: These are sweet, stimulant hormonal, often anti-inflammatory, or diuretic, soapy in water.
- **Tannins**: These are astringent, often antiseptic, checking bleeding and discharges.
- **Volatile oils**: These are aromatic, antiseptic, fungicidal, irritant and stimulant.

Table 1.6 Molecular phytopharmacology of a few herbs and spices

Plant	Active principle	Molecular action	Uses
Piper longum	Piperine	RNA synthesis	Antiviral
Curcuma longa	Curcumin	Protein synthesis	Against Alzheimer's
Mangifera indica	Mangiferin	Macrophage activation	Immunostimulant
Coleus forskohlii	Forshlin	cAMP increase	Against glaucoma

Source: Vaidya (2002).

Selected modern research into the medicinal properties of herbs and spices

The lower incidence of adverse reactions to herbal medicines and decreased cost as compared to conventional pharmaceuticals are driving national healthcare institutions to consider plant medicine as an alternative to synthetic drugs. Pharmaceutical firms recognize the potential of natural products to provide novel drugs as well as templates for the development of improved versions of the existing treatments for human illnesses. The 'natural' movement towards increased use of herbs and spices has also slowly begun to reduce the demand for synthetically derived drugs. 'Bioprospecting' of natural resources has impetus around the world; the search for new and novel molecules as therapeutic agents is extensive. Studies indicate that around 60 % of the antitumour and anti-infective agents in the later stages of clinical trials have plant origin (Singh *et al*., 2000). Many medicinal herbs used in Ayurveda have multiple bioactive principles. It is not always easy to isolate compounds and demonstrate that the efficacy can be attributed to any one of the active principles. However, the active principles and their molecular mechanism of action of some of the medicinal plants are being studied (Table 1.6). Further examples of research into medicinal plants are given below.

Researchers have found a positive linear correlation between phenolic compounds, primarily phenolic acids and flavonoids, and the antioxidant capacity of herbs and spices. As several metabolic diseases and age-related degenerative disorders are closely associated with oxidative processes in the body, the use of herbs and spices as a source of antioxidants to combat oxidation warrants further attention. Immediate studies should focus on validating the antioxidant capacity of herbs and spices after harvest, as well as testing their effects on markers of oxidation. Table 1.7 lists some antioxidants identified in herbs and spices and their extracts. Plant phenols may scavenge free radicals involved in lipid peroxidation as has been documented (Madsen *et al*., 1996). Miguel (2009) has reviewed the antioxidant activity of medicinal and aromatic plants.

In terms of the prevention of cancer, turmeric has been identified as a spice that decreases expression of receptor tyrosine kinases (RTKs) such as epidermal growth factor receptor (EGFR) and HER2 (Aggarwal and Shishodia, 2006). (RTKs are key regulators of normal cellular processes and also play a crucial role in the development and progression of many types of cancer). As described by Aggarwal and Shishodia, 'The activator protein-1 (AP-1) pathway is linked to growth through regulation, cell transformation, apoptosis, cellular proliferation, repression of tumour-suppressor genes, as well as being involved in the stages of tumour

Table 1.7 Important flavour compounds in major culinary and herbal spices

Spices	Important flavour compounds
Culinary spices	
Allspice	Eugenol, ß-caryophyllene
Anise	(E)-anethole, methyl chavicol
Black pepper	Piperine, S-3-carene, ß-caryophyllene
Caraway	*d*-carvone, carone dervatives
Cardamom	α-Terpinyl acetate, 1-8-cineole, linalool
Cinnamon, cassia	Cinnamaldehyde, eugenol
Chilli	Capsaicin, dihydrocapsaicin
Clove	Eugenol, eugeneyl acetate
Coriander	*d*-Linalool, C10-C14-2-alkenals
Cumin	Cuminaldehyde, *p*-1, 3-mentha-dienal
Dill	*d*-Carvone
Fennel	(E)-anethole, fenchone
Ginger	Gingerol, shogaol, neral, geranial
Mace	α-Pinene, sabinene, 1-terpenin-4-ol
Mustard	Ally isothiocynate
Nutmeg	Sabinine, α-pinene, myristicin
Parsley	Apiol
Saffron	Safranol
Turmeric	Turmerone, zingeberene, 1,8-cineole
Vanilla	Vanillin, *p*-OH-benzyl-methyl ether
Herbal spices	
Basil, sweet	Metylchavicol, linalool, methyl eugenol
Bay laurel	1, 8-cineole
Marjoram	*e*- and *t*-Sabinene hydrates, terpinen-4-ol
Oregano	Carvacrol, thymol
Origanum	Thymol, carvacrol
Rosemary	Verbenone, 1-8-cineole, camphor, linanool
Sage, clary	Salvial-4 (14)-en-1-one, linalool
Sage, Dalmatian	Thujone, 1,8-cineole, camphor
Sage, Spanish	*e*- and *t*-Sabinylacetate, 1,8-cineole, camphor
Savory	Carvacrol
Tarragon	Methyl chavicol, anethole
Thyme	Thymbol, carvacrol
Peppermint	*1*-Menthol, menthone, menthfuran
Spear mint	*1*-Carvone, carvone derivatives

metastasis…Quercetin, which is an active component in basil, coriander, cumin, and fennel, as well as curcumin, and capsaicin have been shown to suppress AP-1 activation…Coriander and fennel have been found to decrease expression of both mitogen-activated protein kinase (MAPK) pathway and c-Jun *N*-terminal kinase (JNK), which is another component of MAPK pathways' (Aggarwal and Shishodia, 2006), so may have a role to play in cancer prevention.

The role of curcumin as an antioxidant, anticarcinogenic and anti-HIV agent is fast changing our acceptance of herbal components in pharmaceuticals (Farooqi *et al*., 2000). The antidiabetic and hypocholosterolaemic effect of fenugreek ensures its use in various antidiabetic preparations. Ginger is used as an anti-emetic for cancer chemotherapy. The hypoglycemic and hypocholesterolaemic properties of

black caraway (*Carum carvi*) oil have been reported. Rosemary oil improves chronic circulatory weakness on external application. Extracts of *Ginseng biloba*, hawthorn, ginseng and garlic are good for cardiovascular disorders, such as hyperlipidaemia, cerebral and cardiac insufficiency. Peppermint oil, spearmint oil and extracts have been reported to be effective as inhibitors of helicobacteria. *Andrographis paniculata* is used as an astringent anodyne tonic and is useful in treatment of jaundice due to the andrographolide lactones present in it. *Aloe vera* gel is used for preparing health drinks due to presence of anthrone and polysaccharides (Farooqi *et al*., 2000).

1.3 Safety and efficacy issues: a phytochemical perspective

The potential for a herbal product to cause adverse reactions can be assessed from the perspective of the phytochemical content of the plant. Reports of phytochemicals with adverse reaction profiles are available. Tannin-containing herbs can inhibit trace element and B vitamin absorption. They should therefore not be used in high doses for long periods. Saponins are gastric irritants. Hence, doses of herbs which contain saponins can cause reflux and/or vomiting in sensitive individuals. The alternative is to give them in enteric-coated tablets or with meals. Pungent herbs such as capsicum and ginger (*Zingiber officinale*) may lead to gastroesophageal reflux. In the case of herbs that contain mustard oils such as horseradish (*Armoracia rusticana*), the burning sensation is real and can cause considerable gastric discomfort. High doses of ginger can cause heartburn.

Controlled trials of herbal products are necessary to establish safety and efficacy; manufacturing standards are required to ensure product quality. Ideally, quality control and assurance methods should be defined for each product on the market. Without adhering to standards for purity, potency, disintegration and dissolution, a consistent, high-quality product cannot be produced. Powdered herbs and extracts in oral dosage forms are the most popular with consumers. Specific compounds from a bulk herb may be extracted and prepared for delivery. Extracts concentrate the active components. They may be taken as such or made into a fluid extract or tincture including a solvent. Tinctures are made when the active component is not water soluble. Tinctures and freeze-dried herbs are preferred to those that are dried or encapsulated because there is less loss of potency by oxidation. The chemical stability of these products is complex, making it a challenge to determine expiration or shelf-life dating. Botanicals packaged in dosage forms for medicinal purposes may behave as drugs, even if they are not regulated as such. Pharmacokinetic data remain quite limited (Desmet and Brouwers, 1997).

The American Spice Trade Association (ASTA) Safety Guidelines for Spices Sold in the United States insists that steps need to be taken at every step throughout the process of growing, harvesting, drying, and processing spices to ensure that clean, safe spice is ultimately delivered to the consumer. ASTA advise the following of good agricultural practices (GAP) (to minimize the potential for contamination of spices by heavy metals, mycotoxins, pesticide residues, etc). ASTA also provide guidelines on handling and storage of materials to minimize the contamination risks. The society encourages using the following techniques: good manufacturing

practices (GMP) (processing of spices, facility construction and design, maintenance of the grounds, equipment design pest control, etc.); a hazard analysis critical control point (HACCP) plan (a key analytical tool to allow for the identification of physical, chemical and microbiological risks and the steps to prevent the resulting risks to food safety); microbial reduction techniques (to ensure spices are free of pathogens); and supply chain management (to ensure clean, safe spice).

Future research into the medicinal properties of herbs and spices should focus on identifying the key molecules in the cell signalling network which are affected by components of herbs and spices and elucidation of their mechanisms of action. A range of bioactive compounds in herbs and spices have been studied in animals for their anti-carcinogenic properties (among many others), but the challenge lies in integrating this knowledge to ascertain whether any effects can be observed in humans, and within defined cuisines.

1.4 The structure of this book

This book is the second volume of the series *Handbook of herbs and spices (Second edition)*. A group of introductory chapters address general issues of importance to those using herbs and spices in consumer products, such as antimicrobial and antioxidant properties. The following chapters contain detailed information on particular herbs and spices. The crops are organized alphabetically and range from ajowan to tamarind. Two chapters on a selection of less-commonly encountered herbs and spices complete the volume. It is hoped that the two volumes of the second edition of *Handbook of herbs and spices* will form a useful reference work for all those involved in the study, cultivation, trade and use of herbs and spices.

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2

Herbs, spices and their active components as natural antimicrobials in foods

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Abstract: In the context of the recent upward trend of foodborne illness incidences caused by the consumption of food contaminated with bacteria and the increasing resistance of foodborne pathogens to antimicrobials, this chapter looks at the potential role of essential oils as antimicrobial agents in food preparation. The chapter provides an overview of the published data in this area and presents the methodologies in use for evaluation of their effectiveness. The challenges faced by the food industry in respect of the use of essential oils in food safety and spoilage prevention are discussed and current hypotheses regarding the mode of action of natural preservatives are reviewed.

Key words: essential oil, food preservation, food safety, foodborne pathogens, antibacterial activity, MIC, active packaging, biofilm disinfection, carvacrol, thymol.

2.1 Introduction: a need for 'new' preservatives

Various preservative agents are currently used to ensure that manufactured foods remain safe and unspoiled. The rising number and severity of food poisoning outbreaks worldwide has increased public awareness about food safety issues. In this context, public concern has particularly been stimulated as a result of the recent scares involving BSE in many European countries and *Escherichia coli* O157:H7 in the USA, Japan, Australia and Scotland. The annual healthcare costs associated with selected foodborne pathogens such as *Listeria monocytogenes*, *E. coli* O157:H7 and *Salmonella* spp. are estimated at €5–6 billion per year (WHO, 2002). On the other hand, the excessive use of chemical preservatives, many of which are believed to have potential carcinogenic and/or teratogenic actions as well as having residual toxicity, has resulted in mistrust among European consumers. The increasing resistance of various foodborne pathogens to antimicrobials is also of major concern.

In order to remedy the aforementioned problems, the food industry and European authorities have to demonstrate increased alertness towards food quality and safety issues. Consumers need to feel reassured that the foods they consume are safe; there is therefore increasing pressure on food manufacturers and

authorities to eliminate harmful chemical preservatives from food preparations, as well as to prioritize research activity that may generate alternative – more effective, non-toxic, natural or synthetic – preservatives. However, the development and approval of novel, safer and more potent synthetic chemical preservatives is a time-consuming process; current research interest has therefore focused on the development and application of more 'natural' means of food preservation. In this context, the use of essential oils from edible and medicinal plants, herbs and spices is of great interest, since they constitute a class of very potent, natural antibacterial agents (Nychas *et al.*, 2003). Their use in food systems may be considered as an additional intrinsic component to increase the safety and shelf-life of foods.

The main objective of this chapter is to provide an overview of the published data on the antibacterial activity of natural antimicrobial systems and present the methodologies in use for the evaluation of their effectiveness, both *in vitro* and *in situ.* Moreover, the challenges that the food industry is facing with respect to the use of such substances as antibacterial agents in food safety and spoilage prevention will also be discussed. Current hypotheses regarding the mode of action of natural preservatives and the stress–response mechanisms that they might induce in food-borne pathogens are also reviewed.

2.1.1 History of the use of herbs and spices as preservatives

The use of essential oils as flavouring substances has been known for many years: Greek and Roman historians have reported the use of the essential oil of turpentine (Guenther, 1948). The oil was prepared by steam distillation; a method that was developed by the eastern civilizations of Egypt, India and Persia (Guenther, 1948) and improved on in the ninth century by the Arabs (Bauer *et al.*, 2001), and which still constitutes the main method for essential oil preparation. During the Renaissance, many essential oils were produced in pharmacies and their pharmacological effects were gradually included in pharmacopoeias (Bauer *et al.*, 2001). For example, the use of tea tree oil for medical purposes was well documented at the end of the eighteenth century (Carson and Riley, 1993). The first experimental determination of the bactericidal properties of essential oil vapours was carried out by Dela Croix in 1881 (Boyle, 1955). However, in the course of the nineteenth and twentieth centuries, the role and applications of essential oils in medicine gradually diminished relative to their uses for flavour and aroma purposes (Guenther, 1948).

Currently, essential oils are used in food preparations mainly as flavouring agents; they are also widely used by cosmetic and pharmaceutical industries as fragrances and functional additives (Bauer and Garbe, 1985). The individual components of the essential oils – either extracted from plant material or synthetically manufactured – are also used as food flavourings (Oosterhaven *et al.*, 1995). These natural substances have been suggested for use in foodstuffs (Farag *et al.*, 1989) because they have been found to display a wide range of antimicrobial properties, for example against bacteria, fungi and mycobacteria (Conner and Beuchat, 1984a,b; Galli *et al.*, 1985). For the time being, only a limited number of preservatives containing such natural compounds are commercially available. One example is 'DMC Base Natural', a food preservative produced by DOMCA S.A., Alhendin, Granada, Spain; this preservative comprises a mixture of the essential oils of rosemary, sage

and citrus (50 %) and glycerol (50 %) (Mendoza-Yepes *et al.*, 1997). Another example of these natural preparations is the 'Protecta' range of blended herb extracts produced by Bavaria Corp., Apopka, FL, USA.

2.1.2 Potential applications and barriers to the adoption of flavouring substances as antimicrobials in foods

The pharmaceutical, cosmetic, meat and flavour industries are the main end users of spices, herbs and their compounds. Although the majority of essential oils are classified as generally recognized as safe (GRAS) (Kabara, 1991), their use in foods as preservatives is often limited due to flavour considerations, since effective antimicrobial doses may exceed organoleptically acceptable levels. The potential for using these compounds as natural antimicrobial agents is therefore not well exploited when compared with their use as compounds that enhance the flavouring and antioxidant effect of foods.

This problem could be possibly overcome if research was able to answer the following questions. (i) Can the inhibitory effect of a certain essential oil, be attributed to only a few of its constituents? (ii) Does the essential oil provide a synergy of activity, which simple mixtures of components cannot deliver? (iii) What is the minimum inhibitory concentration of the active compound(s) of the essential oil that will enhance its use in food preservation? (iv) What is the behaviour of such substances in foods of homogeneous (liquid–semi-solid) or heterogeneous (emulsions/mixtures of solid and semi-solid substrate) structure? (v) Can the efficacy of these substances be enhanced by combining their use with traditional (salting, heating, acidification, etc.) and modern (modified atmosphere packaging (MAP), vacuum packaging) methods of food preservation?

In order to answer these questions, the antimicrobial activity of these compounds should be precisely and accurately determined and investigated. This has not been the case so far. The methodologies used for the evaluation of the inhibitory actions of these compounds have certain limitations which have contributed to the contradictory results reported in the literature; these limitations are discussed below. In addition, *in situ* studies are quite laborious to perform and the mode of application of the crude essential oils in foods has a significant effect on the results and conclusions drawn. For instance, immersion, mixing, encapsulation, surface-spraying, evaporating in active packaging are some promising methods of adding these compounds in foods, but their effects on the efficacy of the essential oils have not yet been extensively investigated.

2.2 Chemical composition of flavouring substances produced from herbs and spices

Steam distillation is the method most commonly used to produce flavouring substances on a commercial basis. Extraction by means of liquid carbon dioxide, under low temperature and high pressure, is a more expensive alternative that provides a more natural organoleptic profile (Burt, 2004). Essential oils obtained by distillation and those produced by solvent extraction also have different organoleptic profiles

and chemical compositions; these differences in composition will in turn influence their antimicrobial properties. Literature reports have indicated that essential oils extracted from herbs with hexane exhibit superior antimicrobial activities compared with the corresponding essential oils obtained by steam distillation (Burt, 2004). It must be noted, however, that since all essential oils are volatiles, they have to be stored in airtight containers in the dark to prevent compositional changes.

Analysis of the chemical composition of essential oils obtained from plants has been carried out using gas chromatography and mass spectrometry (Salzer, 1977; Daferera *et al.*, 2000; Juliano *et al.*, 2000; Jerkovic *et al.*, 2001; Delaquis *et al.*, 2002). A typical essential oil scan comprises more than 60 individual components (Senatore, 1996; Russo *et al.*, 1998). The major components account for up to 85 % of the oil, whereas the minor components are present in only trace amounts (Senatore, 1996; Bauer *et al.*, 2001). The major component content of many economically interesting essential oils has been reviewed by Bauer *et al.* (2001) and the main constituents of essential oils that possess significant antibacterial activities are presented in Table 2.1. Despite the presence of many major constituents that display antibacterial activity, the antibacterial properties of some essential oils are mainly attributed to their phenol monoterpenes content (Cosentino *et al.*, 1999). However, there is also much experimental evidence that minor constituents play a critical role in antibacterial activities, possibly via a synergistic effect with other components. This has been examined in detail for the essential oils of sage (Marino *et al.*, 2001), thyme (Paster *et al.*, 1995; Marino *et al.*, 1999) and oregano (Paster *et al.*, 1995).

Table 2.1 Major components of selected essential oils that display significant antimicrobial properties

Essential oil	Major components	Composition (%)	References
Origanum vulgare (oregano)	Carvacrol	0–89	Lawrence, 1984; Prudent *et al.*, 1995; Sivropoulou *et al.*, 1996; Kokkini *et al.*, 1997;
	Thymol	0–64	Russo *et al.*, 1998; Daferera *et al.*, 2000;
	γ-Terpinene	2–52	Demetzos and Perdetzoglou, 2001;
	p-Cymene	0–52	Marino *et al.*, 2001; Chorianopoulos *et al.*, 2004, 2007
Origanum dictamus	Carvacrol	52–55	Chorianopoulos *et al.*, 2004, 2007
	γ-Terpinene	7–14	
	p-Cymene	9–13	
	Linalool	4	
Thymus vulgaris (thyme)	Thymol	10–64	McGimpsey *et al.*, 1994; Cosentino *et al.*, 1999; Marino *et al.*, 1999; Daferera *et al.*, 2000; Juliano *et al.*, 2000;
	p-Cymene	10–56	
	γ-Terpinene	2–31	
	Carvacrol	2–11	

Table 2.1 *Continued*

Essential oil	Major components	Composition (%)	References
Thymus longicaulis	Carvacrol	16–61	Chorianopoulos *et al.*, 2004, 2007
	Geraniol	3–42	
	Thymol	4–32	
	γ-Terpinene	4–13	
	p-Cymene	0–8	
	Geranyl acetate	0–10	
	Borneol	0–8	
	Linalool	0–7	
	β-Caryophyllene	0–6	
Cinnamomum spp. (cinnamon)	*Trans*-cinnamaldehyde	65	Burt, 2004
Syzygium aromaticum (clove)	Eugenol	75–85	Bauer *et al.*, 2001
Satureja thymbra	Thymol	13–41	Chorianopoulos *et al.*, 2004, 2006a
	Carvacrol	4–39	
	γ-Terpinene	11–25	
	p-Cymene	9–18	
	β-Caryophyllene	4–8	
Satureja spinosa	Carvacrol	27–44	Chorianopoulos *et al.*, 2004
	Thymol	15–24	
	γ-Terpinene	6–11	
	p-Cymene	6–9	
	β-Caryophyllene	5–9	
Salvia officinalis (sage)	α-thujone	20–42	Marino *et al.*, 2001
	Camphor	6–15	
	1, 8-Cineole	6–14	
	β-Pinene	2–10	
	α-Pinene	4–5	
Rosmarinus officinalis (rosemary)	1, 8-Cineole	3–89	Daferera *et al.*, 2000, 2003; Pintore *et al.*, 2002
	α-Pinene	2–25	
	Bornyl acetate	0–17	
	Camphor	2–14	

It must also be pointed out here that the chemical composition of any essential oil is very dependent on the cultivation site and harvesting period of the crop (Arras and Grella, 1992; Marotti *et al.*, 1994; McGimpsey *et al.*, 1994; Cosentino *et al.*, 1999; Marino *et al.*, 1999; Juliano *et al.*, 2000; Faleiro *et al.*, 2002; Chorianopoulos *et al.*, 2004, 2006a, 2007). This can be explained by considering the biosynthetic pathways of the major components of the oil. For example, in the case of *Origanum*, *Satureja* and *Thymus* species, *p*-cymene and γ-terpinene constitute the precursors of their phenol monoterpenes carvacrol and thymol (Fig. 2.1; Muller-Riebau *et al.*, 1997; Cosentino *et al.*, 1999; Jerkovic *et al.*, 2001; Ultee *et al.*, 2002; Chorianopoulos *et al.*, 2006a). Research on the essential oils of Greek *Origanum*, *Satureja* and *Thymus* plants has indicated that these monoterpenes always represents the bulk of the

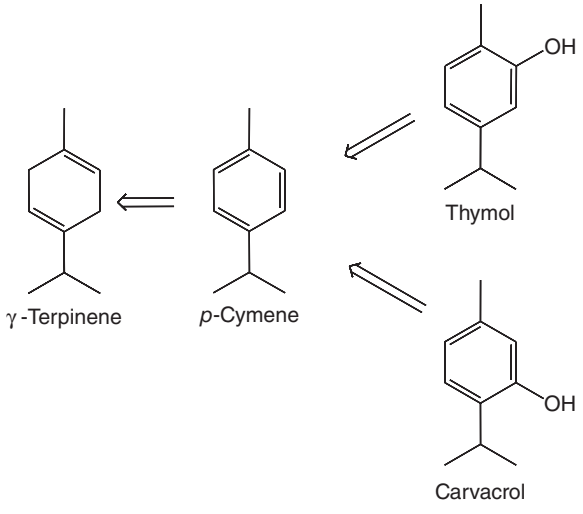

Fig. 2.1 Biosynthesis of monoterpenic phenols (carvacrol and thymol).

essential oil, regardless of cultivation site (Kokkini *et al.*, 1997; Chorianopoulos *et al.*, 2004, 2006a, 2007) and/or harvesting time (Fig. 2.2; Jerkovic *et al.*, 2001; Chorianopoulos *et al.*, 2006a); therefore any changes in the levels of these four constituents will greatly affect the composition of the oil.

Similar findings were obtained for essential oils of *Thymus vulgaris* from Italy (Marino *et al.*, 1999) and Greek *Satureja thymbra* and *S. parnassica* (Chorianopoulos *et al.*, 2006a). Figure 2.2 shows the seasonal variation of these compounds for the essential oil of *S. thymbra* (Chorianopoulos *et al.*, 2006a), indicating that during the premature vegetative stage, γ-terpinene and *p*-cymene constitute the major components of the essential oil. As the flowering period approaches, there is a simultaneous gradual diminishing of monoterpene precursors and their phenolic metabolites. Thus, during the full flowering period, carvacrol prevails as the major component, while the end of the flowering stage delineates a sharp decrease in carvacrol levels and the predominance of thymol as the major component of the essential oil. A few months later, as the premature vegetative stage approaches, the level of monoterpene precursors is restored (Fig. 2.2).

The aforementioned data indicate that the four compounds are biologically and functionally associated, supporting the theory that thymol and carvacrol are biosynthesized from *p*-cymene and γ-terpinene (Fig. 2.1; Kokkini *et al.*, 1997; Chorianopoulos *et al.*, 2006a). In this regard, it is also evident that essential oils obtained during (or immediately after) the plant's flowering season, will exhibit the most significant antimicrobial activities (McGimpsey *et al.*, 1994; Marino *et al.*, 1999; Chorianopoulos *et al.*, 2004, 2006a, 2007). Finally, the composition of essential oils obtained from different parts of the same plant may also vary significantly. For example, essential oil obtained from coriander seeds (*Coriandrum sativum* L.) has a quite different chemical composition to the essential oil of cilantro, which is produced from the immature leaves of the same plant (Delaquis *et al.*, 2002).

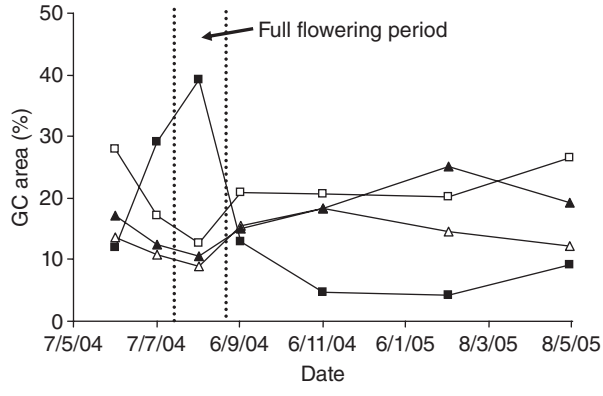

Fig. 2.2 Seasonal variation of the major constituents (■: carvacrol, □: thymol, ▲: γ-terpinene and Δ: *p*-cymene) of *Satureja thymbra* plants; Values at x-axis cover period of 12 months – 7/6/2004 to 7/5/2005 (data from Chorianopoulos *et al*., 2006a, modified accordingly).

2.3 *In vitro* antimicrobial activities of herbs, spices and their components

2.3.1 Methods used to assay antimicrobial activity

The antimicrobial activities of plant-derived compounds against diverse types of microbes, including foodborne pathogens, are well documented in the literature (Davidson and Branen, 1993; Nychas, 1995; Nychas *et al.*, 2003). However, these results are not directly comparable, since various distinct and divergent data have been reported for the same antimicrobial compound and/or mixture (Mann and Markham, 1998; Manou *et al*., 1998; Skandamis, 2001; Skandamis *et al*., 2001, Chorianopoulos *et al*., 2007). Moreover, it is not always clear whether the cited method is capable of assaying the bacteriostatic or bactericidal activities separately, or simply measures the combined result of both activities.

The assay methods reported in the literature use various measurements to record results. The following indices have been measured and reported.

1. the bacterial growth inhibition zone around a paper disc containing the compound (or mixture) tested, on various non-specific substrates (Farag *et al.*, 1989; Aureli *et al.*, 1992; Kim *et al.*, 1995a,b; Senatore *et al.*, 2000; Wilkinson *et al.*, 2003; Chorianopoulos *et al*., 2004, 2007; Proestos *et al.*, 2005);
2. the minimum inhibitory concentration (MIC) that is necessary to inhibit the bacterial growth (Carson *et al.*, 1995a; Wan *et al.*, 1998; Cosentino *et al.*, 1999; Hammer *et al.*, 1999; Lambert and Pearson, 2000; Lambert *et al.*, 2001; Chorianopoulos *et al.*, 2006b);
3. the inhibition of bacterial growth on an agar medium when the tested compound (or mixture) is diffused in agar (Deans and Ritchie, 1987; Smith-Palmer *et al.*, 1998; Wan *et al.*, 1998; Dorman and Deans, 2000);
4. the optical density changes of a growth medium (non-selective broth) to which inoculum and antimicrobial compound(s) have been added (Shelef *et al.*, 1984; Kim *et al.*, 1995a; Sivropoulou *et al.*, 1996; Lambert and Pearson, 2000; Lambert *et al.*, 2001; Chorianopoulos *et al.*, 2006b);

5. the changes in the impedance of a non-specific growth medium (broth), with or without the addition of antimicrobial compounds (Smith-Palmer *et al.*, 1998; Chorianopoulos *et al.*, 2004, 2006a,b);
6. the comparative bacteriostatic activities of the antimicrobial compounds in an agar-diffusion, versus a serial dilution assay (Farag *et al.*, 1989; Stecchini *et al.*, 1993; Juven *et al.*, 1994; Pandit and Shelef, 1994; Quattara *et al.*, 1997; Hammer *et al.*, 1999; Wilkinson *et al.*, 2003).

The results obtained using any of the above methods can be affected by: (i) the sample composition (plant species, geographical location and collection period); (ii) the microorganism investigated (strain, conditions of growth, inoculum, etc.); and (iii) the method used to grow and enumerate the surviving bacteria. In addition, much of the literature data has been based on subjective observations – as in the disc diffusion method or the various rapid techniques such as the measurement of the optical density (turbidimetry). More specifically, in the disc diffusion technique the inhibition area depends on the essential oil being uniformly diffused through the agar medium or the oil vapours being released uniformly onto the bacteria (Nychas *et al.*, 2003). The antimicrobial assay is also influenced by the presence of multiple active components, which at low concentrations display antagonistic, additive or synergistic activities. Thus, the rate of partition of active components between lipid and aqueous phases influences the antimicrobial activity of an essential oil, thereby producing different results when the oil is tested in a complex (e.g. food) or in a simple system (Stecchini *et al.*, 1993, 1998).

Compared with the viable counts (VC) techniques (techniques 1–3 described above), turbidimetry (technique 4) is a rapid, non-destructive, inexpensive and relatively easy to automate method but has the serious disadvantage of low sensitivity. In addition, turbidimetry detects only the upper parts of the growth curves; absorbance differences are evident only when population levels reach 10^6–10^7 cfu/ml. Correction procedures and calibration methods are often required in order to correlate turbidimetric results with viable counts results (Koch, 1981; Bloomfield, 1991; Cuppers and Smelt, 1993; McClure *et al.*, 1993; Dalgaard and Koutsoumanis, 2001; Skandamis *et al.*, 2001). Correlation between absorbance changes and viable counts numbers can only be assessed for identically treated samples, i.e. different calibration curves must be obtained for every differently treated sample. For example, the timing of the addition of the essential oil is crucial: addition of the oil prior to inoculation increases the initial absorbance in relation to controls. The size of bacterial cells, their physiological state (damaged, injured or healthy), the oxidation stage of the essential oil as well as the inadequate dissolution of the compound(s) tested can also affect the absorbance measurement in the growth media.

Unlike the VC techniques, impedance measurements (technique 5 above) record microbial metabolism in a real-time mode. Thus, the impedance method is widely recognized as a promising alternative, rapid technique, not only for screening the biocide activity of novel antimicrobial agents against food spoilage and pathogenic bacteria, but also for the estimation of growth kinetic parameters (Ayres *et al.*, 1993, 1998; Tranter *et al.*, 1993; Tassou *et al.*, 1995, 1997; Johansen *et al.*, 1995; Tassou and Nychas, 1995a,b,c; Koutsoumanis *et al.*, 1997, 1998; MacRae *et al.*, 1997; Lachowicz

et al., 1998; Chorianopoulos *et al.*, 2006b). These measurements can be accomplished by using a medium that offers a sharp detectable impedance change in accordance with bacterial population growth, thus converting the low-conductivity nutrients to highly charged products. It must be noted, however, that – for comparison reasons – a correlation between the standard plating procedure and the corresponding impedimetric data must be established (Dumont and Slabyj, 1993; Koutsoumanis *et al.*, 1998).

Finally, VC – the traditional microbiological method – should remain the gold standard procedure for determining the limitations of each the aforementioned methodologies. The VC method has the major advantage of requiring low capital investment; on the other hand, it is a laborious, material-intensive method that requires long lapse times and often exhibits low reproducibility and therefore there is much interest in the more recent techniques that rely on turbidity and impedance measurements.

2.3.2 Terms used in antimicrobial activity testing

The terms used to describe the outcome of tests investigating the antibacterial activity of the natural substances under consideration in this chapter are described below.

1. **Minimum inhibitory concentration (MIC)**: This has been defined in various ways as: (a) the lowest concentration that is essential to maintain the inoculum viability (Carson *et al.*, 1995a); (b) the lowest concentration that is required for complete inhibition of the test organism for up to 48 hours of incubation (Wan *et al.*, 1998; Canillac and Mourey, 2001); (c) the lowest concentration that inhibits the visible growth of the test organism (Karapinar and Aktug, 1987; Onawunmi, 1989; Hammer *et al.*, 1999; Delaquis *et al.*, 2002); (d) the lowest concentration that results in a significant decrease of inoculum viability (Cosentino *et al.*, 1999); and (e) the concentration above which no growth is observed relative to the control test (Lambert and Pearson, 2000).
2. **Minimum bactericidal concentration (MBC)**: The concentration capable of killing more than 99.9 % of the initial inoculum (Carson *et al.*, 1995b; Cosentino *et al.*, 1999; Canillac and Mourey, 2001).
3. **Non-inhibitory concentration (NIC)**: The concentration above which the inhibitor begins to display a negative effect on growth (Lambert and Pearson, 2000).
4. **Bacteriostatic concentration**: The lowest concentration at which bacteria fail to grow in broth systems but may be cultured when the broth is plated onto agar and/or the bacteria are not cultivable when the broth is plated onto agar with the bactericidal concentration (Smith-Palmer *et al.*, 1998).

2.3.3 Minimum inhibitory concentration (MIC)

It has already been mentioned that for successful incorporation of natural antimicrobials in food products it is necessary to establish a balance between sensory acceptability and antimicrobial efficacy. In this regard, there is an increasing demand for the accurate measurement of the MIC for these natural substances. This measurement can be accomplished by *in vitro* and *in vivo* studies, either separately

or together. The *in vitro* evaluation techniques (e.g. diffusion, dilutions, impedance and optical density methods), and their limitations, have already been delineated in detail (Koutsoumanis *et al*., 1998, 1999; Tassou *et al*., 2000; Dalgaard and Koutsoumanis, 2001; Skandamis *et al*., 2001; Lambert *et al*., 2001; Chorianopoulos *et al.*, 2006b). These reports have indicated that the dilution method provides accurate quantitative results (Manou *et al*., 1998) but that these results are not comparable with the corresponding results obtained by other methodologies (Tassou *et al*., 2000; Lambert *et al*., 2001; Skandamis *et al*., 2001).

The most frequently used methods of measuring MIC enumerate populations using either viable counts or optical density measurements (Burt, 2004). The first method is time-consuming and labour-intensive, while the second is automated but has the disadvantage of not taking into account various crucial parameters such as the possible oxidation of the essential oil, the physiological state of cells, etc. These limitations may possibly alter the results obtained (Nychas *et al.*, 2003). Other widely used methods for the MIC determination include the agar dilution technique (developed by Mann and Markham (1998)) and a novel microdilution technique that uses resazurin (a redox indicator) as the visual indicator for the MIC measurement. The latter is more sensitive compared with the dilution technique but relies on visual measurements (Burt, 2004), which are often subjective. A similar technique, developed by Burt and Reinders (2003) as a modification of the method described in Salvat *et al.*, 2001, uses a patented colour indicator (based on resazurin). This is an automated method that measures the end-point by fluorescence instead of visual means (Burt, 2004). In conclusion, it is evident that in complex systems the traditional methods of MIC assessment are time-consuming, resource-intensive and often introduce subjectivity into the evaluation procedure.

In contrast, a modification of the method developed by Lambert and Pearson (2000) to assay the inhibition of pure compounds (e.g. thymol) enables the quick and efficient measurement of MIC in complex systems and mixtures (Lambert *et al.*, 2001). More specifically, the method evaluates the effectiveness of various concentrations of the tested material through optical density measurements, leading to an MIC determination that is based on 'growth' or 'no growth' information (Lambert *et al.*, 2001). In a recent study (Chorianopoulos *et al.*, 2006b), a novel aspect was introduced to this method when optical density measurements were replaced with conductance measurements. The latter do not depend on either the active state of cells (including their shape and size) or the possible essential oil oxidation stage (Nychas *et al.*, 2003). The conductance measurements were recorded using a Malthus apparatus, which permits the measurement of cell metabolism, thereby displaying the essential oil inhibition effects as a delay in the bacteria metabolism in treated substances compared with the control. The results obtained using the impedance measurements produced better curve fittings compared with those obtained using optical density measurements, especially at concentrations near MIC. This may be rationalized by considering that the inactivated cells induce only absorbance (and not conductance) changes. Thus, the use of impedance measurements in MIC determination has the advantage of maximum sensitivity near the growth boundaries (where the bacterial metabolism is very slow). Table 2.2 summarizes the data available in the literature for MIC assays of natural substances that possess significant antimicrobial properties.

Table 2.2 MICs of essential oils or their components tested *in vitro* against foodborne pathogens (MICs from the references have been converted to % v/v)

Essential oil or component	Bacterial species	MIC range (% v/v)	References
Origanum spp.	*Escherichia coli*	0.05–0.12	Prudent *et al.*, 1995; Hammer *et al.*, 1999; Burt and Reinders, 2003
	Salmonella ser. Typhimurium	0.12	Hammer *et al.*, 1999
	Staphylococcus aureus	0.05–0.12	Prudent *et al.*, 1995; Hammer *et al.*, 1999
Thymus spp.	*Escherichia coli*	0.045–0.125	Farag *et al.*, 1989; Smith-Palmer *et al.*, 1998; Cosentino *et al.*, 1999; Hammer *et al.*, 1999; Burt and Reinders, 2003
	Listeria monocytogenes	0.016–0.045	Firouzi *et al.*, 1989; Smith-Palmer *et al.*, 1998; Cosentino *et al.*, 1999
	Salmonella ser. Typhimurium	0.045–> 2	Cosentino *et al.*, 1999; Hammer *et al.*, 1999
	Staphylococcus aureus	0.02–0.25	Farag *et al.*, 1989; Smith-Palmer *et al.*, 1998; Cosentino *et al.*, 1999; Hammer *et al.*, 1999
Satureja spp.	*Bacillus cereus*	0.049–0.058	Chorianopoulos *et al.*, 2006b
	Escherichia coli	0.054–0.066	Chorianopoulos *et al.*, 2006b
	Listeria monocytogenes	0.035–0.08	Chorianopoulos *et al.*, 2006a, 6b
	Salmonella ser. Enteritidis	0.056–0.094	Chorianopoulos *et al.*, 2006a, 6b
	Staphylococcus aureus	0.035–0.043	Chorianopoulos *et al.*, 2006b
Salvia spp.	*Escherichia coli*	0.35–0.5	Farag *et al.*, 1989; Smith-Palmer *et al.*, 1998; Hammer *et al.*, 1999
	Listeria monocytogenes	0.02	Smith-Palmer *et al.*, 1998
	Salmonella ser. Typhimurium	0.1–0.2	Shelef *et al.*, 1984; Hammer *et al.*, 1999
	Staphylococcus aureus	0.075–0.1	Shelef *et al.*, 1984; Farag *et al.*, 1989; Smith-Palmer *et al.*, 1998; Hammer *et al.*, 1999
Syzygium spp.	*Staphylococcus aureus*	0.04–0.25	Farag *et al.*, 1989; Smith-Palmer *et al.*, 1998; Hammer *et al.*, 1999
	Escherichia coli	0.04–0.25	Farag *et al.*, 1989; Smith-Palmer *et al.*, 1998; Hammer *et al.*, 1999
	Salmonella ser. Typhimurium	> 2	Hammer *et al.*, 1999
Rosmarinus spp.	*Bacillus cereus*	0.02	Chaibi *et al.*, 1997
	Escherichia coli	0.45–> 1	Farag *et al.*, 1989; Smith-Palmer *et al.*, 1998; Hammer *et al.*, 1999; Pintore *et al.*, 2002
	Listeria monocytogenes	0.02	Smith-Palmer *et al.*, 1998
	Salmonella ser. Typhimurium	> 2	Hammer *et al.*, 1999
	Staphylococcus aureus	0.04–1	Farag *et al.*, 1989; Smith-Palmer *et al.*, 1998; Hammer *et al.*, 1999; Pintore *et al.*, 2002
Carvacrol	*Bacillus cereus*	0.019–0.09	Cosentino *et al.*, 1999
	Escherichia coli	0.023–0.5	Kim *et al.*, 1995a; Cosentino *et al.*, 1999
	Listeria monocytogenes	0.038–0.5	Kim *et al.*, 1995a; Cosentino *et al.*, 1999; Pol and Smid, 1999

Table 2.2 *Continued*

Essential oil or component	Bacterial species	MIC range (% v/v)	References
	Salmonella ser. Typhimurium	0.023–0.025	Kim *et al.*, 1995a; Cosentino *et al.*, 1999
	Staphylococcus aureus	0.018–0.045	Cosentino *et al.*, 1999; Lambert *et al.*, 2001
Thymol	*Bacillus cereus*	0.045	Cosentino *et al.*, 1999
	Escherichia coli	0.023–0.045	Cosentino *et al.*, 1999
	Listeria monocytogenes	0.045	Cosentino *et al.*, 1999
	Salmonella ser. Typhimurium	0.005	Cosentino *et al.*, 1999
	Staphylococcus aureus	0.014–0.023	Cosentino *et al.*, 1999; Lambert *et al.*, 2001
Eugenol	*Escherichia coli*	0.1	Kim *et al.*, 1995a
	Listeria monocytogenes	> 0.1	Kim *et al.*, 1995a
	Salmonella ser. Typhimurium	0.05	Kim *et al.*, 1995a
α-Terpineol	*Bacillus cereus*	0.09	Cosentino *et al.*, 1999
	Escherichia coli	0.045–> 0.09	Cosentino *et al.*, 1999
	Listeria monocytogenes	> 0.09	Cosentino *et al.*, 1999
	Salmonella ser. Typhimurium	0.023	Cosentino *et al.*, 1999
	Staphylococcus aureus	0.09	Cosentino *et al.*, 1999

2.4 *In situ* antimicrobial activities of herbs, spices and their components

2.4.1 Assessment of antibacterial activities in food systems

Although there a limited number of food preservatives based on natural compounds that are commercially available, there was relatively little research activity in this field until the early 1990s, with only a few papers published on the topic (Board and Gould, 1991). An overview of the published literature related to the antibacterial effects of essential oils (or their components) in foodstuffs is presented in Table 2.3. It must be noted, however, that all potent antibacterial essential oils – assayed during *in vitro* studies – have to be used in higher concentrations in the foodstuffs themselves in order to produce similar effects (Shelef, 1983; Davidson, 1997; Smid and Gorris, 1999). Several reports have described the effects of essential oils on foodstuff bacteria but have failed to quantify these effects and/or delineate their mechanism of action.

Possible explanations for the activity differences seen in *in vitro* tests and in tests on the foodstuffs themselves may include: (i) the availability of nutrients in large quantities in food preparations, compared with quantities in the laboratory media, which may enable the fast repair of damaged cells by the respective bacteria (Gill *et al.*, 2002); and (ii) the sensitivity of the bacteria may be influenced by both intrinsic (fat/protein/water content, antioxidants, preservatives, pH, salt and other additives) and extrinsic (temperature, packaging in vacuum/gas/air, characteristics of micro-organisms) food properties (Shelef, 1983; Tassou *et al.*, 1995). In general, it is well

Table 2.3 Antimicrobial activity of essential oils – or their components – in various foodstuffs

Foodstuff	Essential oil or component	Bacterial species	Effectiveness*	References
Beef fillets	*Origanum* spp.	*L. monocytogenes*	B/C	Tsigarida *et al.*, 2000
Beef muscle slices	*Origanum* spp.	*L. monocytogenes*, *Pseudomonas* spp.	C	Oussalah *et al.*, 2004
Fresh beef	*Origanum* spp.	Natural flora	C	Skandamis and Nychas, 2002
Ground beef	*Cinnamomum* spp., *Satureja* spp.	Pathogenic microorganisms	C/D	Turgis *et al.*, 2008
Beef	*Rosmarinus* spp.	Natural flora	C	Nerin *et al.*, 2006
Fried meat	*Origanum* spp., *Thymus* spp.	*L. monocytogenes*, *E. coli*	C/D	Du and Li, 2008
Minced sheep	*Origanum* spp.	*Salmonella* ser. Enteritidis	B	Govaris *et al.*, 2010
Meat surfaces	*Origanum* spp.	*E. coli*	C/D	Mosqueda-Melgar *et al.*, 2008
Minced beef	*Thymus* spp.	*E. coli*	B/C	Solomakos *et al.*, 2008a
Minced beef	*Thymus* spp.	*L. monocytogenes*	B/C	Solomakos *et al.*, 2008b
Minced beef	*Origanum* spp.	Natural flora	C/D	Skandamis and Nychas, 2001
Minced beef	*Origanum* spp.	Natural flora	B/C	Ammor *et al.*, 2009
Minced pork	*Thymus* spp.	*L. monocytogenes*, *Pseudomonas* spp.	C/D	Aureli *et al.*, 1992
Minced pork	*Origanum* spp.	*C. botulinum* spores	D	Ismaiel and Pierson, 1990
Minced pork	*Satureja* spp.	Foodborne bacteria	B/C	Carraminana *et al.*, 2008
Pork liver sausage	*Rosmarinus* spp.	*L. monocytogenes*	B/C	Pandit and Shelef, 1994
Pork liver sausage	*Rosmarinus* spp.	*L. monocytogenes*	B	Hayouni *et al.*, 2008
Poultry patties	Thymol, carvacrol	Natural flora	C	Mastromatteo *et al.*, 2009
Coated chicken fillets	*Origanum* spp., *Rosmarinus* spp.	Natural flora	B/C	Ntzimani *et al.*, 2010
Chicken noodles	*Salvia* spp.	*B. cereus*, *S. aureus*, *Salm.* ser. Typhimurium	D	Shelef *et al.*, 1984
Fresh chicken breast	*Origanum* spp.	Natural flora	C	Chouliara *et al.*, 2007
Paté	*Mentha* spp.	*L. monocytogenes*, *Salm.* ser. Enteritidis	D	Tassou *et al.*, 1995
Coated tuna slices	Eugenol, linalool	Natural flora	C	Naidu, 2000
Fresh-water fish	Thyme hydrosol	Natural flora	B/C	Oral *et al.*, 2008
Salmon fillets	*Origanum* spp.	*Ph. phosphoreum*	D	Mejlholm and Dalgaard, 2002

Table 2.3 *Continued*

Foodstuff	Essential oil or component	Bacterial species	Effectiveness*	References
Cod fillets	*Origanum* spp.	*Ph. phosphoreum*	C	Mejlholm and Dalgaard, 2002
Asian sea bass	*Origanum* spp., *Thymus* spp.	Natural flora	C	Harpaz *et al.*, 2003
Sea bream fillets	*Origanum* spp.	Natural flora	B	Goulas and Kontominas, 2007
Sea bream	*Origanum* spp.	*S. aureus*, *Salm.* ser. Enteritidis	C	Tassou *et al.*, 1996
Red grouper fillet	Carvacrol	*Salm.* ser. Typhimurium	A/B	Kim *et al.*, 1995b
Cooked shrimps	*Thymus* spp.	*Ps. putida*	C	Quattara *et al.*, 2001
Taramasalata	*Origanum* spp.	*Salm.* ser. Enteritidis	B	Koutsoumanis *et al.*, 1999
Taramasalata	*Mentha* spp.	*Salm.* ser. Enteritidis, *L. monocytogenes*	D	Tassou *et al.*, 1995
Taramasalata	*Origanum* spp.	*E. coli*	A/B	Skandamis *et al.*, 2002
Semi-skimmed milk	Carvacrol	*L. monocytogenes*	D	Karatzas *et al.*, 2001
Mozzarella cheese	*Syzygium* spp.	*L. monocytogenes*	C	Vrinda-Menon and Garg, 2001
Soft cheese	DMC	*L. monocytogenes*	C/D	Mendoza-Yepes *et al.*, 1997
Yoghurt	*Syzygium* spp., *Cinnamomum* spp.	*Streptococcus thermophillus*	B/C	Bayoumni, 1992
Tzatziki	*Mentha* spp.	*Salm.* ser. Enteritidis, *L. monocytogenes*	B/C	Tassou *et al.*, 1995
Tzatziki	*Origanum* spp.	*E. coli*	A/B	Skandamis *et al.*, 2002
Carrots	*Thymus* spp.	*E. coli*	C	Singh *et al.*, 2002
Carrot broth	Thymol, carvacrol	*B. cereus*	C/D	Valero and Frances, 2006
Carrot broth	Thymol, carvacrol	*B. cereus*	B/C	Valero and Giner, 2006
Lettuce	*Thymus* spp.	*E. coli*	C/D	Singh *et al.*, 2002
Lettuce	BMC	Natural flora	C	Wan *et al.*, 1998
Eggplant salad	*Origanum* spp.	*E. coli*	B/C	Skandamis and Nychas, 2000
Rice	Carvacrol	*B. cereus*	B/C	Ultee *et al.*, 2000
Rice	*Salvia* spp.	*B. cereus*, *S. aureus*, *Salm.* ser. Typhimurium	D	Shelef *et al.*, 1984
Rice	*Ocimum* spp.	Stored-rice pests	B/C	Lopez *et al.*, 2008
Honeydew melon	Carvacrol	Natural flora	C/D	Roller and Seedhar, 2002
Table grapes	Thymol	Fungi	B	Valero *et al.*, 2006
Kiwi fruit	Carvacrol	Natural flora	C	Roller and Seedhar, 2002

* Characterization of the essential oil – or component – effectiveness (extension of lag phase and/or reduction in the final population), where A: intense, B: mediocre, C: slight and D: negligible.

established that the susceptibility of bacteria to the antimicrobial effects of essential oils increases when there is a decrease in parameters such as pH, storage temperature and amount of oxygen within the packaging (Tassou *et al.*, 1995, 1996; Skandamis and Nychas, 2000; Tsigarida *et al.*, 2000). In particular, at lower pH values the hydrophobicity of an essential oil increases, resulting in easier dissolution of the oil in the cell membrane lipids of the target bacteria (Juven *et al.*, 1994).

It is also evident that the presence of large quantities of fat and/or protein in foodstuffs protects the bacteria from the action of the natural antimicrobials (Aureli *et al.*, 1992; Pandit and Shelef, 1994; Tassou *et al.*, 1995). Moreover, foodstuffs contain lower amounts of water – compared with laboratory media – and this hampers the approach of antibacterial agents to the bacterial cell target sites (Smith-Palmer *et al.*, 2001). For example, the presence of mint oil in high-fat products such as paté and fish roe salad produced only a limited antibacterial effect against *L. monocytogenes* and *Salm.* ser. Enteritidis; when the same essential oil was used in cucumber and yoghurt salads (both constitute low-fat and low-pH products) it was much more effective (Tassou *et al.*, 1995). Large quantities of water and/or salt were also found to facilitate the action of essential oils (Shelef *et al.*, 1984; Tassou *et al.*, 1995; Skandamis and Nychas, 2000).

The reaction between carvacrol – a phenolic component of various essential oils – and proteins is considered to be the limiting factor in the antibacterial activity of the oils against *Bacillus cereus* in dairy products (Pol *et al.*, 2001). Furthermore, the presence of carbohydrates in foods does not appear to protect the bacteria from the action of the essential oils, in contrast to the protective role of fats and proteins (Shelef *et al.*, 1984).

Another parameter limiting the antibacterial activity of an essential oil is the physical structure of the foodstuff. Research reports on the performance of oregano oil against *Salm.* ser. Typhimurium, in broth and gelatine gel, have revealed that the gel matrix dramatically reduced the inhibitory effect of the essential oil, presumably because the diffusion was limited by the structure of the gel matrix (Skandamis *et al.*, 2000). In general, the MIC value of an essential oil on a bacterial isolate is slightly lower in broth, as compared with the corresponding value determined in agar (Hammer *et al.*, 1999). Research concerning the growth characteristics of *L. monocytogenes* and *Yersinia enterocolitica* in oil-in-water emulsions indicated that – depending on the mean droplet size of the emulsion – the bacteria may grow in films or colonies, or as planktonic cells (Brocklehurst *et al.*, 1995). It is also well established that the colonial growth restricts the diffusion of oxygen (Wimpenny and Lewis, 1977), while the cells that are situated within the colony are somehow shielded from the emulsion substrates by the outer cells. Thus, by altering the size of the oil droplets in a food emulsion it is possible to promote the bacterial growth within colonies by protecting them from the action of the essential oil.

In meat and meat products (Table 2.3), the essential oils exhibit more pronounced antibacterial activities, as compared with other antibacterial agents used in meat preparations. Carvacrol, oregano and thyme essential oils have already been recognized as effective agents, since they have been found to inhibit pathogens and autochthonous spoilage flora in meat products, by causing a marked initial reduction in cell numbers (Aureli *et al.*, 1992; Stecchini *et al.*, 1993; Tsigarida *et al.*, 2000; Skandamis and Nychas, 2001). In contrast, in the same systems, mint and sage oils

were found to be much less effective (Shelef *et al.*, 1984; Tassou *et al.*, 1995). It is also evident that the high fat contents markedly reduce the activity of the essential oils in meat products (Tassou *et al.*, 1995). In this regard, literature reports have indicated that encapsulated rosemary oil is much more effective against *L. monocytogenes* in pork liver sausage as compared with pure rosemary oil (Pandit and Shelef, 1994). Finally, the activity of oregano essential oil against *Clostridium botulinum* spores was studied in a vacuum packed and pasteurized minced (ground) pork product (Ismaiel and Pierson, 1990).

Chouliara *et al*. (2007) studied the combined effect of oregano essential oil and MAP on the shelf-life extension of fresh chicken meat stored at 4 °C. Based primarily on sensory data, the shelf-life of aerobically packaged fresh chicken meat was 5 days. The addition of 0.1 % oregano essential oil extended the product's shelf-life by 3–4 days, while MAP extended shelf-life by 2–3 days. The combination of both packaging and oregano essential oil extended shelf-life by 5–6 days. Mastromatteo *et al*. (2009) studied the combined effect of thymol, carvacrol and temperature (0–18 °C) on the quality of non-conventional poultry patties (a mix of ostrich, chicken and turkey meats) packaged in air and MAP. Regarding these active compounds, it was reported that thymol was less effective than carvacrol, while the combination of packaging and thymol had an additive effect in reducing total viable counts. In another study, Ntzimani *et al*. (2010) investigated the effect of natural antimicrobial treatments (rosemary and oregano oil) and their combinations on the shelf-life of semi-cooked coated chicken meat stored in vacuum packages at 4 °C. Of the antimicrobial combination treatments examined in this study, the essential oil treatments were the most effective against the growth of gram-negative and gram-positive bacteria. The presence of rosemary oil (0.2 %, v/w) in cooked samples produced a distinct, but acceptable, pleasant odour and taste, well received by the panellists. The application of oregano oil in cooked chicken samples was not as pleasant as rosemary oil. Based on both microbiological and sensory analyses, treatments with essential oils produced a shelf-life extension of 7–8 days compared with control samples.

Studies on the use of essential oils in fish (Table 2.3) have indicated that the high fat content of some fish reduced the effectiveness of antibacterial essential oils. For example, oregano oil was assayed as being more effective against the spoilage organism *Photobacterium phosphoreum* in cod fillets, than it was in salmon which is a fattier fish (Mejholm and Dalgaard, 2002). The same essential oil displayed superior antibacterial activity in fish when compared with mint essential oil, even in fatty fish dishes (Tassou *et al.*, 1995; Koutsoumanis *et al.*, 1999).

Similar experiments on dairy products (Table 2.3) have indicated that the essential oil of mint acts as an effective antibacterial agent against *Salm*. ser. Enteritidis in low-fat yoghurts (Tassou *et al*., 1995). In vegetables (Table 2.3), the antimicrobial activity of the essential oils was enhanced – as was the case in the corresponding meat products – by a decrease in the food's storage temperature and/or pH (Skandamis and Nychas, 2000). It should be noted that, in general, vegetables have a low fat content and this therefore enhances the antibacterial activities of essential oils in vegetables. Consequently, all essential oils (and their components) tested on vegetables were assayed as effective antibacterial agents against the natural spoilage flora and foodborne pathogens (Wan *et al.*, 1998; Singh *et al.*, 2002). More specifically,

oregano oil inhibited the growth of *E. coli* O157:H7 in eggplant salads by considerably reducing its final populations (Skandamis and Nychas, 2000). In rice (Table 2.3), the essential oil of sage was ineffective against *B. cereus*, whereas carvacrol was assayed as being very effective, showing the ability to extend the lag phase and considerably reduce the final population of the bacteria (Shelef *et al.*, 1984; Ultee *et al.*, 2000). Finally, in fruits (Table 2.3), carvacrol was screened as being an effective antibacterial agent that reduces the viable counts of the natural flora on kiwi fruit. The same essential oil was less effective when tested on honeydew melon (Roller and Seedhar, 2002).

2.4.2 Active packaging

Packaging converts foodstuffs into a more convenient form and simultaneously protects them against microorganisms, and biological and chemical changes, assuring a longer shelf-life for the packaged foods (Tsigarida *et al.*, 2000). As a result, packaging has become an indispensable element of the food manufacturing process. Over the past few decades, in order to meet the food industry's growing demands, much research activity has been focused on the development of efficient food packaging techniques. Among the various packaging technologies developed by (and for) the food industry, MAP is responsible for the evolution – over the last 20 years – of fresh and minimally processed food preparations, particularly for meat and meat products (Skandamis and Nychas, 2002).

In such packaging systems, the initial atmosphere is generated either by enabling the air to be enclosed or by injecting a desired initial gas mixture. This blend is then altered by changes in any one or more of the following variables: (i) permeation of oxygen, carbon dioxide and water vapours through the packaging material; (ii) transmission of oxygen, carbon dioxide and water vapour through the seal and defective structural areas; (iii) temperature of the packaging material, which may lead to small changes in permeation; (iv) surface area of the packaging material; and (v) thickness of the packaging material (Tsigarida and Nychas, 2001). Such changes influence/affect the contribution of different elements of the associated microbial population, and consequently affect the shelf-life of the packaged food. It is noteworthy, however, that despite the extended shelf-life of refrigerated products – when stored under vacuum/MAP conditions – there is an increased concern about the growth/survival of microaerophilic psychrotrophic pathogens (Garcia de Fernando *et al.*, 1995). Thus, additional precautions should be taken in order to ensure the safety of such products.

'Smart', 'interactive' and 'active' packaging (Labuza, 1996; Han, 2000; Katz, 2000; Skandamis and Nychas, 2002; Kerry *et al.*, 2006; Nerin *et al.*, 2006; Winther and Nielsen, 2006; Ammor *et al.*, 2009) are terms that have been used to describe some innovative concepts in packaging structures. Various packaging types rely on changes in packaging conditions to extend the shelf-life of a foodstuff or improve its safety and/or sensory characteristics, without affecting the quality of the food. Since most food packaging systems consist of the packaging material, the preserved food and the headspace in the package, antimicrobial agents may either be initially incorporated into the packaging materials and migrate into the food through diffusion and/or partition or be released through evaporation in the headspace. The latter may be

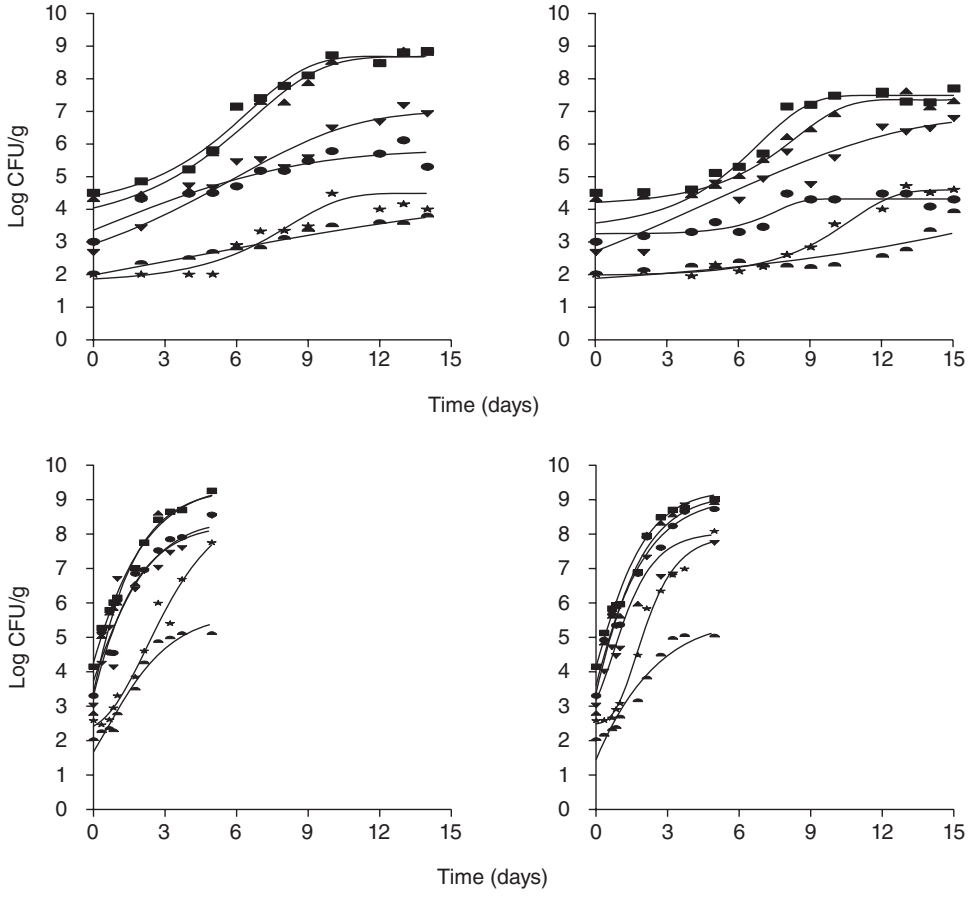

Fig. 2.3 Changes of microbial association of meat stored under 40 % CO_2 / 30 % O_2 / 30 % N_2 modified atmosphere packaging conditions at 5 °C (top diagrams) and 15 °C (bottom diagrams) with (right diagrams) or without (left diagrams) the presence of volatile compounds of oregano essential oil (active packaging) where, total viable count: ■, pseudomands: ▲, *Br. thermosphacta*: ▼, lactic acid bacteria: ●, enterobacteriaceae: ★ and yeasts: ◓ (data from Skandamis, 2001, modified accordingly).

accomplished through the use of antibacterial essential oils which are volatile and are regarded as 'natural' alternatives to chemical preservatives (Skandamis and Nychas, 2002). In addition, their use in foods meets the current demand from consumers for mildly processed or natural products (Nychas, 1995). For the time being, the practical applications of these essential oils are limited because of their strong aroma and/or their decreased effectiveness when interacting with the food's ingredients or structure. Nevertheless, their application in active packaging is of great importance (Juven *et al.*, 1994; Skandamis and Nychas, 2002; Ammor *et al.*, 2009). Figure 2.3 demonstrates the effect of volatile compounds of oregano essential oil (active packaging) on microbial populations in meat stored under 40 % CO_2–30 % O_2–30 % N_2 MAP conditions at 5 and 15 °C (Skandamis and Nychas, 2002).

2.4.3 Natural antimicrobials for biofilm disinfection

The use of essential oils as antimicrobial agents is well known in the literature (Nychas *et al.*, 2003; Burt, 2004). Limited information is available, however, on the comparative evaluation of essential oils and/or their by-products (eg. hydrosols) as disinfectants against bacterial biofilms. For example, polytoxinol, a topical essential oil-based formulation, displays potent antibacterial activity against biofilm-forming strains of coagulate-negative staphylococci (Al-Shuneigat *et al.*, 2005). In addition, the effectiveness of various essential oil components has been demonstrated against biofilm strains of *E. coli* and *Pseudomonas* spp. (Niu and Gilbert, 2004), while a recent report of Lebert *et al.* (2007) indicated that the essential oil of *S. thymbra* is effective against spoilage and pathogen bacteria on monoculture and mixed-culture biofilms that are associated with traditional fermented sausages. Furthermore, Mattos de Oliveira *et al.* (2010) applied different disinfectant solutions based on the essential oils of *Cimbopogon citratus* and *Cimbopogon nardus* against *L. monocytogenes* bacterial biofilm.

A recent study by Chorianopoulos *et al.* (2008) showed that chemical sanitizers such as lactic acid, HCl, ethanol and NaOH failed to eliminate biofilms from stainless steel surfaces efficiently. In contrast, the essential oil of *S. thymbra* – and/or its hydrosol – when tested as natural sanitizers on the same surfaces, was found to possess very potent disinfectant activities against bacterial species grown as monocultures or as mixed-culture biofilms (Chorianopoulos *et al.*, 2008). In most experiments the natural sanitizers caused the maximum possible log-reduction in the bacterial population of the biofilms. It must be noted, however, that despite the biofilm damage caused by these natural sanitizers, the corresponding data for conductance measurements revealed that small biofilm residues still remained on the surfaces (Chorianopoulos *et al.*, 2008). This indicates the usefulness of the impedance method as a tool to quantify the remaining biofilm (Giaouris *et al.*, 2005) that may exist following the incomplete sanitation.

Despite the strong antimicrobial activity of essential oils on biofilms, their practical application is hampered by limitations related to their strong smell, when used at effective doses (Davidson, 1997), and the difficulty in flushing them from surfaces satisfactorily. In this regard, the hydrosols of the essential oils constitute aqueous solutions which can easily be rinsed from surfaces and still possess potent antimicrobial activities (Sagdic, 2003; Sagdic and Ozcan, 2003). Additional advantages of hydrosols are the fact that they are currently a non-useful by-product of essential oils production by steam distillation, and the fact that they have a light odour compared with the initial essential oil. Thus, the food industry should devote more research effort towards the application of these hydrosols in surface cleaning, since they represent a promising tool in food safety procedures with obvious financial benefits (Chorianopoulos *et al.*, 2008).

2.5 Mode of antimicrobial action

Although the antimicrobial effects of essential oils (and their active components) are well established, their mechanism of action is poorly understood (Nychas and Tassou, 2000). There is much published research reporting investigations into the

mode of action of bacteriocins and weak acids, while there are very few reports discussing possible mechanism of actions for essential oils (or their active components). One generally accepted view is that the mode of action of an essential oil is dependent on the concentration of its active components (Prindle and Wright, 1977). More specifically, at low concentrations, they affect the activity of enzymes associated with energy production, while in higher concentrations they cause the precipitation of proteins. It must be noted, however, that this argument is the subject of dispute. For example, Judis (1963) questioned whether the alleged damage caused to cell membranes is directly related to the amount of active antibacterial compounds that act directly on cells or whether the effect proceeds as a result of the initially small damage caused, which is then followed by the cell breakdown.

Nowadays, there are many different mechanisms proposed in the literature to delineate the antimicrobial activities of essential oils (or their active components). For example, it has been reported that the inhibitory action of essential oils is achieved through the impairment of various enzyme systems, including those involved in energy production and structural component synthesis. Investigations on the antimicrobial action of essential oils and their components have indicated that they have deleterious effects on cellular membranes, for example on their permeability, and cause structural–functional damage to plasma membranes. This membrane impairment is mainly reflected by the dissipation of the two components of the proton motive force: the pH gradient (ΔpH) and the electrical potential ($\Delta\psi$) (Sikkema *et al*., 1995; Davidson, 1997; Ultee *et al*., 1999, 2000, 2002). In particular, carvacrol – the active component of many essential oils – is reported to act not only through the destabilization of the cytoplasmic membrane by disintegrating the outer membrane, but also as a 'proton exchanger' which results in a further reduction of the pH gradient across the cytoplasmic membrane (Helander *et al*., 1998; Lambert *et al*., 2001; Ultee *et al*., 2002). This proton motive force collapse and the depletion of the adenosine triphosphate (ATP) pool leads eventually to cell death (Ultee *et al*., 2002).

These detrimental effects of antimicrobial compounds on proton motive force are strongly correlated with the leakage of specific ions (Kroll and Booth, 1981; Bakker and Mangerich, 1981). Indeed, the action of various preservatives – including essential oils, phenols and bacteriocins – on the permeability barrier of the cytoplasmic membrane results in the leakage of various substances, such as ions, ATP, nucleic acids and amino acids, e.g. glutamate, etc. (Tranter *et al*., 1993; Gonzalez *et al*., 1996; Tahara *et al*., 1996; Ultee *et al*., 1999; Helander *et al*., 1998; Cox *et al*., 1998; Tassou *et al*., 2000). In this regard, it is reported that the essential oils of tea and mint, as well as carvacrol, are capable of causing leakage of cellular material, e.g. material absorbing at 260 nm and K^+ (Cox *et al*., 1998; Gustafson *et al*., 1998; Ultee *et al*., 1999). Further cell damage may also be related to nutrient uptake, nucleic acid synthesis and ATPase activity, etc. Several reports have demonstrated that most essential oils (at approximately 100 mg/l) impair the respiratory activity of different bacteria or yeasts (e.g. *Saccharomyces cerevisiae*) (Conner *et al.*, 1984a,b; Denyer and Hugo, 1991; Tassou *et al*., 2000).

Unlike many antibiotics, essential oils are capable of gaining access to the periplasm of gram-negative bacteria, through the porin proteins of their outer membrane (Helander *et al*., 1998), since the cell membrane permeability depends on:

(i) the hydrophobicity of the solution which has to cross the membrane and (ii) the membrane composition (Sikkema *et al*., 1995; Helander *et al*., 1998; Ultee *et al*., 2002). Temperature is another parameter that also affects the activity of essential oils, since at low temperature their solubility decreases, thereby hampering membrane penetration (Wanda *et al*., 1976). Furthermore, the partition coefficient of an essential oil on cell membranes constitutes a crucial determinant of its inhibitory effectiveness, since it is an indication of whether the cell wall plays an important role in the relative resistance to whole-cells lysis. This latter point explains the quantitative differences reported in the literature in the activities of various essential oils against bacteria (degree of sensitivity), especially when bacteria with different gram-staining responses were examined.

The solubility of trace elements present in the essential oils – for example iron, which in many bacteria is as an enzyme co-factor that permits their oxygenation – is negatively affected by the antimicrobial activity of these natural products. The presence of these oils has been shown to influence the growth rate of aerobic or facultative aerobic bacteria. Finally, the reaction of ferrous iron with phenolic compounds can indirectly cause damage to the cells through oxidative stress (Friedman and Smith, 1984; Nagaraj, 2001).

Another possible mode of action might include interactions (formation of complexes) between the antimicrobial constituents (e.g. phenolic constituents) and the bacterial membrane components (proteins) involved in cell membrane biosynthesis. Thus, highly reactive aldehyde groups of various natural compounds (e.g. citral, salicyldehyde, etc.) form Schiff bases that modify (or prevent) cell wall biosynthesis (Friedman, 1996, 1999; Patte, 1996). The previously described molecular mechanisms of antimicrobial activity against pathogens may be inhibited or enhanced, depending on the complexity of the microbial ecology and the food environment in which the antimicrobial is added.

Antimicrobial agents, including antibiotics and/or related chemical or medicinal substances, have substantially reduced the threat from various infectious diseases. These compounds have greatly contributed to the increased life expectancy at the end of the twentieth century, but their benefits are currently under scrutiny because of the emergence and spread of microbes that have gained resistance to the antimicrobials in common use. Since many of these bacteria may also contaminate foodstuffs, it is also necessary to consider the development of resistance to the 'natural antimicrobials'. In this regard, there is no adequate information on the mechanisms involved in this resistance process in microorganisms.

The ability of phenolic and phytoalexin components of the essential oils to affect many cell types has been extensively discussed above, and the conclusion is that they mainly act by causing membrane perturbations which lead to cell malfunction. This kind of activity is desirable since it is difficult for microbes to evolve resistant strains (Weinstein and Albersheim, 1983). In this regard, it has been reported that essential oil phenolics and phytoalexins cause static rather than outright toxic effects (Tokutake *et al*., 1992); cell membranes that leak or function poorly would not necessarily lead to cell death but would most probably cause a deceleration of certain metabolic processes such as cell division (Darvill and Albersheim, 1984; Kubo *et al*., 1985). There is a wide range in the sensitivities of different types of bacteria to these essential oils. In general, gram-positive bacteria were more sensitive than

gram-negative bacteria to the antimicrobial compounds found in spices (Dabbah *et al*., 1970; Farag *et al*., 1989; Shelef, 1983; Tassou *et al*., 1995). However, a broad variation in the rate (or extent) of inhibition is also evident among the gram-negative bacteria. For example, *E. coli* is less resistant than *Pseudomonas fluorescens* or *Serratia marcescens* when tested with sage, rosemary, cumin, caraway, clove and thyme essential oils (Farag *et al*., 1989). Inhibition of growth using citrus oils ranged from 88 % for *Aerobacter aerogenes* to 100 % for *Alcaligenes faecalis* (Dabbah *et al*., 1970). On the other hand, *Salm*. ser. Typhimurium was found to be more sensitive than *P. aeruginosa* to oregano and thyme essential oil (Paster *et al*., 1990).

Deans and Ritchie (1987), who studied the effect of 50 plant essential oils against 25 genera of bacteria, concluded that gram-positive and gram-negative organisms were both susceptible to the essential oils and there was no evidence that the degree of sensitivity to the oils was reflected in the gram reaction of the organism. However, this finding is now under dispute since the resistance or tolerance of bacteria to essential oils has been attributed, among others factors, to membrane characteristics. It is likely that multiple mechanisms are responsible for the susceptibility of bacteria to plant antimicrobials or antibiotics. Most naturally-occurring small organic antimicrobial biomolecules are hydrophobic and contain aromatic structures similar to those found in some solvents (e.g. toluene) and classical preservatives (e.g. benzoic acid). Different studies on *P. putida* and *P. aeruginosa* have shown that these bacteria are relatively tolerant to essential oils; the contribution of outer membrane or efflux pumps are considered to be possible mechanisms for this resistance (Pattnaik *et al*., 1995a,b; Isken and de Bond, 1998; Mann *et al*., 2000). Similar reports on the resistance of *E. coli* and *Staphylococcus aureus* strains and other microorganisms to inactivation by essential oils have been published (Moken *et al*., 1997; Nelson, 2000). In particular, mutants of *E. coli* and sub-populations of *S. aureus* have been found to be resistant to pine oil and to tea-tree oil, respectively.

2.6 Legislation and labelling

Currently, the use of natural antibacterials derived from plant species in food preparations is not well enough established to have widespread application in the food industry. However, essential oils are being incorporated in various food preparations such as salad dressings, sauces, fermented sausages, various ethnic foods, etc. The recent interest in the consumption of more 'natural' foods, in connection with the negative consumer perceptions regarding artificial preservatives, has produced a positive trend towards the use of natural antimicrobials derived from plant herbs and spices (Beuchat and Golden, 1989).

To date, most of the research efforts on food preservatives have focused on the development of chemically defined substances. Thus, more research is required to enable the development of natural compound mixtures as food preservatives and the exploitation of their potential toxicological effects – at present, the data on toxicological effects in foods do not correlate with the corresponding toxicological data that is used by the cosmetics industry (Table 6.4). In this regard, these compounds have to be recognized by the food industry as safe (GRAS) food additives. In addition, various regulatory authorities (e.g. Commission of the European Union)

Table 2.4 Lethal dose (LD_{50}) of essential oils determined in rats

Plant/herbs	LD_{50} (g/Kg)	Plant/herbs	LD_{50} (g/Kg)
Prunus amygdalus	A	*Juniperus communis*	D
Angelica archangelica	C/D	*Laurus nobilis*	C
Pimpinella anisum	C	*Lavandula angustifolia*	C/D
Ocinum basilicum	B	*Citrus limonum*	D
Pimenta racemosa	B	*Origanum marjorana*	C
Citrus bergamia	D	*Pistacia lentiscus*	D
Cinnamomum camphora	C	*Citrus aurantium*	D
Anethum graveolens	C	*Origanum vulgare*	B
Allium sativum	D	*Petroselinum sativum*	B/C
Anthemis nobilis	D	*Piper nigrum*	D
Cinnamomum zeylanicum	C	*Rosemarinus officinalis*	D
Daucus carota	D	*Menta viridis*	C
Cinnamomum cassia	C	*Salvia officinalis*	C
Syzygium aromaticum	B/C	*Thymus vulgaris*	C
Eucalyptys globules	C	*Citrus reticulata*	D
Foeniculum vulgare	C	*Coriandrum sativum*	C
Zingiber officinale	D	*Camellia sinensis*	C

A: < 1.0 g/kg; B: 1–2 g/kg; C: 2–5 g/kg; D: > 5 g/kg.
Source: Data from Skandamis (2001) modified accordingly.

should amend and maintain list(s) of the essential oils which are as recognized additives with antibacterial and/or antioxidant properties. The authorities must also define their purity criteria and appropriate daily intake quantities. Furthermore, they should compose and update a catalogue with active ingredients and evaluation protocols, in order to produce a list of authorized additives for food preservation. Finally, new legislation is needed to ensure that a full list of ingredients appears on food labels; thus ensuring that consumers are fully informed of a foodstuff's composition. Consumers who – for health or ethical reasons – have (or want) to avoid certain ingredients will then be able to decide which products they wish to buy. Another problem to be resolved is that of carryover additives, since the essential oils may contain ingredients that act as allergens when the oils are misused or in cases of overdose. In this case, the indication of an additive name and category on the label would be sufficient to enable susceptible consumers to avoid the consumption of such products.

In conclusion, clear and binding labelling rules need to be introduced to ensure that consumers have all the information they require – in terms of product characteristics such as composition, storage and use – to enable them to make suitable food choices.

2.7 Future trends

2.7.1 Food quality and safety

Throughout the food chain (e.g. production, processing and distribution at retail level), preservation methods should maintain raw material quality, physicochemical properties and functionality while providing safe products that have a low spoilage

potential. According to Gould (1995), the technologies employed to maintain the microbiological quality and safety of foods should: (i) prevent the microorganisms from accessing the foods; (ii) inactivate the microorganisms, if they have gained access; and (iii) slow down, suppress or prevent the growth of microorganisms that have not been inactivated. These stages should be achieved through product-specifict processing techniques. In addition, combination processes, such as mild heat treatment in conjunction with a low concentration of preservatives, have been developed and are in use by the food industry. Alternative physical treatments such as ultra-high pressure (UHP) or pulsed electric fields (PEF) are now being investigated as possible replacements for the classical heat treatments. The potential use of some of the novel, 'natural' preservatives derived from plants, as discussed in this chapter, in combinations with physical treatments (i.e. mild-heat, UHP, PEF), has not been extensively evaluated; further research into these combination treatments may lead to the development of novel mild preservation regimes tailored to the organoleptic quality needs of individual products. Indeed, several reports have shown that UHP treatments 'denature' microbial cell wall proteins such that access of 'natural' preservatives to the cell wall and membrane can be greatly facilitated (Pagan and Mackey, 2000).

2.7.2 Areas for future research

In order to increase our knowledge of the effectiveness of the use of natural, plant-derived antimicrobial biomolecules (biological, 'natural' preservatives) in conjunction with other food preservation systems, the following areas can be identified as priorities for research.

- To understand whether microorganisms die, survive, adapt or grow when treated with plant-derived natural antimicrobials and to identify the physiological and molecular mechanisms (signal transduction, stress proteins induced, energy cost, activation of specific pathways, etc.) within the cells that result in these phenotypes.
- To study the effect of the food matrix on the antimicrobial efficiency of plant-derived antimicrobials in conjunction with other hurdles, by evaluating and controlling the spatial distribution of ingredients and the physicochemical properties of the food.
- To establish the effect of the food matrix on the organisms and specifically the role of surface adhesion on the microbial physiology with or without the presence of natural preservatives.
- To determine which of the emerging food preservation technologies may act synergistically with the use of natural antimicrobials to enhance the safety and extend the shelf-life of specific products.
- To develop mathematical models for the prediction of shelf-life or the true physicochemical conditions pertinent to either survival or inactivation of pathogenic bacteria in food products.
- To validate (at a global level and by end users in the food industry) the procedures and mathematical models, and also to develop software for dissemination of results.

- To understand and research consumer attitudes and quality perceptions. The combined application of such models and methods will offer a novel alternative to the conventional evaluation (assessment) of the safety and quality of specific products.

Finally, more emphasis should be given to determining the prevalence of pathogenic microorganisms following the use of natural antimicrobials; during all stages of production and in the finished products at the plant and supermarket levels. The inclusion of several factors (e.g. food matrix, physiological status of microorganisms under stress responses leading to adaptation or survival) into mathematical models describing microbial growth and death would represent a significant advance compared with the empirical, descriptive models of microbial growth, with limited predictive capability (Fig. 2.3), that are currently used in the food industry (Zwietering *et al.*, 1994; Cuppers *et al.*, 1997; Koutsoumanis *et al.*, 1998; Skandamis and Nychas, 2000; Skandamis *et al.*, 2002).

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3

The effect of natural antioxidants in herbs and spices on food shelf-life

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Abstract: Herbs and spices contain flavouring substances and antioxidants which are partially changed by hydrolysis, oxidation and polymerization. Foodstuffs can also contain smaller quantities of antioxidants, but their main reactive components are proteins, sugars and sulfur compounds. Spices interact with food components only slowly at room temperature, but the rate and degree of interaction rises with increased heat during food processing for consumption. Antioxidant activity usually decreases due to this interaction, although it can also increase. Future trends are likely to see the introduction or further development of methods to preserve herbs and spices during storage.

Key words: spices and foods interactions, effect of food processing, heating foods.

3.1 Introduction

Herbs and spices modify the appearance and flavour of food, and are especially used in tropical and subtropical countries, though their use is also now widespread in Central Europe and North America – the type of spices used often being native to the particular country concerned. In addition to their use as flavourings, their role as antioxidants is also very important, as they can help prolong the shelf-life of final foods.

The most effective antioxidants in herbs used as spices are the phenolic substances, possessing at least two hydroxylic groups in the ortho or para positions, e.g. caffeic acid or most flavones or catechins (Table 3.1). Most of the phenolics are weak polar compounds and therefore appear in plants in the form of more polar (hydrophilic) derivatives, mostly as *O*-glycosides. Some components may even be volatile, such as eugenol. Lignans, such as sesamol, also have antioxidant properties. Several substances derived from essential oils are active, such as those derived from basil, oregano or thyme. Sulfur-containing inhibitors of oxidation are often used in European additives (in garlic or onion, for example) and are discussed further below.

Table 3.1 Examples of active antioxidants in herbs and spices

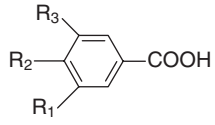
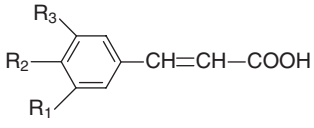
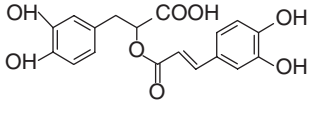
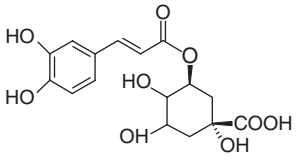
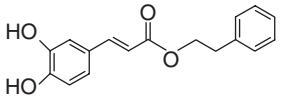
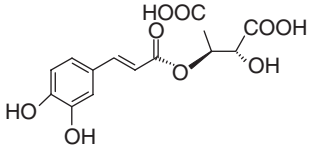
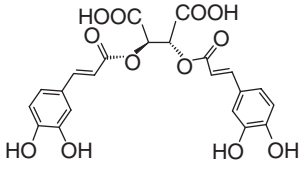
Group of compounds	Important compounds	Example of sources	
Phenolic acids			
Benzoic acid derivatives 	R_1=R_2=OH, R_3=H Protocatechuic acid R_1=R_2=R_3=OH Gallic acid	Common herbs	
Cinnamic acid derivatives 	R_1=R_3=H, R_2=OH *p*-Coumaric acid R_1=R_2=OH, R_3=H Caffeic acid R_1=OCH_3, R_2=OH, R_3=H Ferulic acid R_1=R_3=OCH_3, R_2=OH, Sinapic acid	Common herbs	
Caffeic acid derivatives			
	Rosmarinic acid	Thyme Sage Rosemary Oregano Basil Marjoram	*Thymus vulgaris* L. *Salvia officinalis* L. *Rosmarinus officinalis* L. *Origanum vulgare* L. *Ocimum basilicum* L. *Majorana hortensis* Moench
	Chlorogenic acid	Sage Rosemary Oregano Dill Bay leaf	*Salvia officinalis* L. *Rosmarinus officinalis* L. *Origanum vulgare* L. *Anethum graveolens* L. *Laurus nobilis* L.
	Caffeic acid Phenylethylester	Basil	*Ocimum basilicum* L.
	Caftaric acid	Basil	*Ocimum basilicum* L.
	Cichoric acid	Basil	*Ocimum basilicum* L.

Table 3.1 *Continued*

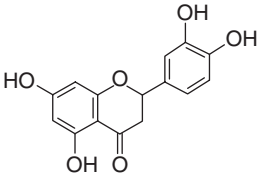
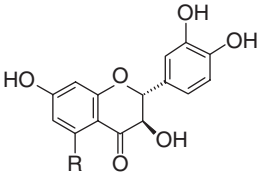
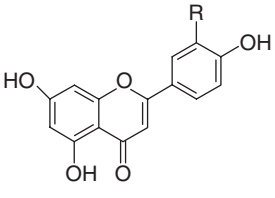
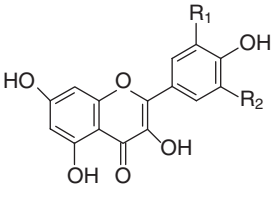
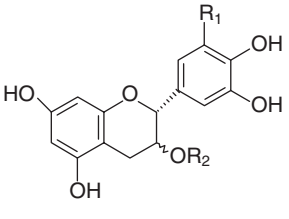
Group of compounds	Important compounds	Example of sources	
Flavonoids and catechins			
Flavanones 	Eriodictiol	Sumac	*Rhus* spp. L.
Flavanonoles 	R=OH Taxifolin R=H Fustin	Sumac	*Rhus* spp. L.
Flavones 	R=H Apigenin R=OH Luteolin	Parsley Celery Garlic Onion Thyme Rosemary	*Petroselinum crispum* A.W.Hill *Apium graveolens* L. *Allium sativum* L. *Allium cepa* L. *Thymus vulgaris* L. *Rosmarinus officinalis* L.
Flavonoles 	R_1=R_2=H Kaempferol R_1=OH, R_2=H Quercetin R_1=R_2=OH Myricetin R_1=OCH_3, R2=H Isorhamnetin	Celery Garlic Onion Chive Dill Bay leaf	*Apium graveolens* L. *Allium sativum* L. *Allium cepa* L. *Allium schoenoprasum* L. *Anethum graveolens* L. *Laurus nobilis* L.
(+)-Catechins and (−)-Epicatechins 	(+)-Catechin and (−)-Epicatechin (+)-Gallocatechin and (−)-Epigallocatechin and its gallates	Dill Bay leaf Oregano Savory	*Anethum graveolens* L. *Laurus nobilis* L. *Origanum* spp. L. *Satureja* spp. Mill.

Table 3.1 *Continued*

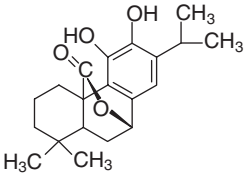
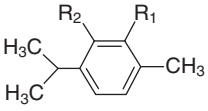
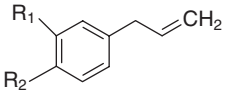
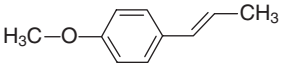
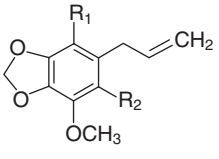
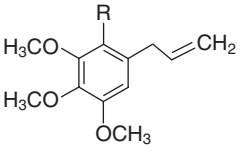
Group of compounds	Important compounds	Example of sources	
Phenolic diterpenes			
	Carnosol Related compounds: carnosic acid, rosmanol, epirosmanol, isorosmanol	Rosemary Sage	*Rosmarinus officinalis* L. *Salvia officinalis* L.
Volatile phenols			
	R_1=OH, R_2=H Carvacrol R_1=H, R_2=OH Thymol		*Lamiaceae* family *Origanum* spp. L. *Satureja* spp. Mill. *Thymus* spp. L. *Thymbra* spp. L.
	R_1=H, R_2=O-CH_3 Estragol R_1=O-CH_3, R_2=OH Eugenol	Basil Star-anise Clove Cinnamon Anise Tarragon	*Ocimum basilicum* L. *Illicium verum* Hook *Eugenia caryophyllata* (*Syzigium aromaticum* L.) *Cinnamomum verum* J. Presl (*C. zeylanicum* Blume) *Pimpinella anisum* L. *Artemisia dracunculus* L.
	(*E*)-Anethol	Dill Caravay Coriander Star-anise	*Anethum graveolens* L. *Carum carvi* L. *Coriandrum sativum* L. *Illicium verum* Hook
Polyalkoxybenzenes			
	R_1=O-CH_3, R_2=H Apiol R_1=H, R_2=O-CH_3 Dillapiol R_1=R_2=H Myristicin	Parsley Celery Dill Caraway Bay leaf	*Petroselinum crispum* A.W.Hill *Apium graveolens* L. *Anethum graveolens* L. *Carum carvi* L. *Laurus nobilis* L.
	R=H Elemicin R=O-CH_3 Allyltetramethoxybenzene		

Table 3.1 *Continued*

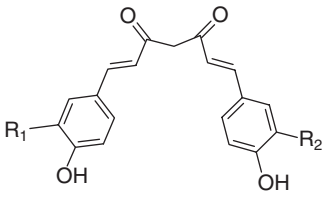
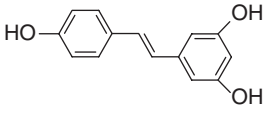
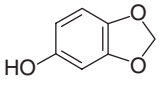
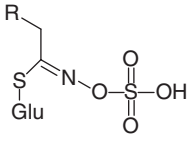
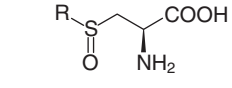
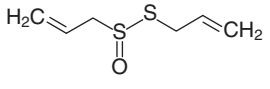
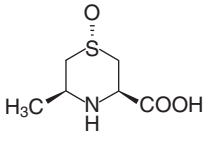
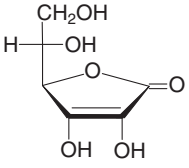
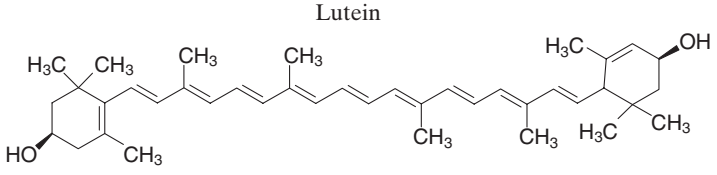
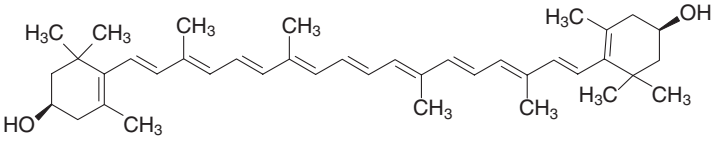
Group of compounds	Important compounds	Example of sources	
Other phenols			
Curcuminoides 	R_1=R_2=O-CH_3 Curcumin R_1=O-CH_3, R_2=H Demethoxycurcumin R_1=R_2=H Bisdemethoxycurcumin Related compounds: cyclocurcumin, calebin, etc.	Turmeric (curcuma) Galangal	*Curcuma* spp. L. *Alpinia galanga* (L.) Willd.
Resveratrol 		Grape wine Bay leaf	*Vitis vinifera* L. *Laurus nobilis* L.
Lignans			
	Sesamol	Sesame	*Sesamum indicum* L.
Sulphuric compounds			
Thiols	Cysteine Homocysteine N-acetylcysteine Glutathione γ-Glutamylcysteine	Ginger Mustard Fenugreek Turmeric Cardamom	*Zingiber officinale* Roscoe *Sinapis alba* L. *Trigonella foenum-graecum* L. *Curcuma longa* L. *Elettaria cardamomum* (L.) Maton
Glucosinolates 	R = 4-hydroxybenzyl Sinalbin	Mustard	*Sinapis alba* L.
Alliin and related compounds   	R=CH_2=CH-CH_2 Alliin R=CH_3-CH=CH Isoalliin R=CH_3 methiin Allicin Cycloalliin	Onion Garlic Leek Chive	*Allium* spp. *Allium cepa* L. *Allium sativum* L. *Allium ampeloprasum* var. *porrum* (L.) J. Gay *Allium schoenoprasum* L.

Table 3.1 *Continued*

Group of compounds	Important compounds	Example of sources
Ascorbic acid 	L-ascorbic acid	Common herbs
Carotenoids Lutein  Zeaxanthin 		
Parsley		*Petroselinum crispum* A.W.Hill
Celery		*Apium graveolens* L.
Basil		*Ocimum basilicum* L.
Coriander		*Coriandrum sativum* L.
Dill		*Anethum graveolens* L.

Interesting and little-known compounds have been found in the polar phenolic fraction of oregano (*Oreganum vulgare* L.) and marjoram (*Majorana hortensis* Moench). Fractions containing highly active esters of phenolic acids, especially caffeic acid, have also been found. Typical of these is rosmarinic acid, though its 4-hydroxybenzyl derivative was also found. Significant antioxidant activity was also detected in the 4-hydroxybenzyl ester of protocatechuic acid, with its glycosidically bound glucopyranose.

Two glycosides of protocatechuic acid derivatives – 4′-*O*-β-D-glucopyranosyl-3′, 4′ -dihydroxybenzyl protocatechuate (GDBP) and methyl 4′-*O*-β-D-glucopyranosyl-3′, 4′-dihydroxybenzyl-4-*O*-methyl protocatechuate (GDBMP) – were identified in the methanolic extract of oregano (*O. vulgare* L.) in addition to other better-known compounds. Both of these compounds demonstrate an ability to scavenge DPPH radicals, GDBP's effectiveness in this respect being comparable to quercetin or rosmarinic acid, while the methyl derivative (GDBMP) has a much lower efficiency. GDBP content was found to be 3.8 mg per gram of dry leaves, which demonstrates

that that the contribution of the compound to the total antioxidant capacity of oregano leaves is quite significant.

Some esters of sinapic acid with choline and malic acid (called sinapines) have been found in mustard. Volatile aromatic compounds such as thiocyanates and/or isothiocyanates are formed after damage of mustard seeds by enzymatic hydrolysis of glucosinolates by myrosinase. These compounds are the main aromatic compounds of mustard preparations. Plants of the *Allium* genus, in particular onion (*A. cepa* L.) and leek (*A. ampeloprasum* var. *porrum* (L.) J. Gay), contain other important lignans including pinoresinol, lariciresinol and secoisolariciresinol. As well as sesamol, sesame seeds also contain other lignans, e.g. sesamin, sesaminol, saminol and other related compounds, and pinoresinol or piperitol.

The less well-known spices galangal (*Alpinia galanga* (L.) Willd.) and lesser galangal (*A. officinarum* Hance) contain high levels of various 4-hydroxyphenyl alcohols, which have a strong antioxidant capacity.

The most significant lipophilic antioxidants are the tocopherols (mainly α- and/or γ-tocopherol) which occur mainly in oilseeds e.g. in sesame seeds, which contain up to 500 mg of tocopherols per kilogram. Bay leaves (*Laurus nobilis* L.) contain significant quantities of vitamin E (Nakatani, 2000; Ly *et al*., 2003; Matsuura *et al*., 2003; Conforti *et al*., 2006; Kivilompolo and Hyötyläinen, 2007; Yanishlieva *et al*., 2006; Moazzami *et al*., 2007; Hounsome *et al*., 2008; Lin *et al*., 2008; Hahm *et al*., 2009; Lee and Scagel, 2009).

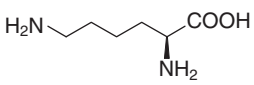
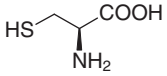
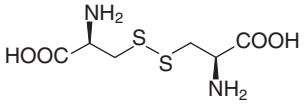
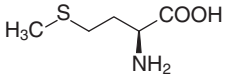
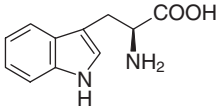
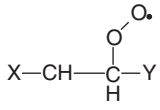
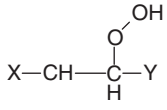
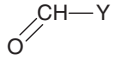
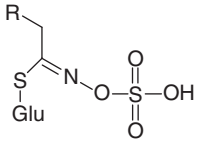
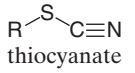
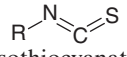
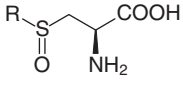
Foodstuffs also contain phenolic antioxidants, though in smaller quantities than occur in spices. The main reactive substances in foodstuffs are amino acids, peptides and proteins, e.g. lysine, cysteine, cystine, tryptophan, sugars and polar lipids (Table 3.2). Active components in garlic and onion should also be included here. Another important food component is ascorbic acid (vitamin C), possessing an antioxidant activity of its own, or acting as a synergist for other ingredients. Carotenoids and lycopene (found in tomatoes) are also antioxidants, albeit with lower activity. Some food components contain enzymes which have low activity, even with a natural moisture content. These enzymes may act as pro-oxidants (e.g. peroxidase) or as antioxidants (destroying peroxides). Often, a mixture of antioxidants does not have the same effect as they each do if added separately.

3.2 Reactions of spice antioxidants with natural food components

The antioxidants in spices react most easily with proteins, especially with the lysine-terminated amino group, with cysteine and cystine, with the free hydroxyl groups of tyrosine, and with tryptophan. Free amino acids and peptides are more reactive, but are present only in smaller quantities.

The terminated groups of free amino acids are very reactive, while the reactivity of the α-amino group is significantly less reactive and, for non-enzymatic reactions, require high activation energy. Peptides with a free terminating group, e.g. glutathione (γ-*L*-glutamyl-*L*-cysteinyl-glycin) are similarly as reactive as the free amino acids. Native proteins with a quarter structure are bound to these reactive groups by various physical bonds (e.g. hydrogen bridges). However, as a result of

Table 3.2 Reactive components of food materials

Component	Reactive substance	Reactive functional group
Proteins	Lysine 	Terminate amino-group
	Cysteine 	–SH group
	Cystine 	–S–S group
	Methionine 	–S group
	Tryptophan 	Heterocyclic ring
Sugars and Maillard products	Simple sugars and carbohydrates with free poloacetal group	Free poloacetal group
	Reductones arising in Maillard reaction	Reactive carbonyl- and/or amino-groups
Oxidized lipids	Peroxyl radicals 	
	Hydroperoxides 	
	Carbonyl derivatives 	
Sulfur-containing foods	Thiols Cysteine, glutathione, etc.	Thiol group -SH
	Glucosinolates 	Sulfo group Enzymatic degradation products  thiocyanate  isothiocyanate
	Alliin and related compounds 	Sulfoxy group
Enzymes	Oxidoreductases Polyphenoloxidases	*o*-Diphenol:O_2 oxidoreductase *p*-Diphenol:O_2 oxidoreductase

denaturation during food processing, the quarter structure is destroyed and the reactivity of these free groups rapidly increases.

The lysine-terminated amino group easily reacts (via a Maillard reaction) with the free carbonyl group of various compounds (including quinines) formed during oxidation of phenolics. Consequently, this reaction leads to a reduction in the nutrition value of the protein. Oxidation is the most important reaction of the other reactive amino acids. Products of sulfuric amino acid oxidation, for example by hydroperoxides of fatty acids or by peroxyl radicals (Fig. 3.1a), are either poorly- or non-utilizable. A similar oxidation of tryptophan leads to the formation of cynurenine and its related compounds (Fig. 3.1a), which have a demonstrable mutagenic activity. Therefore, any inhibition of oxidation reactions of proteins is significant for food quality. These reactions are also relevant to organisms, and consequences for health can be severe. Today, problems caused to organisms by glycation and other adverse reactions are important areas of study (Davídek *et al*., 1990; Panek *et al*., 1995; Kim and Kim, 2003; Edeas *et al*., 2010; Robert *et al*., 2010).

The free semiacetal group of carbohydrates is very reactive. The enzyme-catalyzed condensation of the free semiacetal group of sugars with the free hydroxy group of phenolic antioxidants leads to the formation of glycosidic bonds between these components. Most phenolic antioxidants are weak polar antioxidants, and poorly soluble in water. As a result, plants develop phenolic glycosides as water-soluble compounds. However, the antioxidant activity of phenolic glycosides is significantly lower than the activity of lower polar aglycones. During food processing and digestion of glycosides, most of the glycosidic bonds are destroyed and the antioxidant capacity of phenols increases.

Reductones (Fig. 3.1b), arising in food via Maillard reactions, are very reactive compounds which can play an important role in the antioxidant status of many processed foods. Reactive enol- and/or amino-groups can quickly reduce any oxidative forms of various compounds (ascorbic acid, tocopherols, quinones) and protect these compounds against oxidation (Justesen and Knuthsen, 2001; Dragland *et al*., 2003; Amigo-Benavent *et al*., 2010; Chrpova *et al*., 2010; Peinado *et al*., 2010). Another reactive class of food components are the natural phenolic antioxidants, which are subject to similar changes as the antioxidants in herbs and spices.

Peroxyl radicals and hydroperoxides of fatty acids are very strong oxidants which can oxidize practically all components in food (including practically all phenolic compounds) and usually have a strong negative effect on food quality and shelf-life. These compounds can oxidize proteins (mainly sulfuric amino acids and tryptophan – see above), some vitamins (e.g. ascorbic acid, tocopherols, retinol) and also other minor compounds. For example, various natural food colourings (such as anthocyans, carotenoids or haeme colours) are very sensitive to oxidation. The negative impact of this oxidation on food quality is evident. Quinones, which are formed due to oxidation, can consequently condense to brown polymeric compounds. These reactions also deteriorate food quality.

Carbonyl derivatives of fatty acids, arising as a secondary product of chain autoxidation, have a negative effect on the flavour of fat-containing foods. These compounds are also important donors of the very reactive carbonyl group to non-enzymatic browning reactions (Pokorný, 1991; Beddows *et al*., 2000; Govaris *et al*., 2004; Yanishlieva *et al*., 2006; Rohlik *et al*., 2010).

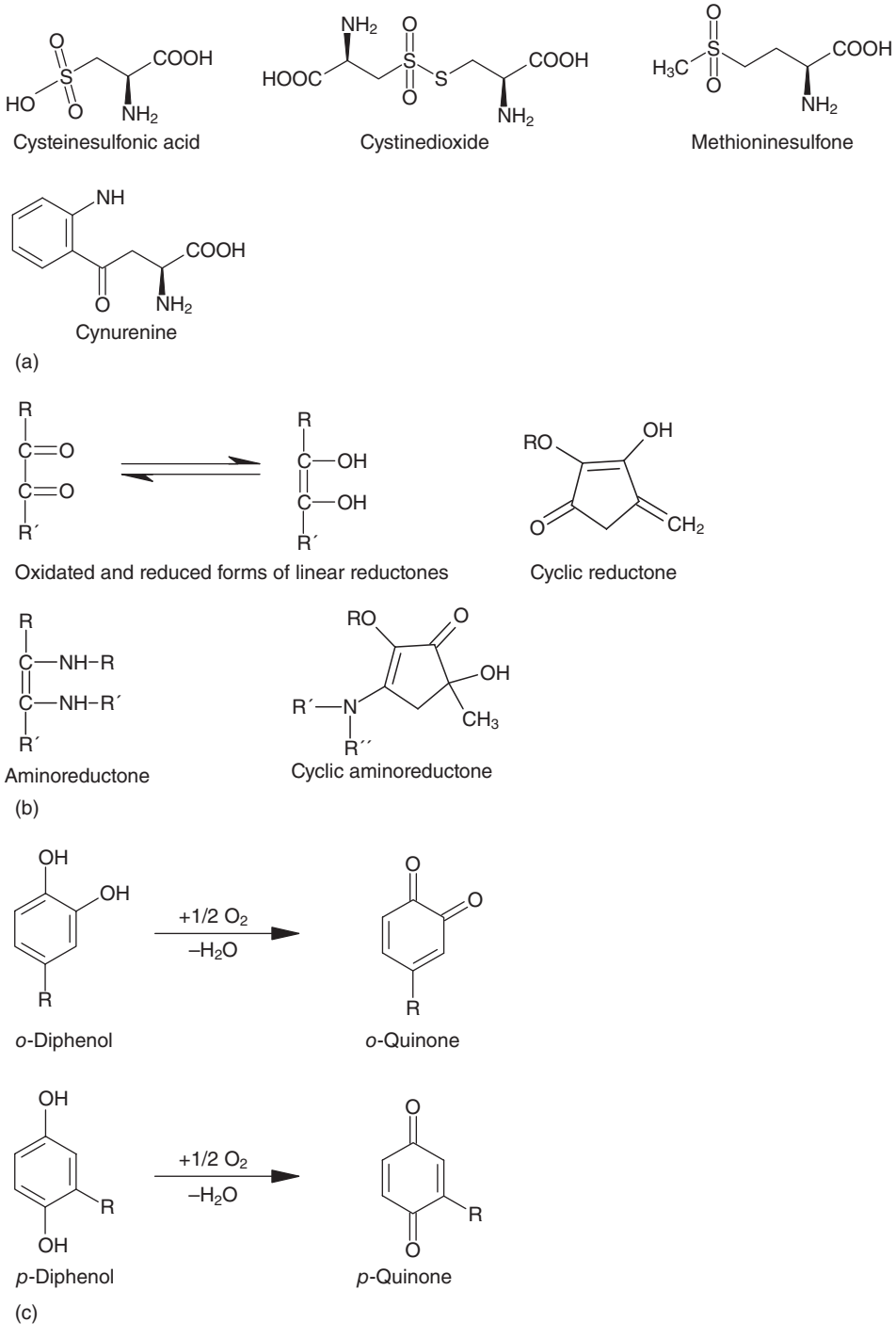


Fig. 3.1 Changes of reactive compounds of food. (a) Products of oxidation of sulfuric amino acids and tryptophan. (b) Examples of reductones formed due to non-enzymatic browning reaction. (c) Enzymatic oxidation of phenols.

Sulfur compounds in garlic and onion (alliin and its related compounds, and allicin) bind to the labile groups of the flavones. Allylisothiocyanate (a product of the hydrolysis of sinigrine) is the main spicy compound in black mustard (*Brassica nigra* L.), while 4-hydroxybenzylisothiocyanate can occur due to sinalbine hydrolysis in mustard pasta produced from white mustard (*Sinapis alba* L.).

Phospholipids and glycolipids bind heavy metal traces (iron and copper), which otherwise act as pro-oxidants. Polyphenoloxidases oxidize various compounds with an ortho- or para-diphenol structure. The oxidation schema is described in Fig. 3.1c. Phenolic acids (mainly caffeic acid and its derivatives, catechins and tyrosol derivatives) are the main substrates for these enzymes. Oxidation of other flavonoids occurs only partially, whilst other phenolics barely oxidize at all.

3.3 Main changes in herb and spice antioxidants under different conditions

3.3.1 Main changes in herb and spice antioxidants and food components in storage before application

Spices are often stored a long time before being processed and then used, so that their components, which are very reactive, may be subject to profound changes affecting their activity. The most important of these are summarized in Table 3.3.

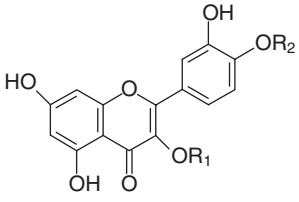
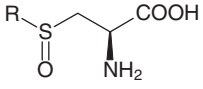
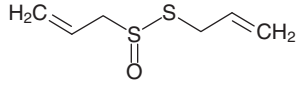
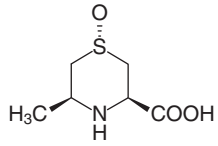
Phenolic antioxidants are often present as glycosides, and their reactivity is therefore improved by hydrolysis. They are generally easily oxidized, producing free radicals or quinones. In either case, the oxidation products easily form polymers, the antioxidant activity of which may be greater or slower than that of the original substances.

Flavonoids and some other phenolic compounds are bound in plants with various sugars, mainly as glucosyl derivatives. Glycosidic bonds are formed by an enzymatic condensation reaction between some hydroxyl groups of phenol and the reactive semiacetal group of sugars. Flavonoids usually have *O*-glycosides in positions 3 and 4, with glycosides in positions 7 and 5 being less common, and *C*-glycosides being even scarcer. Reverse phase high-performance liquid chromatography (HPLC) is usually used for the analytical determination of phenol glycosides. Separation is judged by the polarity of molecules, with bonded sugars increasing polarity and the less polar aglycons being separated with the highest retention time. The glycosidic group is not very stable and, during food processing, it is partially hydrolyzed, with hydrolysis contining during digestion.

Biosynthesis using condensation reactions in plants leads to the formation of caffeic acid esters, e.g. rosmarinic or chlorogenic acids. However, depside bonds are very unyielding and compounds are stable against hydrolysis under normal conditions.

Hydrolysis of glucosinolates in plants of the *Brassicaceae* family leads to the formation of sensory active thiocyanates and isothiocyanates. It is these compounds which give the typical aroma to these plants (Dorman *et al.*, 2004; Vågen and Slimestad, 2008; Lee and Scagel, 2009; Pérez-Gregorio *et al.*, 2010; Pokorný and Schmidt, 2010). Plants of the *Allium* family contain sulfur amino acids (*S*-alk(en)yl-L-cysteine

Table 3.3 Changes of antioxidants of herbs and spices before their application to foods

Process	Example
Hydrolysis	Hydrolysis of glycosidic bounds of phenol glycosides; typical example – glucoside derivatives of quercetin in onion  $R_1 = R_2 = H$ quercetin R_1 = Glc, $R_2 = H$ quercetin-3-glucoside $R_1 = H$, R_2 – Glc quercetin-4′-glucoside $R_1 = R_2$ = Glc quercetin-3,4′-diglucoside Hydrolysis of glucosinolates
Other enzymatic dissociative reactions	Decomposition of S-alk(en)ylcysteinesulfoxides to aromatic compounds in *Allium* plants   $R = CH_2 = CH\text{-}CH_2$ allin $R = CH_3\text{-}CH = CH$ isoaliin $R = CH_3$ methiin 
Oxidation	Enzymatic oxidation of *o*- and/or *p*-diphenols – see above. Oxidation of phenols by air oxygen, metal ions, hydrogen peroxide or peroxyl radicals. Oxidation of flavonoids to furan derivatives.
Polymerization	Consequent nucleophilic reactions of quinones with other food components. Nucleophilic addition of diphenols to quinones.

sulfoxides), the most significant of which are alliin, isoalliin and methiin. When the plant tissues are damaged, volatile aromatic allied substances are formed due to the action of the C-S lyases. Allicin is the most important of the aromatic compounds from this group, and allicin content in plants of this family varies from 160 to 350 mg per 100 g; all these substances show rather significant antioxidative properties. If the plant is damaged, and at the same time as isoalliin cyclization, there is also an increase in cycloalliin (Fig. 3.2). These parallel reactions may lead to an increase in pigments, leading to discoloration (Yin and Cheng 1998; Kubec *et al.*, 2004; Ichikawa *et al.*, 2006; Corzo-Martinez *et al.*, 2007).

The enzymatic or non-enzymatic oxidation of ortho- or para-diphenols leads to the formation of unstable quinones. These compounds have a slightly darker colour in comparison to original diphenols. Quinones can be recovered to original phenols by other diphenols (as a result of their redox potential) or by ascorbic acid. For

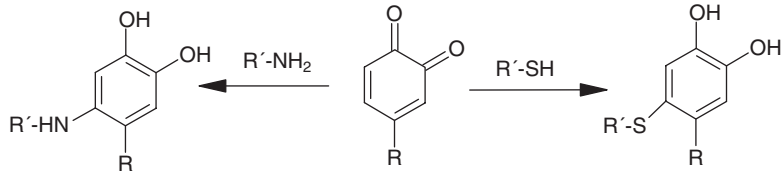


Fig. 3.2 Reaction of quinones with food amino acids.

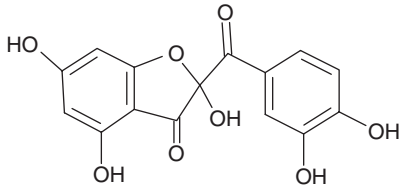


Fig. 3.3 Product of quercetin oxidation in onion.

example, quinones formed by oxidation of caffeic acid derivatives can be recovered to their original compounds by catechins. Consequently, quinones react easily with the lysine-terminated amino group or with the free thiol group of cysteine (and relevant peptides), and substituted diphenols are created (Fig. 3.2). These compounds are also unstable, and brown pigments are formed after their oxidation and consequent polymerization.

Flavonoids can also be oxidized by different mechanisms resulting in the formation of furan derivatives with a similar structure to natural aurones. The degradation product quercetin identified in onion is described in Fig. 3.3 (Jungbluth and Ternes, 2002). Similar reactions can occur in food components during storage after harvest and before their use for food preparation. This period can sometimes be as long as a year, due to seasonal production. Proteins are partially oxidized or react with reducing sugars, including ascorbic acid, forming Maillard colourless intermediary substances converted into deep brown non-enzymic browning products.

In addition to antioxidants, various inactive substances, called synergists, increase the activity of antioxidants. By contrast, antagonists decrease antioxidant activity.

3.3.2 The effect of hydrolysis and polymerization during food preparation on food product shelf-life

As noted above, phenolic antioxidants are often present as glycosides. Although the bonds can be broken, the antioxidant activity usually remains the same, or nearly the same. Hydrolysis, when it proceeds further, splits flavones into simpler compounds or cleaves off phenols bound onto more complicated phenolic compounds. However, the reaction can also proceed in the opposite direction, i.e. leading to the formation of glycosides or condensed phenols. Polymer formation can proceed even

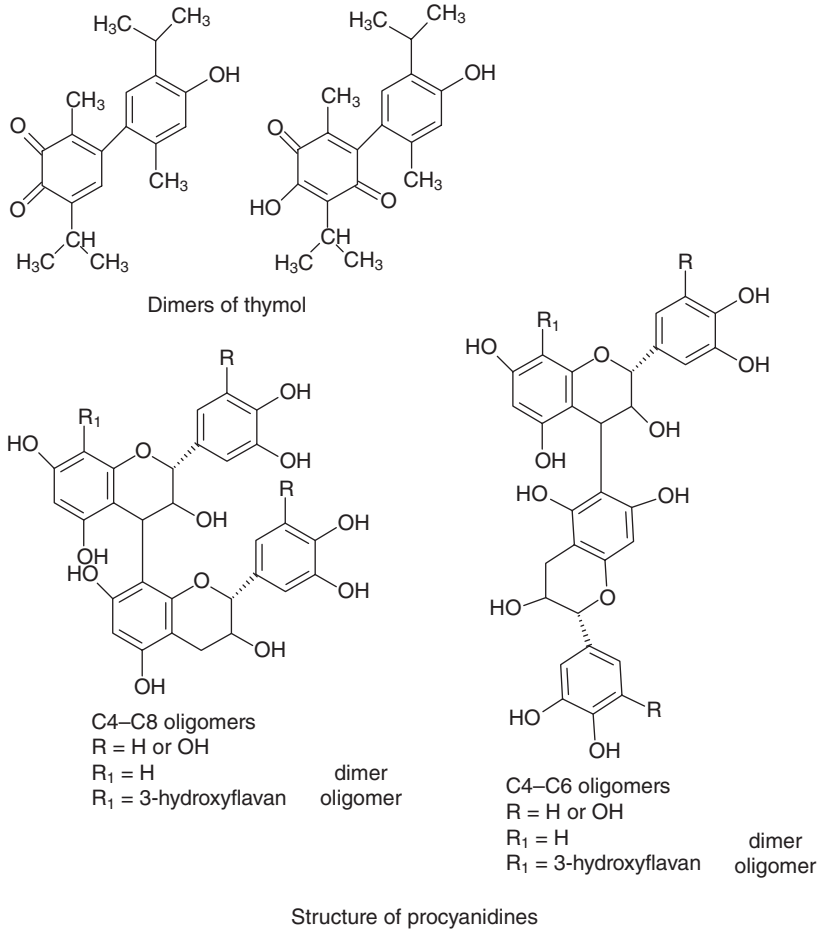


Fig. 3.4 Structure of some oligomers with high antioxidant effect.

in phenolic compounds which have not been combined before the polymer formation. The changes in the phenolic substances can sometimes affect antioxidant activities (see the example in Fig. 3.4).

The addition of diphenols to quinones in food and also in plants *post mortem* usually leads to the formation of inactive products with high molecular mass, which are various shades of brown in colour. However, plants often use these products as precursors for the biosynthesis of dimers or higher oligomers with a similar antioxidant effect to the original compounds. For example, the addition of gallic acid to quinone and consequent reactions leads to the formation of ellagic acid, which is a very effective antioxidant e.g. found for example in strawberry leaves. Similar pathways lead, for example, to the formation of non-volatile dimers of thymol (Fig. 3.4) (Nakatani, 2000) which has a significant antioxidant capacity, and also to the formation of procyanidines from flavanoles (important antioxidants, mainly in fruits – see Fig. 3.4).

3.3.3 Oxidation of herb and spice antioxidants and their reactions with food components

Phenolic antioxidants are easily oxidized in storage, and always found with inoxidized components. Traces of heavy metals, such as iron or copper, increase the rate of oxidation. Oxidation increases the reactivity of antioxidants, as additional free radicals or quinones react with the natural phenolic substances in food, oxidize sulfur amino acids bound in proteins and the sulfur compounds of garlic and onion, and destroy carotenes and related substances. By contrast, ferrous ions are oxidized into ferric ions, which are less active as pro-oxidants.

Being only plants and their by-products, the safety of spices has, to some extent, been proven by generations of our ancestors and questions of acute toxicity are therefore not relevant. On the other hand, all these substances may, in addition to their undeniable positive effects, also bring some negative consequences. Natural phenolic antioxidants are usually present in foods as herbs or herb extracts, but they also contain a number of other compounds, making it impossible to estimate the exact chemical safety of these substances. The long-term use of many substances (even at concentrations which are well below health limits) and related potential problems of chronic toxicity are currently major priorities for biological research (part 3.2.).

The possible toxicological impacts of some phenols have already been described. –SH–S–CH_3 and –NH_2 bonds in groups of amino acids with quinones (part 3.2.) reduce the biological value of protein. At the same time, the body uses these products to produce so-called AGEs (advanced glycation end-products) which contribute to ageing. Furthermore, unoxidized phenols (mainly phenolic acids) in organisms can bind to proteins with hydrogen bonds and partly change their properties. For example, it has been shown that increases in concentrations of ellagic acid act as a precipitable factor in blood. On the other hand, these bonds in microorganisms and viruses can increase the antimicrobial and antiviral activity of phenols (Singleton, 1981; Pánek *et al*., 1995; Pokorný, 2006; Frankel, 2007).

3.3.4 Effect of interactions in storage at room temperature or in cold storage

Changes of antioxidant activity at room temperature are only slight, especially if enzymes have been previously inactivated, even after a few months' storage. This allows spices to be added even before packaging and distribution in supermarkets. Storage in refrigerated rooms reduces the changes still further.

In spiced foods stored in a frozen state, it is recommended that they be frozen in inert gas or in a vacuum, as the process of food oxidation and antioxidant destruction will otherwise very slowly continue. In spiced foods dried before packing, any microscopic tubes which have contained water remain exposed to air, so that drying in inert gas or in a vacuum is advisable (Frankel, 2007; Shahidi and Ho, 2007; Pokorný and Schmidt, 2010).

3.3.5 Changes in spiced foods when heated

When baking bread, the inside temperature of dough is under 100 °C and, therefore, losses of antioxidants are low. Surface temperature, however, is 150–200 °C

and non-enzymic browning (Maillard) reactions are formed, which act as synergists and bind pro-oxidative traces of iron or copper salts. Fortunately, the weight of the crust is low, so that the dough inside can continue to rise despite the outer browning.

Cocoa beans are rich in flavanols so that antioxidant content remains high in spite of the high roasting temperature used. During Dutch processing of cocoa (in an alkalized medium to obtain a stronger colour and flavour), the higher pH value causes a rapid destruction of flavanols and other polyphenols (Min and Smouth, 1989; Frankel, 2007; Shahidi and Ho, 2007; Pokorný and Schmidt, 2010).

During boiling, food is placed in boiling water at a temperature of approximately 90–110 °C, and oxygen access is negligible. Some losses of volatile antioxidants may be caused by distillation with water vapour. Losses of ascorbic acid occur when cooking green or red cabbage. Antioxidants bound as glycosides are partially hydrolyzed, but the activity of glycosides and free phenols is almost the same. By contrast, however, whilst boiling broccoli, flavonoid and other phenolic compound content is increased by their liberation from insoluble complexes.

Microwave heating is now frequently used both in households and in small restaurants. Heating is faster than in a conventional oven and almost no changes in antioxidant activity are observed. Extrusion cooking occurs at temperatures above 100 °C, but the heating time is short and losses of antioxidant activity are moderately decreased as a result of tocopherol losses, in spite of partial liberation of phenolic substances from complexes with proteins.

3.3.6 Changes as a result of pan or deep fat frying

Traditional pan frying allows free access to air and, even though the heating time is short, losses of phenolic components are high. Oxygen access to antioxidants is partially prevented by the addition of polysiloxane. In deep-fat frying, at a similar temperature to that of pan frying (160–180 °C), there is a thick lipid layer of 100–200 mm. Losses of phenolic antioxidants are higher when frying foods rich in fat, such as meat or fish or full-fat cheese, as the lipid fraction containing non-polar antioxidants is in part subject to diffusion into the frying oil. Phenolic derivatives are efficient at inhibiting polymerization, particularly of oxidized lipids, and their losses of activity are dependent on the degree of their effect on polymerization and the synergistic activity of the food components. In all cases, it is recommended that spices are added after frying (Min and Smouth, 1989; Frankel, 2007; Pokorný and Dostálová, 2011; Pokorný and Schmidt, 2010).

3.3.7 Testing antioxidant activity in foods containing herbs and spices

With the many liquid foods containing herbs and spices, such as flavoured edible oils, it is relatively easy to use the methods specified in the ISO standards (e.g. the Schaal Oven test) to determine antioxidant activity. With solid foods, however, their composition is too complicated so that shelf-life has to be determined experimentally (except for groups of foods of identical or nearly identical composition, such as batches of the same materials prepared for packing). If longer-term storage of packed foods is foreseen, it is safer to test the shelf-life packed, before distribution to the market. Sensory analysis (using standard ISO methods) is very

useful for this purpose. For chemical examination, it is possible to isolate the lipid fraction by extraction using organic solvents (ISO standard), then determining the peroxide and acid values using common methods.

3.4 Future trends and conclusions

In the near future, it is likely that herbs and spices will be kept in optimum conditions and in optimum packaging to minimize losses by volatilization and negative changes before their application. In some cases, it could be useful to include them in special non-reactive small packs directly inside the packed food. This procedure would be particularly useful in the case of liquid flavourings. Losses can be minimized further by adding ascorbic acid to spices.

Shelf-lives are now unnecessarily long in modern supermarkets, as spiced foods are often sold and consumed within a few days. It should be possible to reduce the shelf guarantee for some foods.

Spiced foods are often heated before consumption. In modern ovens, heating time and/or temperature can be reduced which requires less spice to be added before heat processing.

Spice antioxidants are partially oxidized during food preparation; some antioxidants can be saved if oxidation is prevented or at least minimized – something which will become possible in more modern cooking devices, where food is protected by water vapour or by carbon dioxide.

With most foods, the addition of herbs and spices is a good idea and can improve shelf-life to various degrees, depending on the type of spice, type of food and on the treatment during food preparation; the exact prolongation of shelf-life can only be determined by experiment. Generally some losses occur, especially at high temperatures, such as during frying. The type of device used for food processing is also important, but modern devices usually result in lower losses.

This chapter has discussed the possibility of oxidation of antioxidants and the consequent occurrence of dimers or higher oligomers in plants. Products usually have an antioxidant capacity similar to that of their original compounds, as shown in the example of gallic acid dimerization to ellagic acid. However, non-enzymatic oxidation (for example, by lipid peroxyl radicals) and consequent dimerization and other reactions usually lead to a decrease in or loss of antioxidant capacity. However, these reactions are very important because they lead to the inactivation of reactive antioxidant radicals (a diagram showing lipid peroxidation by free radicals or other reactive oxygen species and antioxidant action is presented in Fig. 3.5).

Oxidation of phenols by peroxyl radicals can be connected with a loss of antioxidant activities. For example, catechins and other flavonoids can create higher polymers, called condensed tannins. By contrast, polymerization of phenolic acids can lead to the breaking of benzene rings and to the formation of inactive compounds. These reactions are shown in Fig. 3.6, with the example of gallic and caffeic acids. (Jungbluth and Ternes, 2002; Pokorný, 2006; Frankel, 2007; Pokorný and Schmidt, 2010).

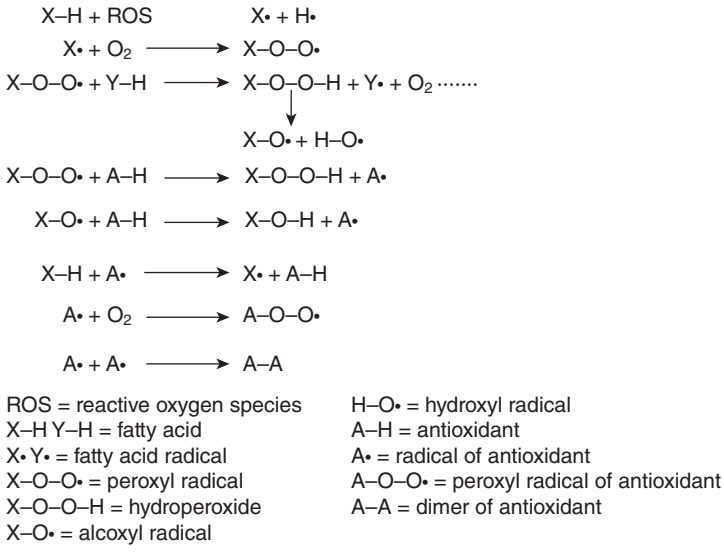


Fig. 3.5 Scheme of lipid peroxidation and function of antioxidants.

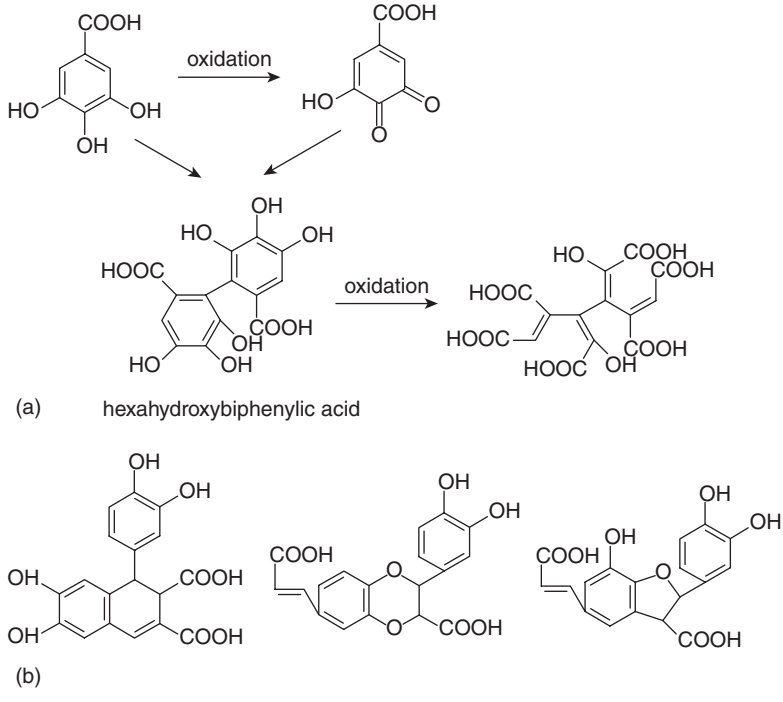


Fig. 3.6 (a) Oxidation and consequent degradation of gallic acid and (b) some dimers created from caffeic acid.

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4

Health benefits of herbs and spices

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Abstract: This chapter discusses the different health aspects of herbs and spices. It begins by introducing the historical uses of herbs and spices, in diet and in traditional medicine. The phytochemical classes associated with herbs and spices are also discussed. The following section goes on to review the potential for herbs and spices in chemoprevention of cancer through antioxidant activity, antimicrobial activity, anti-inflammatory activity, alteration of biotransformation enzyme activity, antitumorigenic mechanisms and antimutagenic and apoptotic activity. The next section discusses the health effects of herbs and spices on the cardiovascular system, diabetes, osteoarthritis and inflammatory response, obesity, gastro-intestinal and hepatoprotection, neuro-degenerative disorders, as well as infections and parasitic diseases. Safety and toxicity concerns from a medicinal perspective are briefly discussed, followed by future trends.

Key words: antioxidant activity, antimicrobial activity, anti-inflammatory activity, alteration of biotransformation enzyme activity, antitumorigenic mechanisms and antimutagenic and apoptotic activity.

4.1 Introduction

Herbs and spices have a rich tradition of use as preservatives, colorants, flavor enhancers and as potential therapeutic agents. Increased use of herbs and spices as flavorings in foods is a major trend globally, with an estimated growth rate of 20–30 %. Trends in spice and herb usage vary substantially since regions around the world often have distinct ethnic cuisines. Many ethnic cuisines are notable for this use of spices and herbs. Turmeric in Indian cuisine; basil, garlic and oregano in Italian and Greek cuisines; and lemongrass, ginger, cilantro and chilli peppers in Thai food illustrate the diverse use of herbs and spices around the world. A study which identified the most commonly used spices involving 36 countries found the most commonly used spices worldwide were onions, garlic, ginger and some peppers (Kaefer and Milner, 2008).

Efforts to assess dietary intake of spices and herbs are complicated because their use is so varied, and because they are consumed in conjunction with other foods and in trace amounts. Concentrations of herbs and spices in finished foods frequently fall within the range of 0.5–1 % (Kaefer and Milner, 2008). Population-wide, average dietary intake of common spices per person per day is estimated at 0.5 g in Europe, 1.0 g in New Zealand, 4 g in the USA and 1.5 g of turmeric alone

in India (Lampe, 2003). Garlic usage has increased more than six-fold in the US (USDA-ERS, 2011).

The beneficial properties in herbs and spices are due to presence of phytochemicals. The major phytochemical classes associated with herbs and spices include a diverse array of compounds such as terpenes and terpene derivatives (Lampe, 2003). Other compounds include glycosides, alkaloids like piperine, chavicene, capsaicinoids (pepper fruit), saponins like trigonelline, allyl sulphur, adenosine, (onions and garlic), quercetin (onions), curcumin (turmeric) and isothiocyanates (cruciferous vegetables) (Halvorsen *et al*., 2002; Keservani *et al*., 2010). Both *in vitro* and *in vivo* studies have suggested that dietary spices and herbs maintain human health by their antioxidative, chemopreventive, antimutagenic, anti-inflammatory and immune modulatory effects (Conn, 1995). Dietary guidelines refer to the usefulness of herbs as good sources of antioxidant compounds and as salt substitutes (Tapsell *et al*., 2006).

Culinary herbs and spices were historically used for both culinary and health benefits. Herbal medicine was practised by ancient cultures in Asia, Africa, Europe and the Americas. In Ancient Egypt, papyri from 1555 BC mention coriander, fennel, juniper, cumin, garlic and thyme. In India, the traditional medicine, Ayurveda, evolved more than 5000 years ago in the Himalayas, with knowledge transmitted orally until it was written down in Sanskrit poetry – the Vedas – around 1500 BCE. Ayurveda focuses on the use of diet in disease prevention (Thatte and Dahanukar, 1986). Spices and herbs are important ingredients in the prescriptions of Indian systems of medicine including Ayurveda, Siddha and Unani (Kochhar, 2008).

As early as 460–377 BCE, Hippocrates began recording medicinal uses for over 300 herbs and spices (including garlic, cinnamon and rosemary) in Ancient Greece. Roughly 500 years later, a Greek physician and botanist Galen published information on over 600 herbs with description on their usage (Bellamy and Pfister, 1992). In China, two legendary emperors, Sheng Nong and Huang Di, are credited with discovering and recording the medicinal properties of herbs. Arabic medicine (500–1300 CE) was developed based on the knowledge of Galen. The spread of Islamic culture into North Africa blended their knowledge with that from China and India. During the 11th century, the knowledge of Arabic medicine filtered back to Europe and, by the 13th century, trade with Africa and Asia brought in new herbs and spices. Around this time, for example, galangal was called the 'spice of life' and garlic was used during outbreaks of plague. As a result, spices and herbs have long been used used for a variety of ailments the world over (Table 4.1).

4.2 Cancer preventive properties of herbs and spices

A number of *in vitro* and *in vivo* models indicate the potential for herbs and spices in chemoprevention of cancer (Lampe, 2003; Kaefer and Milner, 2008; Bhattacharjee and Sengupta, 2009; Aravindaram and Yang, 2010). Mechanisms in herbs and spices related to inhibition of mutagenesis and epigenesis of DNA include antioxidant activity, alteration of biotransformation enzyme activity and antibacterial and antiviral effects (Lampe, 2003; Kaefer and Milner, 2008). As a result, clinical trials have shown the safety of herbs such as turmeric and curcumin in the treatment of

Table 4.1 Medicinal properties of spices and herbs

Spices/herbs	Medicinal properties
Ajowan	Digestive, antispasmodic, stimulant, carminative, expectorant
Allspice	Stimulant, digestive, carminative, anodyne against rheumatism and neuralgia
Aniseed	Mild expectorant, stimulating, carminative, diuretic, diaphoretic, in asthma powders, in veterinary medicine
Basil	Stomachic, anthelmintic, diaphoretic, expectorant, antipyretic, carminative, stimulant, diuretic, demulcent, in skin diseases, asthma, opthalmia
Bay leaves	Stimulant, in sprains, narcotic and in veterinary medicine
Caper	Diuretic, aspirant, expectorant, emmenagogue, tonic and in scurvy, rheumatism, gout, afflictions of liver and spleen
Capsicum	Digestive, thermogenic, carminative, stimulant, cardiotonic, antipyretic, serdorific, rubefacient and sialagogue
Caraway	Stomachic, carminative, anthemintic, lactagogue, adjuvant/ corrective for nauseating and griping effects of medicines
Cardamom (small)	Stimulant, tonic, diuretic, carminative, digestive, expectorant, cardiotonic and used in several pharmaceutical preparations
Cardamom (large)	Hypnotic, appetizer, astringent to bowels, tonic to heart and liver
Cambodge	Astringent, digestive, thermogenic, constipating, used in haemorrhoids, diarrhoea and to control obesity
Cassia	Astringent, stimulant, carminative, germicidal, for checking nausea and vomiting
Celery	Stimulant, tonic, diuretic, carminative, emmenagogue, anti-inflammatory
Cinnamon	Astringent, diuretic, carminative, aphrodisiac, deodorant, expectorant, febrifuge, stomachic
Clove	Refrigerant, ophthalmic, digestive, carminative, stomachic, stimulant, antispasmodic, antibacterial, expectorant, rubefacient, aphrodisiac, appetizer, emollient
Coriander	Carminative, diuretic, tonic, stimulant, stomachic, refrigerant, aphrodisiac, analgesic, anti-inflammatory
Cumin	Digestive, carminative, astringent, anti-inflammatory, constipating, diuretic, revulsive, galactogogue, uterine and nerve stimulant
Curry leaf	Astringent, anthelmintic, febrifuge, stomachic, appetizing, carminative, constipating, anti-inflammatory, antiseptic, used in skin diseases, and in diarrhoea, ulcers
Dill	Carminative, stomachic, antipyretic
Fennel	Stimulant, carminative, stomachic, emmenagogue, refrigerant, cardiac stimulant, antiemetic, aphrodisiac, anthelmintic
Fenugreek	Carminative, tonic, aphrodisiac, emollient, antibacterial, used in vomiting, fever, anorexia, colonitis
Garlic	Anticholesterol, antifungal, tonic, rubefacient, stimulant, thermogenic, aphrodisiac, used in cough, asthma, cardiopathy
Ginger	Digestive, carminative, emollient, appetizer, stomachic, rubefacient, anodyne, expectorant, anthelmintic, stimulant
Greater galangal	Carminative, expectorant, digestive, vulnerary, febrifuge, stimulant, depurative, used in skin diseases, rheumatism, asthma, wounds, fever, haemorrhoids
Horse radish	Thermogenic, appetizing, digestive, stomachic, laxative, anti-inflammatory, anodyne, refreshing, antibacterial
Hyssop	Stimulant, carminative, pectoral, used in nervous disorders, toothache, pulmonary and uterine troubles
Juniper berry	Carminative, stimulant, diuretic, useful in dropsy, leucorrhoea, urino-genital disorders
Kokam	Cooling, anthelmintic, cardiotonic, astringent, emollient, useful in piles, dysentery, heart complaints, bilious affections

Table 4.1 *Continued*

Spices/herbs	Medicinal properties
Mace	Cooling, febrifuge, expectorant
Marjoram	Carminative, expectorant, tonic, astringent
Mint	Stimulant, stomachic, carminative, antiseptic, digestive, antispasmodic, contraceptive, used in vomiting, skin diseases, amenorrhoea, dental caries
Mustard	Thermogenic, anodyne, anti-inflammatory, carminative, digestive, anthelmintic, sudorphic, tonic, emetic, used in vomiting, abdominal colic, dyspepsia, flatulence, skin diseases
Nutmeg	Astringent, sweet, thermogenic, aphrodisiac, anti-inflammatory, anodyne, deodorant, digestive, expectorant, narcotic, anticonvulsant, antiseptic, constipating
Oregano	Stimulant, carminative, stomachic, diuretic, diaphoretic, emmenagogue
Parsley	Stimulant, diuretic, carminative, emmenagogue, antipyretic, anti-inflammatory, emetic, aphrodisiac, alexipharmic, refrigerant
Pepper	Anthelmintic, carminative, alterant, antiperiodic, diuretic, digestive, emmenagogue, rubefacient, stimulant, stomachic, used in fever, asthma, cough, dyspepsia, flatulence, arthritis
Pepper long	Expectorant, thermogenic, diuretic, tonic, purgative, stomachic, digestive, emollient, antiseptic, used in bronchitis, fever, asthma
Pomegranate	Astringent, cooling, tonic, aphrodisiac, laxative, diuretic, cardiotonic, used in pectoral diseases, dysentery, diarrhoea, vomiting
Poppy seeds	Expectorant, sudorific, sedative, nervine tonic, constipating, aphrodisiac, used in internal haemorrhages, diarrhoea, dysentery
Rosemary	Astringent, nervine tonic, stomachic, antibacterial, protistocidal, rubefacient, used in headaches and heavy menstruation
Saffron	Stimulant, tonic, stomachic, aphrodisiac, anodyne, antispasmodic, emmenagogue, diuretic, laxative, used in bronchitis, fever, epilepsy, skin diseases, decolouration of skin
Sage	Mild tonic, astringent, carminative, deodorant, insecticidal, antipyretic, used in gingivitis, dentifrice, mouthwash, gargles
Savory	Antispasmodic, anticatarrhal, astringent, carminative, laxative, diuretic, stomachic, sudorific, vermifuge
Star anise	Astringent, carminative, deodorant, expectorant, digestive
Sweet flag	Thermogenic, constipating, emmenagogue, intellect promoting, emetic, carminative, stomachic, expectorant, sudorific, antipyretic, resuscitative, tranquilizing, sedative, nervine tonic
Tamarind	Refrigerant, digestive, carminative, laxative, antiscorbutic, febrifuge, ophthalmic, useful in gastropathy, datura poisoning, alcoholic intoxication, scabies, constipation
Tarragon	Aperient, stomachic, stimulant, febrifuge
Tejpat	Carminative, used in colic, diarrhoea
Thyme	Antispasmodic, carminative, emmenagogue, anthelmintic, spasmodic, laxative, stomachic, tonic, vermifuge
Turmeric	Thermogenic, emollient, anodyne, anti-inflammatory, vulnerary, depurative, antiseptic, appetizer, carminative, expectorant, stomachic, anthelmintic, stimulant, ophthalmic, tonic, used in skin diseases, dyspepsia, asthma, cough, bronchitis, inflammations, ulcers, worms, skin discolouration
Vanilla	Aphrodisiac

Source: Spices Board, India (2012).

conditions such as colorectal cancer (Holt *et al*., 2005; Kaefer and Milner, 2008; Krishnaswamy, 2008). However, it is important to note that other studies have shown limited effectiveness of herbs and spices such as garlic, ginger and cinnamon (Janssen *et al*., 1996; Rompelberg *et al*., 1996; McNulty *et al*., 2001).

4.2.1 Antioxidant activity

Oxidant–antioxidant balance is critical in the human body because it maintains cell membrane integrity and functionality, cell proteins and nucleic acids. Oxidant–antioxidant imbalance has been linked to impaired cell functions, cell death, impaired immunity and DNA damage which can lead to cancer (Knight, 2000). Antioxidants, by virtue of their capability to quench free radicals, can prevent oxidative damage to DNA. Epidemiological evidence indicates a correlation between increased dietary intake of antioxidants and a lower incidence of morbidity and mortality (Devasagayam *et al*., 2004). A positive linear correlation between phenolic compounds, primarily phenolic acids and flavonoids, and the antioxidant capacity of herbs and spices has been reported (Zheng and Wang, 2001). Cancer chemopreventive activities have been shown in both *in vitro* and *in vivo* animal models (Surh *et al*., 1999; Murakami *et al*., 2004).

The largest published study to date on the presence of antioxidants in foods is that of Carlsen *et al*. (2010) which analysed more than 3100 foods. Samples of 425 spices and herbs from 59 different manufacturers/countries revealed 27 products in the range 100–465 mmol/100 g, with a variation from 0.08 mmol/100 g in raw garlic paste procured in Japan to 465 mmol/100 g in dried and ground clove purchased in Norway. Clove has the highest mean antioxidant value, followed by peppermint, allspice, cinnamon, oregano, thyme, sage, rosemary and saffron, with mean values in dried herbs ranging from 44–277 mmol/100 g. When analysed in fresh compared to dried samples, oregano, rosemary, thyme, basil, chives, dill and parsley have lower values, in the range 2.2–5.6 mmol/100 g.

Clove, ginger, cinnamon, turmeric, allspice, black pepper and cumin among spices and oregano, sage, peppermint, thyme, rosemary, dill and marjoram among the herbs, were also reported to contain the greatest antioxidant capacity in several studies (Zheng and Wang, 2001; Dragland *et al*., 2003; Wu *et al*., 2004; Ninfali *et al*., 2005; Halvorsen *et al*., 2006). Variation in antioxidant capacity between studies reported may be attributed to lipophilic/hydrophilic fractions, genotypic and environmental differences within species, parts of the plants studied, season and analytical methods used (Wu *et al*., 2004; Shan *et al*., 2005).

4.2.2 Antimicrobial activity

Several strains of *Helicobacter* species (*H. pylori*, *H. cholecystus*, *H. pullorum*, *H. bilis*, and *H. hepaticus*) may facilitate the invasion and progression of cancer, especially in the stomach, liver, gallbladder and intestine. It has been estimated that bacterial, viral and parasitic mediated cancer deaths range from 20–25 % in developing countries and 7–10 % in developed countries (Schottenfeld and Beebe-Dimmer, 2006). Herbs and spices which possess antimicrobial activity (especially against

gram-positive bacteria) include those containing simple phenols and phenolic acids, coumarins, terpenoids and alkaloids. Spices and herbs which inhibit *H. pylori* have potential in anti-*H. pylori* therapies (Bergonzelli *et al.*, 2003). In a declining order, turmeric, cumin, ginger, chilli, borage, black caraway and oregano figure in the bactericidal activity (Al Mofleh, 2010).

4.2.3 Anti-inflammatory

Estimates suggest that approximately 15 % of all cancers are linked to pro-inflammatory mediators, such as cytokines, chemokines, prostaglandins (PGs), nitric oxide (NO) and leukotrienes, which disrupt normal signalling within cells (Surh *et al.*, 2005). *In vitro* studies indicate that bioactive components in several herbs and spices can inhibit inflammatory activity (Hollman and Katan, 1999). These components which inhibited COX-2 expression include curcumin, delphinidin (abundant in pomegranate) and salicylic acid (in chilli, paprika, turmeric and cumin) (Kaefer and Milner, 2008; Aravindaram and Yang, 2010). NO is an inflammatory mediator implicated in cancer development which is inhibited by bioactive components within herbs and spices. Rosemary has been found to be most effective in blocking NO formation, followed by tarragon, cinnamon, oregano, basil, marjoram, allspice and thyme (Tsai *et al.*, 2007). NO is closely linked to the nuclear transcription factor κB (NF–κB) pathway. Quercetin, a bioactive compound in basil, cumin and fennel, ursolic acid (found in basil and rosemary), gingerol (ginger), capsaicin and curcumin inhibits the NF–κB pathway *in vivo* and *in vitro* (Singh and Aggarwal, 1995; Shishodia *et al.*, 2003; Kim *et al.*, 2004; Comalada *et al.*, 2005; Aggarwal and Shishodia, 2006).

4.2.4 Alteration of biotransformation enzyme activity

Biotransformation enzymes, also referred to as xenobiotic or drug-metabolizing enzymes, play a major role in regulating the toxic, mutagenic and neoplastic effects of chemical carcinogens. Phase I enzymes have an important role in the activation of procarcinogens; equally important in disease prevention are the phase II enzymes, involved in the body's natural detoxification process. Compounds in garlic (diallyl sulphide, diallyl sulfone and diallyl sulfoxide, pepper (piperine), rosemary (1,8-cineole), turmeric (curcumin) and cinnamon appear to influence phase I and phase II enzymes (Debersac *et al.*, 2001; Zhou *et al.*, 2003; Shen *et al.*, 2006). Coriander, citral from lemongrass and myristicin from parsley alter biotransformation enzyme activities (Lampe, 2003; Tapsell *et al.*, 2006).

4.2.5 Antitumorigenic mechanisms

Tumorigenesis, the loss of controlled growth regulation, is a factor in the development of cancer. Turmeric, curcumin, capsaicin and quercetin-containing spices such as basil, cumin, coriander and fennel suppress growth signalling pathways that are crucial in providing proliferation signals to cells (Aggarwal and Shishodia, 2006). Peppermint (McKay and Blumberg, 2006), geraniol in lemon grass, myristicin from

parsley (Tapsell *et al*., 2006), piperine or black pepper (Singletary, 2010a) and thyme and cinnamon essential oils (Zu *et al*., 2010) inhibited tumour growth in *in vitro* and rodent models. Rosemary extract had antiproliferative effect on human leukaemia and breast carcinoma cells (Faixova and Faix, 2008).

4.2.6 Antimutagenic and apoptotic activity

Inhibiting the activity of mutagens can be one of the most important ways to prevent initiation of carcinogenic processes. Extracts of caraway, coriander and black pepper seeds, juice of coriander and mustard, essential oils from nutmeg, ginger, cardamom, celery, xanthoxylum, black pepper, cumin, coriander and eugenol (in cloves and cardamom) have been observed to inhibit mutagenicity (Bhattacharjee and Sengupta, 2009).

Induction of apoptosis in tumour cells, a form of physiological death in unwanted or dysfunctional cells, is a promising therapeutic approach. Natural mediators of apoptosis may play an important role in the prevention of cancer. Allicin has been shown to induce apoptosis of several human non-leukaemia malignant cells including breast, bladder, colorectal, hepatic, prostate cancer, lymphoma and skin tumour cell lines (Bhattacharjee and Sengupta, 2009). Ajoene has inhibited proliferation and induced apoptosis of human leukaemia cells (Rahman, 2007). Cinnamaldehyde (cinnamon), 1,8-cineole (bay leaves and cardamom), diosgenin, protodioscin (PD), isothiocyanates (mustard seeds) and garlic extract are effective in inducing cell apoptosis in a number of human cancer cells (Bhattacharjee and Sengupta, 2009). Apigenin, a flavonoid from parsley, celery and lettuce, diallyl sulphide from onion and garlic and allicin from garlic, and curcumin has also been seen to induce apoptosis in human cancer cells (Aravindaram and Yang, 2010).

4.3 Other health effects of herbs and spices

4.3.1 Cardiovascular effects

Cardiovascular diseases are caused by a great number of factors such as high cholesterol, hypertension and increased platelet aggregation. A reduction in low-density lipoprotein (LDL)-cholesterol levels is considered important to reduce the risk of heart attack (Rahman, 2007). Several spices and their active principles have been associated with lowering of LDL cholesterol, including garlic, ginger, lemon grass, cinnamon, fenugreek, onion, capsaicin and curcumin (Akhani *et al*., 2004; Srinivasan *et al*., 2004; Srinivasan, 2006; Tapsell *et al*., 2006; Sance *et al*., 2008). Garlic has also been associated with reducing blood pressure (Rahman, 2007). There is evidence to suggest that curcumin has the potential to prevent stroke and lessen vascular inflammation and cerebral vasospasm following haemorrhagic stroke (Singletary, 2010 b).

4.3.2 Effects on diabetes

Spices and herbs are known to improve symptoms of diabetes by delaying gastric emptying, improving insulin sensitivity and enhancing antioxidant defences. Cumin

seeds, ginger, mustard, curry leaves, coriander, curcumin and fenugreek have been reported to have hypoglycemic effects (Akhani *et al.*, 2004; Srinivasan, 2006; Kochhar, 2008; Singletary, 2010b). Rosemary and, in particular, cinnamon have been seen to reduce blood glucose levels, although trials involving cinnamon supplementation in patients with type 2 diabetes have produced contrasting results (Broadhurst *et al.*, 2000; Khan *et al.*, 2003; Anderson *et al.*, 2004; Vanschoonbeek *et al.*, 2006; Hlebowicz *et al.*, 2007; Pham *et al.*, 2007; Bakirel *et al.*, 2008; Dearlove *et al.*, 2008; Cao *et al.*, 2010). The use of cinnamon as an adjunct to the treatment of type 2 diabetes mellitus is particularly promising but, as with other spices, well-controlled clinical studies are needed before definitive recommendations can be made (Gruenwald *et al.*, 2010).

4.3.3 Osteoarthritis and inflammatory response

Inflammation plays an important role in various diseases such as rheumatoid arthritis and osteoarthritis. A diet rich in herbs and spices may contribute to the reduction of the inflammatory response and related diseases (Mueller *et al.*, 2010). Herbs and spices such as turmeric, curcumin, rosemary, spearmint and peppermint have exerted anti-inflammatory and anti-atherosclerotic effects (Faixova and Faix, 2008; Krishnaswamy, 2008; Singletary, 2010b; Yi and Wetzstein, 2010). Epidemiological studies have indicated that populations that consume foods rich in polyphenols such as ginger have lower incidences of inflammatory disease. Animal models have been used for *in vitro* and *in vivo* testing of the use of ginger (Yasujiro, 2002; Tapsell *et al.*, 2006; Chien *et al.*, 2008), thymoquinone from black cumin (Tekeoglu *et al.*, 2007) and curcumin (Singletary, 2010b) in reducing arthritic symptoms and inflammatory bowel disease in humans (Hanai and Sugimoto, 2009). The anti-inflammatory activity of approximately 30 plant extracts and several plant compounds has suggested the highest anti-inflammatory potential for chilli pepper, followed by plants such as allspice, basil, bay leaves, black pepper, liquorice, nutmeg, oregano, sage and thyme (Mueller *et al.*, 2010).

4.3.4 Obesity

Obesity is one of the most pressing public health problems worldwide. Although weight gain is essentially caused by an imbalance between energy input and energy output, there are several factors that predispose individuals to obesity. Genetics, fat absorption, metabolic rate and appetite influence weight gain. Herbs and spices have a number of properties that make them potential agents to help prevent and combat obesity. They act by suppressing the appetite, reducing fat absorption and fat deposition and increasing the metabolic burning off of fat (Ludy and Mattes, 2011).

Although results have been mixed, hydroxycitric acid (HCA), derived from *Garcinia cambogia*, has helped to lower body weight and reduce fat mass in humans (Heymsfield *et al.*, 1998; Preuss *et al.*, 2004). Capsaicin, the pungent principle in hot red peppers, has been reported to reduce hunger, stimulate thermogenesis and alter substrate oxidation in humans (Westerterp-Plantenga *et al.*, 2005; Smeets and Westerterp-Plantenga, 2009), and capsiate, a non-pungent capsaicin analogue,

appears to be promising for non-users who abstain from hot pepper due to sensory burn (Ludy and Mattes, 2011). Dietary fenugreek has been found to be hypocholesterolemic (Srinivasan *et al*., 2004). Garlic, ginger and curcumin have also shown lipid-lowering potential in experimental animals as well as in clinical trials (Shashikanth *et al*., 1986; Woo *et al*., 2007; Yadav and Bhatnagar, 2007; Al Mofleh, 2010). In a recent study, 30 spices were subjected to bioactivity screening with several anti-obesity related bioassays. Sesame seed, red chilli, nutmeg, mace, black pepper, turmeric, pimento, turmeric and black onion were identified as the most promising (Yuliana *et al*., 2011).

4.3.5 Gastrointestinal effects and hepatoprotective properties

The role of spices such as fenugreek, black pepper and peppermint in aiding digestion is a combination of their influences on salivary, gastric, biliary and pancreatic secretions and the terminal digestive enzymes present on the mucosa of the small intestine (McKay and Blumberg, 2006; Srinivasan, 2006; Singletary, 2010a). Spices such as ginger and capsaicin also possess potential anti-ulcer properties (Shukla and Singh, 2007; Al Mofleh, 2010).

Liver diseases are a major worldwide health problem in which herbs and spices have a potential therapeutic role (Adewusi and Afolayan, 2010). Several studies have shown the hepatoprotective effects of herbs and spices such turmeric (curcumin), rosemary, larger cardamom, oregano, *Angelica glauca* and *Garcinia kola* (Soni *et al*., 1992; Farombi, 2000; Morikawa, 2007; Joshi *et al*., 2008; Parmar *et al*., 2009; Al-Jassabi and Saif-Ali, 2010; Ibrahim *et al*., 2010). Adewusi and Afolayan (2010) identified 107 plants and 58 compounds (glycosides, flavonoids, triterpenes and phenolic compounds) with hepatoprotective activity. The spices and herbs with hepatoprotective activity include celery, fenugreek, fennel, sweet basil and caraway. The essential oil of caraway appears promising for safe use as a hepatoprotective agent (Samojlik *et al*., 2010).

4.3.6 Benefits for neurodegenerative disorders

According to World Health Organization (WHO), about 450 million people suffer mental, neurological or behavioural problems at some time in their life. Conditions range from epilepsy to Alzheimer's disease. Extensive research on plants and their derivatives has taken place in recent years that could provide new alternative treatments and therapeutic approaches for diseases of the central nervous system (de Almeida *et al*., 2011). Epidemiological studies have shown a relationship between levels of antioxidants, dementia and cognitive impairment. Herbs and spices may influence cognitive decline through the neuroprotective action of antioxidants (Tapsell *et al*., 2006).

Although several spices such as turmeric, rosemary, sage, cardamom, lemon and garlic may have the potential to prevent and treat several neurological diseases, saffron has been one of the first to be tested as a treatment of mild depression in clinical trials (Abe and Saito, 2000; Hosseinzadeh and Khosravan, 2002; Akhondzadeh *et al*., 2005). It may also have potential in the treatment of

Alzheimer's disease (Pellegrini *et al*., 2006). Rosemary essential oil has been found to cause moderate inhibition of acetylcholine esterase, considered one of the treatment strategies against several neurologic disorders, including Alzheimer's disease, senile dementia and myasthenia gravis (Faixova and Faix, 2008). Animal studies suggest that piperine may have an antidepressant-like action (Singletary, 2010a). There is also evidence that curcumin may lessen the development and progression of Alzheimer's disease, improving memory function and cognition (Jyoti *et al*., 2009; Singletary, 2010b). A literature-based survey identified spices and herbs with anticonvulsant activity such as *Ocimum basilicum*, *Laurus nobilis*, *Lippia alba*, *Nigella sativa*, *Myristica fragrans*, *Syzygium aromaticum*, *Pimpinella anisum*, *Cymbopogon citratus*, *C. winterianus*, *Cuminum cyminum*, *Ferula gumosa*, *Acorus calamus* and *Acorus gramineus* (de Almeida *et al*., 2011).

4.3.7 Infections and parasitic diseases

The emergence of parasites resistant to current chemotherapies highlights the importance of plants, their extracts and essential oils as novel antiparasitic agents. Herbs and spices which possess antimicrobial activity include those containing simple phenols and phenolic acids, coumarins, terpenoids and alkaloids (McNulty *et al*., 2001). Essential oils extracted from herbs and spices are more effective as bactericidal agents when compared to their primary constituents, which indicates that there may be unknown minor components in the essential oils with antimicrobial activity, or there may be synergistic or antagonistic effects between all the bioactive compounds combined (Bergonzelli *et al*., 2003; Anthony *et al*., 2005). Garlic oil has been found to be antibacterial, antifungal, antiviral and antiparasitic (Cellini *et al*., 1996; Ankri and Mirelman, 1999; Bakri and Douglas, 2005). Lemon balm, oregano, cinnamon and thyme oils also showed activity against more than one parasite. Activity against coccidia and flagellates was shown by garlic, oregano and cinnamon oils. Basil and black seed showed activity against helminths while African guinea pepper and garlic were effective against *Plasmodium* species (Anthony *et al*., 2005). Essential oils from clove and rosemary alone and in combination exerted a significant antimicrobial effect against *Staphylococcus*, *Escherichia coli* and *Candida albicans* and methanol extracts of curry leaf against *Staphylococcus* spp. (Fu *et al*., 2007; Shihabudeen *et al*., 2010). Curcumin has shown potential as antitubercular, antimalarial and antibacterial agent (Singletary, 2010b).

4.4 Safety and toxicity

Kaefer and Milner (2008) explain the issue of herb and spice safety and toxicity clearly as follows:

> Herbs and spices are 'Generally Recognized As Safe' (GRAS) by the FDA, at least at concentrations commonly found in foods (0.5–1 %). However, many herbs, spices, and their bioactive components, being investigated for potential disease prevention and treatment at concentrations often exceed those commonly used in food preparation.

The NCI/NTP has reviewed the safety of several herbs and spices, suggesting some evidence of carcinogenic activity in spices such as turmeric when very high doses are consumed (NTP, 1992, 1993, 2004).

The German Commission E Monographs are probably the most widely known resource on herbal medicines (Blumenthal, 1998). Commission E currently has published more than 400 monographs on herbs and spices, including pharmacological and toxicology studies. The first category of monographs consists of herbs and spices that are 'unapproved' i.e. where 'no plausible evidence of efficacy' was available or when safety concerns outweighed potential benefits associated with the product's use. Basil, lemongrass, marjoram, nutmeg and saffron are included in this category.

Herbs and spices included in the 'approved' monographs include caraway oil and seed, cardamom seed, cinnamon bark, cloves, coriander seed, dill seed, fennel oil and seed, garlic, ginger root, liquorice root, mint oil, onion, paprika, parsley herb and root, peppermint leaf and oil, rosemary, sage, thyme, turmeric root, and white mustard seed. The recommendations for the various essential oils range from 3–6 drops per day for caraway and mint oils to 10–20 drops for rosemary. The recommended dose for seeds and other herbs and spices range from 1.5 g/day for caraway and cardamom seeds to 50 g/day of fresh onion or 20 g dried onion.

It has been suggested that excessive consumption of garlic can cause a range of problems (Amagase *et al*., 2001; Imada, 1990), though extensive testing by the FDA in the USA supports the Commission E finding that garlic is generally safe (Rahman, 2007). Reviews of the safety of some other herbs include fenugreek (Rao, 1996; Muralidhara, 1999), nigella (Ali and Blunden, 2003; Al Mofleh, 2010), turmeric and curcumin (Singletary, 2010b).

4.5 Future trends

Much still needs to be learnt about the potential health benefits of herbs and spices. There is a lack of standardization and comparability between individual studies which makes it hard to draw conclusions (Kaefer and Milner, 2008). There is a need for placebo-controlled clinical trials to determine safety and optimal dosage, bioavailability and bioefficacy of herbs and spices (Tapsell *et al*., 2006). There is also a need to understand the effect of spices within the context of the total diet and to establish appropriate guidelines which may then need to be tailored to individual genetic profiles (Lampe, 2003; Kaefer and Milner, 2008). In cases where the *in vivo* efficacy in humans at typical dietary intakes is constrained by poor bioavailability as in curcumin, strategies to improve absorption are warranted (Singletary, 2010b).

4.6 References and further reading

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5

Methods of analysis of herbs and spices

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Abstract: The quality of spices depends largely on the chemical signatures of the essential oils and oleoresins contained within them. Every importing country has developed quality requirements for spices and in this context standard analytical techniques for quality assessment of spices have been established. This chapter describes some of these methodologies, discussing general analytical methods, distillation and extraction techniques, and methods of identifying the constituents of essential oils and of estimating the oleoresins in spices. Methods for quantifying antioxidant capacity and estimation of crude fibre are also described.

Key words: volatile oil, oleoresin, piperine, curcumin, capsaicin, anthocyanins, phenols, carotenoids, antioxidant, crude fibre.

5.1 Introduction

Spices are storehouses of many chemical constituents that impart flavour, fragrance and piquancy. Most spices owe their flavouring properties to volatile oils and in some cases to a combination of oils and resinoids, which are known as oleoresins. No single compound is responsible for the characteristic flavour of a spice; but a combination of different chemical constituents such as alcohols, phenols, aldehydes, esters, terpenes and alkaloids in various proportions impart flavour. The quality of spices is assessed by intrinsic and extrinsic characteristics of spices. The former consists of chemical quality while the latter emphasizes the physical quality. This includes the appearance, shape, texture, colour, presence or absence of extraneous things, etc. In addition to this, certain quality standards of spices, namely, pesticide residue, aflatoxin, heavy metals, solvent residues, sulphur dioxide and microbiological quality are stipulated. The physicochemical qualities remain the ultimate attribute while considering the export requirement of spices. The physicochemical characteristics vary depending on the variety, agroclimatic conditions existing in the area of cultivation, harvest and post-harvest operations.

Every importing country has developed quality requirements for spices. Cleanliness specifications for spices of ASTA (American Spice Trade Association) are a universally adopted manual for the assessment of physical quality of spices. The US Food and Drug Administration (USFDA) has also provided quality specifications

for spices. Besides this, Indian agencies namely, AGMARK and Bureau of Indian Standards (BIS), have also developed quality regulations. Similarly, European Spice Association (ESA), British Standard Institution (BSI), International Standards Organization (ISO), Essential Oil Association (EOA) are other reputed agencies providing quality specifications for spices. This underlines the importance of adopting standard analytical techniques for quality assessment of spices.

5.2 General analytical methods

5.2.1 Sieve analysis

Purpose: To determine the particle size distribution of prepared samples.

Apparatus required:
Shaking device, vibratory sieve – Model VS1 OR VE1, or equivalent. Sieves, US Standard or equivalent, 20 cm in diameter, with cover and bottom pan (clean and free of oil and other contamination). Faucet washers, rubber, srewcap.

Procedure:
Obtain the tare weight of each sieve containing three faucet washers and nest the sieves in order of decreasing fineness; attach the cover and tared bottom pan. Weigh 50.0 g sample into tared 240 ml screw cap jar. Weigh 1.0 g of Syloid 244, or equivalent, and add it to the contents of the jar. Cap the jar tightly and mix by inversion until a uniform or homogeneous mixture results. Transfer the mixture quantitatively to the top sieve. Mount the nested sieves in the shaking device and shake 2 min. plus additional 2 min. for each sieve (do not include the pan). Reweigh each sieve with washers and pan and then subtract the tare weight to obtain the fraction retained (ASTA, 1997).

Calculation:

$$\frac{\text{grams retained/screen} \times 100}{\sum \text{g retained on screens} + \text{pan}} = \%\ \text{retained on each sieve}$$

5.2.2 Bulk index/bulk density (manual method)

Purpose: To determine bulk index and bulk density.

Apparatus required:
Nalgene graduated cylinders, 250 ml, 500 ml and 1000 ml capacity. Ring stand and ring clamp.

Procedure:
Weigh 100 g of sample and place in graduated cylinder of appropriate size. Weigh 50 g of parsley flakes or other bulky materials exceeding 1000 ml volume. Place cylinder on ring stand and adjust ring clamp so that, when base of cylinder is raised to touch the ring, the bottom surface of the cylinder is exactly 1 in. from the base

of ring stand. Drop cylinder (1 in) 50 times and record to nearest 2 ml for 250, 5 ml for 500, or 10 ml for 1000 ml graduated cylinder (ASTA, 1997).

Calculation:

$$\text{bulk index} = \frac{\text{volume of product (ml)}}{\text{sample weight (g)}} \times 100$$

$$\text{bulk density (g/ml)} = \frac{\text{sample weight (g)}}{\text{volume of product (ml)}}$$

5.2.3 Bulk index/bulk density (machine method)

Purpose: To determine bulk index and bulk density.

Apparatus required:
Nalgene graduated cylinders, 250 ml, 500 ml and 1000 ml capacity. Stop watch. Bulk index machine.

Procedure:
Weigh 100 g of sample and place in graduated cylinder of appropriate size. Weigh 50 g of parsley flakes or other bulky materials exceeding 1000 ml volume. Clamp cylinder in bulk index machine and allow tapping to proceed for 60 seconds. Record volume to nearest 2 ml for 250, 5 ml for 500 or 10 ml for 1000 ml graduated cylinder (ASTA, 1997).

Calculation:

$$\text{bulk index} = \frac{\text{volume of product (ml)}}{\text{sample weight (g)}} \times 100$$

$$\text{bulk density (g/ml)} = \frac{\text{sample weight (g)}}{\text{volume of product (ml)}}$$

5.2.4 Moisture (distillation method)

Purpose: To determine the moisture content by distillation with toluene.

Apparatus required:
Round-bottomed flask, receiver trap, condenser.

Reagent:
Toluene, analytical grade.

Procedure:
Weigh 20–40 g of spice or sample sufficient to yield 2–5 ml. of water. Place the weighed sample in a distilling flask and add sufficient toluene to cover the sample completely (never less than 75 ml). Insert the attiring bar when magnetic stirring is to be used. Assemble the apparatus and fill the receiver trap with toluene by pouring

it through the condenser until the toluene begins to overflow into the distillation flask. Insert a loose cotton plug in the top of the condenser to prevent condensation of atmospheric moisture in the tube. Bring to boil and slowly distill at *c*. two drops per second until most of the water is collected in the trap, then increase the distillation rate to *c*. four drops per second. Continue distillation until two consecutive readings taken at 15 min. intervals show no change of water volume in the trap, dislodge any water droplets in the condenser tube with the brush or wire loop and rinse with *c*. 5 ml of toluene. Continue the distillation for 3–5 min. and cool the receiver trap to room temperature either by allowing it to stand in air or by immersing it in water. The toluene and water layers should now be clear; if not, allow to stand until clearing occurs, then read the volume of water, estimating the nearest 0.01 ml.

Calculation:

$$\text{moisture}\,(\%) = \frac{\text{volume of water (ml)} \times 100}{\text{weight of sample (g)}}$$

5.2.5 Total ash

Purpose: To determine, as ash, the residue remaining after ignition.

Apparatus required:
Muffle furnace, flat-bottomed crucible.

Procedure:
Ignite the flat-bottomed dish or crucible to a dull redness, cool to room temperature in desiccator and weigh. Weigh accurately 2 g of well-mixed sample in the tared flat-bottomed dish or crucible. Place the dish or crucible in the entrance of the open muffle furnace until the sample is well carbonized. The sample must not catch fire. Place the carbonized sample in the furnace at 600 °C ± 2° for 2 hours. If carbon remains, leach ash with hot water, filter through quantitative filter paper, wash paper thoroughly, and transfer paper and contents to the original dish. Dry and ignite in the muffle furnace at 600 °C until the ash is white. Cool dish, add filtrate, evaporate to dryness on steam bath and heat in muffle for 30 mins. When carbon-free ash is obtained, place the dish in desiccator, cool and weigh (ASTA, 1997).

Calculation:

$$\text{ash}\,(\%) = \frac{\text{weight of ash (g)} \times 100}{\text{weight of sample (g)}}$$

5.3 Extraction techniques: determining essential oil content of plant material

A laboratory distillation of essential oil from plant material is often necessary in order to evaluate raw material to be used for large-scale commercial distillation.

The determination of essential oil may be conveniently carried out in a special apparatus devised by Clevenger. The apparatus consists of specially designed oil traps and a small condenser of the cold-finger type. Two types of traps are supplied; one, for oils lighter than water and the other for oils heavier than water.

Volatile oils give the aroma and the oleoresins impart the taste. Aroma compounds play a significant role in the production of flavourants, which are used in the food industry to flavour, improve and increase the appeal of their products. They are classified as per the functional groups, *viz*; alcohols, aldehydes, amines, esters, ethers, ketones, terpenes, thiols and other miscellaneous compounds. In spices, the volatile oils constitute these components (Parthasarathy *et al.*, 2008).

Essential oil is a complex mixture of the following primary compounds:

- terpene hydrocarbons: monoterpene hydrocarbons, sesquiterpenes;
- oxygenated compounds, phenols, alcohols;
- monoterpene alcohols;
- sesquiterpene alcohols;
- aldehydes, ketones, esters, lactones, coumarins, ethers, oxides.

Therefore gas chromatography (GC) coupled with flame ionization detection (FID) or mass spectroscopy (MS) is used to characterize the components in an essential oil.

Depending up on the nature of the constituents, polar and non-polar columns are used.

5.3.1 The modified Clevenger method

Purpose: To determine the amount of steam volatile oil (most adopted laboratory method).

Apparatus required:

Boiling flask, short neck, 1 or 2 l, with T.S. 24/40 ground joint. Suitable electric heating mantle or oil bath. Volatile oil traps, Clevenger with T.S. 24/40 ground joints, either

- designed for lighter-than-water oils, or
- designed for heavier-than-water oils.

West condenser, 400 mm length with drip tip and T.S. 24/40 ground joints.

Procedure:

Weigh accurately sufficient size sample to yield 2–5 ml of oil and transfer quantitatively to flask – using water if necessary. Add about 500 ml of water. If magnetic stirring is to be used, insert stirring bar. Assemble apparatus, select the trap depending upon the density of the oil to be trapped. Heat the flask to boiling and maintain a reflux rate of one to two drops per second. Reflux until two consecutive readings taken at 1 hour intervals show no change of oil volume in the trap. Cool to 20 °C either by allowing to stand in air or immersing trap in a suitable water bath. If the calculated volume of oil is below 2 ml or above 5 ml, the test should be repeated with appropriate adjustment to the amount of sample used (AOAC, 2007).

Calculation:

$$\text{volatile oil \% (v/w)} = \frac{\text{volume of oil (ml) 20 °C}}{\text{weight of sample (g)}} \times 100$$

For oils heavier than water when xylene is used, calculate the volatile oil by:

$$\frac{\text{volume of oil (ml)} - \text{volume of xylene in blank}}{\text{weight of sample (g)}} \times 100$$

Note: Antifoam agent (containing no volatile oil) and sodium alkylbenzene sulphonate can also be used if severe frothing is observed.

The Clevenger method is the most popular method for extraction of oil. Other related methods are also:

- Supercritical fluid extraction (SFE), mainly by supercritical carbon dioxide (SC-CO_2), can be used to extract volatile oils from natural products and does not produce substantial thermal degradation or organic solvent contamination (Chempakam and Parthasarathy, 2008).
- Solvent-free microwave extraction (SFME) (Lucchesi *et al.*, 2004) a combination of microwave heating and dry distillation, performed at atmospheric pressure without the use of any solvent or water, is a new development in the field. SFME was compared with hydrodistillation, for the extraction of essential oil from three aromatic herbs, namely basil (*Ocimum basilicum* L.), garden mint (*Mentha crispa* L.) and thyme (*Thymus vulgaris* L.), and it was found that the essential oils extracted by SFME for 30 min. were similar to those obtained by conventional hydrodistillation for 4.5 hours, in terms of quantity (yield) and quality (aromatic profile) (Lucchesi *et al*., 2004). The SFME method yielded an essential oil with higher amounts of oxygenated compounds, and substantial savings of costs, in terms of time, energy and plant material. SFME is a green technology and appears as a good alternative for the extraction of essential oils from aromatic plants.

5.3.2 The Lee and Ogg method

Purpose: To determine the amount of water-insoluble steam volatile oil.

Apparatus required:

One litre, T.S. 24/40 short neck, round-bottom flask. Volatile oil traps, either

- lighter-than-water designed for oils with densities less than that of water, or
- heavier-than-water designed for oils with densities greater than water.

Wet condenser, 400 mm in length, with T.S. 24/40 drip tip.

Procedure:

Weigh a sample that will yield 0.5–1.5 ml of oil and transfer quantitatively to the 1 l flask. Add about 500 ml water and place the flask in the heating mantle. Assemble the apparatus using the proper trap. Heat the flask with occasional shaking and maintain a distillation rate of 1 to 1½ drops per second. If foaming occurs, reduce

the voltage, and increase the temperature gradually until the proper distillation is obtained. Distill until two consecutive readings taken at half hour intervals show no change of oil volume in the trap. Cool to the room temperature, allow to stand until the oil layer is clear and read the volume of the oil collected, estimated to the nearest 0.05 ml. If the calculated volume of oil is below 0.5 ml or above 1.5 ml, the test should be repeated with appropriate adjustment to the amount of sample used (AOAC, 2007).

Calculation:

$$\text{volatile oil \% (v/w)} = \frac{\text{volume of oil (ml)}}{\text{weight of sample (g)}} \times 100$$

When xylene is used, calculate the volatile oil by:

$$\frac{\text{volume of oil (ml)} - \text{volume of xylene in blank}}{\text{weight of sample (g)}} \times 100$$

5.3.3 Estimation of steam volatile oil in cassia

Purpose: To determine the amount of water insoluble steam volatile oil in cassia.

Apparatus required:

One litre, T.S. 24/40 short neck, round-bottom flask. ASTM D322 crankcase dilution trap, 5 ml capacity. West condenser with T.S. 24/40 drip tip.

Procedure:

Cleaned trap is rinsed with acetone and then rinsed well with water. Weigh 35 g sample to nearest 0.01 g and quantitatively transfer to the 1 l flask. Add 500 ml of 10 % sodium chloride solution. Add small amount of water to the trap followed by 2.00 ml xylene using the volumetric pipette. Assemble the apparatus, using a small amount of non-volatile stopcock grease on the ground joints. Heat the flask and maintain a distillation rate of 30 drops/minute for 5 hours after boiling begins. Cool the trap to 20 °C by placing it in suitable water bath and hold until the oil layer is clear. Read the trap to the nearest 0.01 ml. The volume of the oil is obtained by deducting the blank for xylene (ASTA, 1997).

Calculation:

$$\text{volatile oil (\%) (v/w)} = \frac{\text{vol. of oil (ml)} - \text{vol. of xylene in blank}}{\text{weight of sample (g)}} \times 100$$

Analysis of essential oil constituents

Essential oil of any spice consists of terpenes and hydrocarbons with a boiling range of 70–210 °C. It can be separated using GC–FID. Zachariah *et al.* (2010) conducted detailed analysis of leaf and berry constituents of black pepper oil by GC and GC–MS. The instrument techniques are same for all spice essential oils.

5.4 Identifying the physical properties of essential oils

5.4.1 Specific gravity

Specific gravity is an important criterion of the quality and purity of an essential oil. Specific gravities of essential oils vary between 0.696 and 1.188 at 15 °C; in general, the value is less than 1.000.

The specific gravity of an essential oil at 15 °C/15 °C may be defined as the ratio of the weight of a given volume of oil at 15 °C to the weight of an equal volume of water at 15 °C. Pycnometers offer the most convenient and rapid method for determining specific gravity.

Apparatus required:

Pycnometer: A conical-shaped pycnometer having a volume of about 10 ml with a ground-in thermometer and a capillary side tube with a ground glass cap proves very satisfactory.

Procedure:

Clean the pycnometer by filling it with a saturated solution of chromium trioxide in sulphuric acid and allow it to stand for at least three hours. Empty the pycnometer and rinse thoroughly with distilled water. Fill the pycnometer with recently boiled distilled water which has been cooled to a temperature of about 12 °C and place it in a water bath, previously cooled to 12 °C. Permit the temperature to rise slowly to 15 °C. Adjust the level of water to the top of the capillary side arm, removing any excess with a blotter or cloth, and put the ground glass cap in place. Remove the pycnometer from the water bath, dry carefully with a clean cloth, permit it to stand for 30 min. and weigh accurately. Empty the pycnometer, rinse several times with alcohol and finally with ether.

Remove the ether fumes with the aid of an air blast and permit the pycnometer to dry thoroughly. Weigh accurately after standing 30 min. The 'water equivalent' of the pycnometer may be found by subtracting the weight of the empty pycnometer from its weight when full.

Fill the clean, dried pycnometer with the oil previously cooled to a temperature of 12 °C. Following the same procedure as above, place the pycnometer in a water bath and permit it to warm slowly to 15 °C. Adjust the oil to the proper level, put the cap in place and wipe the pycnometer dry. Accurately weigh after 30 min.

The weight of the oil contained in the pycnometer divided by the water equivalent gives the specific gravity of the oil at 15 °C/15 °C. For a given pycnometer the water equivalent need be determined only once; therefore, it is important that the determination be performed with great care and accuracy.

5.4.2 Optical rotation

Most essential oils when placed in a beam of polarized light possess the property of rotating the plane of polarization to the right. The extent of the optical activity of oil is determined by a polarimeter and is measured in degrees of rotation. The angle of rotation is dependent upon the nature of the liquid, the length of the column through which the light passes, the wavelength of the light used and the temperature.

Both the degree of rotation and its direction are important as criteria of purity. In recording rotations, it is customary to indicate direction by the use of a plus sign (+) to indicate dextrorotation (rotation to the right), or a minus sign (−) to indicate laevorotation (rotation to the left).

Since the scale reading for an optically active liquid is directly proportional to the length of the transmitting column of liquid, it is necessary to use standard tube, 100 mm long. It has become customary in polarimetric work to use sodium light.

Liquids:
The oil or liquid should be free from suspended material. Often oils are hazy owing to the presence of small amounts of water; such oil should be dried with anhydrous sodium sulphate and filtered before a determination is attempted.

Procedure:
Place the 100 mm polarimeter tube containing the oil or liquid under examination in the trough of the instrument between the polarizer and the analyser. Slowly turn the analyser until both halves of the field, viewed through the telescope, show equal intensities of illumination. At the proper setting, a small rotation to the right or to the left will immediately cause a pronounced inequality in the intensities of illumination of the two halves of the field.

Determine the direction of rotation. If the analyser was turned counterclockwise from the zero position to obtain the final reading, the rotation is laevo(−); if clockwise, dextro(+).

After the direction of rotation has been established, carefully readjust the analyser until equal illumination of the two halves of the field is obtained. Adjust the eyepiece of the telescope to give a clear, sharp line between the two halves of the field. Determine the rotation by means of the protractor; read the degree directly, and the minutes with the aid of either of the two fixed verniers; the movable magnifying glasses will aid in obtaining greater accuracy. A second reading should be taken; it should not differ by more than ±5° from the previous reading.

5.4.3 Refractive index

When a ray of light passes from a less dense to a more dense medium, it is bent or 'refracted' towards the normal. If e represents the angle of refraction and i the angle of incidence, according to the law of refraction:

$$\frac{\sin i}{\sin e} = \frac{N}{n}$$

where n is the index of refraction of the less dense, and N the index of refraction of the more dense medium. Refractrometers offer a rapid and convenient method for the determination of this physical constant.

5.4.4 Solubility

Solubility in alcohol
Since most essential oils are only slightly soluble in water and are miscible with absolute alcohol, it is possible to determine the number of volumes of dilute alcohol

required for the complete solubility of one volume of oil. The determination of such a solubility is a convenient and rapid aid in the evaluation of quality of an oil. Oils rich in oxygenated constituents are more readily soluble in dilute alcohol than oils rich in terpenes.

Adulteration with relatively insoluble material will often greatly affect the solubility. The solubility of an oil may change with age. Polymerization is usually accompanied with a decrease in solubility. Alcohols of the following strengths are customarily used in determining solubilities of essential oils:

50 %– 60 %–70 %–80 %–90 %–95 % and occasionally 65 % and 75 %

In preparing dilute alcohols, it is convenient to weigh the alcohol and the distilled water to give the proper volume percentage. The strength of the alcohol should be checked by determining specific gravity at 15.56 °C/15.56 °C.

Solubility in non-alcoholic media
Several solubility tests have been introduced for the rapid evaluation of oils.

- **Carbon disulphide solubility for the presence of water**: Oils rich in oxygenated constituents frequently contain dissolved water. Such oils fail to give a clear solution when diluted with an equal volume of carbon disulphide or chloroform. This is the basis of a rapid test to ascertain whether or not an oil has been sufficiently dried.
- **Potassium hydroxide solubility for phenol-containing oils**: Phenolic isolates and synthetics as well as oils consisting almost exclusively of phenolic bodies may be evaluated rapidly by dissolving 2 ml of the oil in 20 or 25 ml of a 1 N aqueous solution of potassium hydroxide in 25 ml glass-stoppered graduated cylinder. It is well to examine critically the odour of the solution or any insoluble portion, whereby additions of foreign, odour-bearing substances may be detected.
- **Sodium bisulphite solubility for aldehyde-containing oils**: Oils and synthetics and isolates may reveal impurities by their incomplete solution in dilute bisulphite solution. This test is usually carried out in a 25 ml glass-stoppered, graduated cylinder: shake 1 ml of the oil with 9 ml of a freshly prepared saturated solution of sodium bisulphite and then add 10 cc of water with further shaking. The odour of the resulting solution should be carefully examined.

Congealing point
The congealing point offers distinct advantages over the melting point and the titre, in the case of mixtures such as essential oils. In determining the congealing point, the oil is super-cooled so that, upon congelation, immediate crystallization with liberation of heat occurs. This results in a rapid rise of temperature, which soon approaches a constant value and remains at this temperature for a period of time. This point is the congealing point.

5.4.5 Evaporation residue

An important criterion of purity is the evaporation residue; i.e., the percentage of the oil which is not volatile at 100 °C. A determination of the evaporation residue

is of special value in the case of the citrus oils; a low value for an expressed oil suggests the possibility of the addition of terpenes, or other volatile compounds; a high value may indicate the addition of foreign material.

It is important to study the odour of an oil as it volatilizes during the heating. The consistency of the residue, both when hot and cool, and the colour sometimes indicates the presence of particular adulterants.

The fact that essential oils are complex mixtures makes an exact determination of the non-volatile residue very difficult.

5.5 Estimation of oleoresin in spices

The total extracts or oleoresins reflect the flavour profile more closely than the volatile oil. The oleoresins also extract non-aromatic fats, waxes, resinoids, colour and other components soluble in the chosen extracting solvent. Oleoresins freed from fat components and prepared as concretes are useful in perfume industries.

5.5.1 Determining the non-volatile ether content of spices and contents

Purpose: To extract with diethyl ether, removal of the volatile fractions, removal of the insoluble substances, drying of the non-volatile residue and weighing.

Reagent:
Diethyl ether, anhydrous.

Apparatus required:
Apparatus for continuous extraction (Soxhlet apparatus).

Procedure:

- **Test portion:** Weigh, to the nearest 1 mg, approximately 2 g of the test spice sample.
- **Determination:** Extract the test portion with the diethyl ether in the continuous extraction apparatus for 18 hours. Eliminate the diethyl ether by distillation, using the extractor flask connected to a rotary evaporator. Dry the flask at 110 °C, until the difference in mass between two successive weighings is not more than 2 mg. By shaking gently, mix the residue in the flask with 2–3 ml of the diethyl ether at laboratory temperature, allow to settle and decant the supernatant solution. Repeat the extraction procedure and eliminate the diethyl ether as before, until no more of the residue dissolves. Dry the flask again as before until the difference in mass between two successive weighings is not more than 2 mg.

Calculation:
The non-volatile ether extract, expressed as a percentage by mass on the dry basis, is given by

$$\frac{m_1 - m_2}{m_0} \times 100 \times \frac{100}{100 - II}$$

where:

m_0 is the mass, in grams, of the test portion;
m_1 is the mass, in grams, of the flask plus residue obtained after drying;
m_2 is the mass, in grams, of the flask and the insoluble residue obtained;
II is the moisture content expressed as a percentage by mass, of the sample as received.

5.5.2 Analysis of constituents: determining the piperine content of pepper

Piperine is the major constituent of pepper oleoresin. Investigations have demonstrated, that piperine is the major pungent principle and chavicine is a mixture of piperine and several minor alkaloids. The presence of chavicine and isopiperine has not been confirmed in pepper extracts while isochavicine is shown to occur as an artifact of photolytic transformation of piperine. Five minor alkaloids possessing a degree of pungency have been identified in pepper extracts. They are piperettine, piperylin, piperolein A and B and piperanine (Zachariah and Parthasarathy, 2008). Piperine is a yellow crystalline substance having a melting point of 128–130 °C. Piperine, $C_{17}H_{19}O_3N$, was shown to be a weak base which, on hydrolysis with aqueous alkali or nitric acid, yielded a volatile base $C_5H_{11}N$, later identified as piperidine. Wood *et al.* (1988) developed the reversed-phase high-performance liquid chromatographic (HPLC) method for piperine determination in black pepper and its oleoresins. It employs C18-bonded stationary phase (ODS-2) and acetonitrile aqueous acetic acid mobile phase with UV detection. As the spectrophotometric method which invariably yields higher results because of the contributions from other alkaloids such as piperyline and piperettine, the HPLC method relates more to piperine.

Principle:
Extraction with ethanol under reflux, then determination of piperine by HPLC.

Reagents:
Use only reagents of recognized analytical grade and distilled or demineralized water or water of equivalent purity.

Reference substance:
Piperine of at least 98 % purity, ethanol, 96 % (v/v), acetonitrile, acetic acid, 1 % (v/v) aqueous solution.

Elution solvent:
Mix 52 volumes of acetic acid solution and 48 volumes of acetonitrile.

Calibration method:

1. **Reference solution:**
   Prepare for immediate use a 1 g/L stock solution of the piperine in ethanol.

2. **Calibration curve:**
   (a) From the reference solution prepare at least three standard solutions of piperine with concentrations ranging from 0.05 g/L to 0.2 g/L. Inject each solution into the chromatograph. Repeat the determination at least once.
   (b) Plot the calibration curve, i.e. the mass of piperine injected versus the peak area.
   (c) Define the mean slope of the curve.

Calculation of the response factor, *K*:
Calculate the response factor K using the following formulae:

$$K = m'/A$$

$$m' = m \times P_1$$

where:
m is the mass of piperine, in milligrams;
A is the area of the piperine peak, in integrator units;
P_1 is the purity of the reference piperine;
m' is the corrected mass of piperine, in milligrams.

Calculation – peppers, whole or ground:
Calculate the piperine content, as a percentage by mass, using the following formula:

$$A \times K \times \frac{25}{10} \times \frac{100}{m_x} \times 100$$

where:
A is the area of the piperine peak, in integrator units;
m_x is the mass of the sample, in milligrams;
K is the response factor determined for the reference substance.

Calculation – oleoresins of pepper
Calculate the piperine content, as a percentage mass, using the following formula

$$A \times K \times \frac{50}{10} \times \frac{100}{m_x} \times 100$$

where A, m_x, and K have the same meaning as in the above equation.

5.5.3 Analysis of constituents: determining the curcumin content of turmeric

Turmeric oleoresin is the organic extract of turmeric and is added to food items as a spice and colouring agent. Oleoresin yield ranges from 7.0–14.0 %. Curcumin, the principal colouring matter, forms one-third of a good-quality oleoresin. The rhizomes contain curcuminoids (2.5–6 %) and are responsible for the yellow colour. Curcuminoids comprises Curcumin I (diferuloylmethane), Curcumin II (demethoxycurcumin) and Curcumin III (bisdemethoxycurcumin) which are found to be natural antioxidants (Chempakam and Parthasarathy, 2008).

Purpose: To determine the percentage of curcumin present in turmeric preparations by spectrophotometric procedure.

Apparatus required:
Spectrophotometer, visible (tungsten) light source, capable of accurately measuring absorbance at 415–425 nm cuvettes, 1 cm square, silica. Erlenmeyer flask, 125 ml, TS 24/40, amber. Condenser, West type, 400 mm, TS 24/40. Volumetric flasks, 100 and 200 ml, TS, amber.

Reagents:
acetone, reagent grade.

Preparation of sample:
- Raw turmeric rhizome powder with a composite mixture of mother, primary and secondary rhizome (20:60:20) of 40 mesh size.
- Oleoresins – stir well, use as is.

Procedure:
- **Raw turmeric rhizome powder:**
  a. Weigh to nearest 0.01 g, 1 g of sample into 125 ml Erlenmeyer flask; add *c*. 75 ml of acetone and stir bar; gently reflux on stirring hot plate with West condenser for 1 hour.
  b. Cool to room temperature and filter quantitatively into 250 ml volumetric flask. Transfer extracted residue to filter with acetone. Wash thoroughly and dilute with acetone.
  c. Pipette 1 ml of (b) solution in to a 100 ml volumetric flask; dilute to volume with acetone and mix.
  d. Compare the absorbance with a pure standard curcumin preparation (0.0025 g/L).
- **Turmeric oleoresins:**
  a. Weigh to the nearest 0.001 g appropriate weight of well-mixed sample and transfer into 100 ml volumetric flask. Dissolve in acetone and mix.
  b. Pipette 1 ml in to a 100 ml volumetric flask; dilute to volume with acetone and mix.
  c. Compare the absorbance with a pure standard curcumin preparation.

Calculation:

$$\%\ \text{curcumin} = \frac{D_s}{100} \times \frac{A_s}{W_s \times 1650} \times 100\%$$

where:
D_s is dilution volume of sample (ml) which, if using the dilution schedule as presented in this method, is 20000 for raw spice and 10000 for oleoresins;
W_s is weight of sample (g);
A_s is absorbance of sample;
1650 = E_{1m} for curcumin.

5.5.4 Analysis of constituents: determining the gingerol and shogaol content of ginger

Ginger oleoresin should contain predominantly the aroma and pungency contributed mainly by the volatile oils, gingerols and related compounds. The pungent group includes gingerols, shogaols, paradols and zingerone that produce a 'hot' sensation in the mouth. The gingerols, a series of chemical homologues differentiated by the length of their unbranched alkyl chains, were identified as the major active components in the fresh rhizome. The major constituents were found to be condensation products of zingerone with saturated straight-chain aldehydes of chain length 6, 8 and 10. These compounds were named [6]-, [8]- and [10]-gingerols and their dehydration products [6]-, [8]- and [10]-shogaols, according to the length of the aldehyde unit, and the relative abundance of these compounds in the sample was estimated as 53:17:30, respectively (Zachariah, 2008).

Principle:

From ground and dried ginger, extraction of the pungent components by methanol at atmospheric temperature and concentration at reduced pressure of part of the extract. The oleoresins are dissolved in methanol. The resulting solutions are analysed directly by reversed phase HPLC on octadecyl-silyl silica column, with a mixture of acetonitrile and aqueous acetic acid as the mobile phase and by UV detection at 280 nm.

The quantification is done by external standardization with nonanoic acid vanillyl amide (NVA) which has a retention time comparable to that of [6]-gingerol.

Procedure:

Refer to ISO/ ASTA manual.

Note the area of the peak of NVA for each chromatogram and calculate the mean value for each concentration of NVA (about 0.2 mg/ml and 0.4 mg/ml).

Calculation:

Calculate the NVA as follows:

$$K_{\mathrm{NVA}} = \frac{C_{\mathrm{NVA}}}{A_{\mathrm{NVA}}} \times 100 \qquad \mathrm{mg/100\ ml/unit\ area}^2$$

where:

C_{NVA} is the concentration of NVA (mg/ml);

A_{NVA} is the mean area of the peak of NVA at that concentration.

For a linear response, the values of K_{NVA} calculated for the two concentrations must not differ by more than 2 %.

$$K_{[6]\text{-G}} = K_{\mathrm{NVA}} \times \frac{\text{molecular weight of [6]-G (294.38)}}{\text{molecular weight of NVA (293.41)}}$$
$$= K_{\mathrm{NVA}} \times 1.003\ \mathrm{mg/100\ ml/unit\ area}$$

In the same way,

$K_{[8]\text{-G}} = K_{\mathrm{NVA}} \times 1.099$ mg/100 ml/unit area

$K_{[10]\text{-G}} = K_{\mathrm{NVA}} \times 1.194$ mg/100 ml/unit area

$K_{[6]\text{-S}} = K_{\text{NVA}} \times 0.942$ mg/100 ml/unit area
$K_{[8]\text{-S}} = K_{\text{NVA}} \times 1.037$ mg/100 ml/unit area
$K_{[10]\text{-S}} = K_{\text{NVA}} \times 1.133$ mg/100 ml/unit area

Expression of results:
Calculate the concentration of each gingerol or shogaol in the sample of dried ginger or oleoresin as illustrated below for [6]-gingerol:

$$\text{content of [6]-G in the sample} = \frac{A_{[6]\text{-G}} \times K_{[6]\text{-G}}}{C} \% \text{ (m/m)}$$

where:

$A_{[6]\text{-G}}$ is the area of the peak of [6]-G in the chromatogram of the sample;
$K_{[6]\text{-G}}$ is the response factor for [6]-G (mg/100 ml/unit area);
C is the concentration of dried ginger or of oleoresin in the sample solution (mg/ml).

5.5.5 Analysis of constituents: determining the carotenoid content of capsicum

The colour of chilli spice powder is due to the presence of red-pigmented carotenoids. The main pigments are capsanthin, capsorubin, zeaxanthin and cryptoxanthin. Carotenoids are very stable in intact plant tissue. However, when chillies are processed by drying and grinding into spice powder, the carotenoids easily autooxidize due to effects of heat, light and oxygen. This leads to a more orange and less intense colouration that devalues the spice powder. Carotenoids control pod colour with approximately 20 carotenoids contributing to the colour of the powder. Carotenoid compounds are yellow to red pigments of aliphatic or alicyclic structures composed of isoprene units, which are normally fat-soluble colours. The ketocarotenoids, capsanthin, capsorubin and cryptocapsin are unique *Capsicum* carotenoids. The major red colour in chilli comes from the carotenoids capsanthin and capsorubin, while the yellow-orange colour is from β-carotene and violaxanthin. Capsanthin, the major carotenoid in ripe fruits, contributes up to 60 % of the total carotenoids. The total carotenoid content of the ripe fruits was about 3.2 g/100 g DW of which capsanthin constituted 42 %, zeaxanthin 8 %, cucurbitaxanthin A 6.6 %, capsorubin 3.2 % and β-carotene 7 % (Zachariah and Gobinath, 2008).

The colour of chilli powder can be measured either as extractable red colour or surface colour. Extractable colour is estimated by ASTA (ASTA, 1997) in international trade. Generally, in trade the lower limit allowable for chilli powder is 120 ASTA units and for non-pungent paprika 160–180 ASTA units.

Extractable colour in capsicums and their oleoresins
Purpose: To determine the extractable colour in capsicums and their oleoresins by measuring the absorbance of an acetone extract at 460 nm.

Apparatus required:
Spectrophotometer capable of accurately measuring absorbance (A) at 460 nm. Glass referenced standard: NIST SRM 2030 OR 930, glass filter with absorbance specified by NIST in range 0.4–0.6 AT 465 nm, or equivalent. Volumetric flasks 100 ml with ground glass stoppers.

Reagents:
Acetone.

Preparation of sample:
- Capsicums–ground samples.
- Oleoresins – mix the sample well by shaking.

Procedure:
- **Capsicums:** Accurately weigh 70–100 mg of ground capsicum sample and transfer quantitatively to a 100 ml volumetric flask. Dilute to volume with acetone, and stopper tightly. Shake the flask and let it stand for 16 hours at room temperature in a dark area. Shake the flask and allow sufficient time for the particles to settle. Fill the spectrophotometer cell with acetone. Set the wavelength on the spectrophotometer to 460 nm. Place the acetone-filled cell in the instrument. Zero the spectrophotometer. Measure the absorbance at 460 nm. Avoid transferring particles to absorption cell. Determine the absorbance of the glass filter at 465 nm.
- **Oleoresins:** Accurately weigh 70–100 mg of ground capsicum sample and transfer quantitatively to a 100 ml volumetric flask. Dilute to volume with acetone, shake and let stand for 2 min. Pipette 10.00 ml of this extract into another 100 ml volumetric flask. Dilute to volume with acetone and shake. Transfer a portion to the spectrophotometer cell and measure the absorbance at 460 nm. Determine the absorbance of standard glass filter at 465 nm (ASTA, 1997).

Calculation:
- Capsicums extractable colour:

$$\text{ASTA colour} = \frac{\text{absorbance at } 460\text{ nm} \times 16.4}{\text{sample weight (g)}}$$

- Oleoresins extractable colour:

$$\text{ASTA colour} = \frac{\text{absorbance at } 460\text{ nm} \times 164}{\text{sample weight (g)}}$$

- Instrument correction factor (I_f):

$$I_f = \frac{\text{NIST declared absorbance for glass filter at } 465\text{ nm}}{\text{Lab observed absorbance of glass filter at } 465\text{ nm}}$$

- Corrected ASTA colour:

$$\text{ASTA colour (corrected)} = \text{ASTA colour} \times I_f$$

5.5.6 Analysis of constituents: determining the capsaicinoid content of capsicum

Pungency of chilli is produced by the capsaicinoids, a group of alkaloid compounds that are found only in the plant genus, *Capsicum*. The nature of the pungency has been established as a mixture of seven homologous branched-chain alkyl vanillylamides. They often are called capsaicin after the most prevalent one.

Dihydrocapsaicin is usually the second most prevalent capsaicinoid, while the other five compounds, norcapsaicin, nordihydrocapsaicin, nornordihydrocapsaicin, homocapsaicin and homodihydrocapsaicin, are considered minor capsaicinoids because of their relative low abundance in most natural products. Capsaicin is a powerful and stable alkaloid that can be detected by human taste buds in solutions of ten parts per million. Capsaicin's composition ($C_{18}H_{27}NO_3$) is similar to piperine ($C_{17}H_{19}NO_3$) that gives black pepper its bite (Zachariah and Gobinath, 2008). The most common instrumental method of analysis is HPLC. It provides accurate and efficient analysis of content and type of capsaicinoids present in a chilli sample. HPLC analysis has become the standard method for routine analysis by the processing industry. A spectrophotometric method is also available.

Determining the total capsaicinoid content
Purpose: To determine total capsaicinoid content from methanolic extracts of chillies or chilli oleoresins at wavelengths of 248 nm and 296 nm.

- **Chillies in powder form:** Extraction with tetrahydrofuran, then determination of the capsaicinoids by spectrometry.
- **Whole chillies:** Preparation by grinding the sample, then extraction with tetrahydrofuran, followed by determination of the capsaicinoids by spectrometry.
- **Oleoresins of chillies:** Dilution of the oleoresin in methanol, then determination of the capsaicinoids by spectrometry.
- **Reagents:** Carbon black, for analysis. Methanol, spectrometric grade. Methanol solution, obtained by mixing 70 parts by volume of methanol with 30 parts by volume of water. Hydrochloric acid solution, freshly prepared, 1 mol/L. Tetrahydrofuran, freshly distilled or spectrometric grade.

Procedure – Preparation of sample:

- **Chillies in powder form:** Check that all of the powder passes through the 500 μm sieve. Weigh, to the nearest 0.01 g, about 10 g of homogenized powder, and transfer it quantitatively to the continuous extraction apparatus. Extract for 8 hours using 100 ml of tetrahydrofuran. Evaporate the solvent to the maximum extent possible in the rotary vacuum evaporator under reduced pressure in a 250 ml round-bottomed flask on the water bath.
- **Whole chillies:** Grind the chillies until the powder obtained passes entirely through the 500 μm sieve. Homogenize the powder after sieving. Weigh, to the nearest 0.01 g, about 10 g of homogenized powder, and transfer it quantitatively to the continuous extraction apparatus. Extract for 8 hours using 100 ml of tetrahydrofuran. Evaporate the solvent to the maximum extent possible in the rotary vacuum evaporator under reduced pressure in a 250 ml round-bottomed flask on the water bath.
- **Oleoresins of chillies:** Thoroughly homogenize the oleoresin. Weigh, to the nearest 0.0001 g, 0.5–1 g of the homogenized oleoresin in a 250 ml volumetric flask with a ground glass stopper.

Procedure – Preparing test solutions:

- **Chillies, whole or in powder form:** To the extract add 0.05–0.1 g of carbon black so as to maintain a ratio of the order of 10 between the extract and carbon black.

Add about 90 ml of methanol solution. Agitate on the magnetic stirrer for 30 min. Allow the solution to stand for 5 min. Filter through the membrane filter into a 100 ml volumetric flask. Dilute to the mark with methanol solution. The filtrate shall be clear.

- **Oleoresins of chillies:** To the test portion, add 0.05–0.1 g of carbon black so as to maintain a ratio of the order of 10 between the oleoresin and carbon black. Add about 90 ml of methanol solution. Agitate on the magnetic stirrer for 30 min. Allow the solution to stand for 5 min. Filter through the membrane filter into a 100 ml volumetric flask. Dilute to the mark with methanol solution. The filtrate shall be clear (ASTA, 1997; Sadasivam and Manickam, 2008).

Preparing dilutions for spectrometric measurement:

1. Transfer the following to a 25 ml volumetric flask: 3 ml of water, 2 ml of hydrochloric acid. Dilute to the mark with methanol. This solution is the 'blank acid solution' (A).
2. Transfer the following to a 25 ml volumetric flask: 3 ml of water, 2 ml of sodium hydroxide solution. Dilute the mark with methanol. This solution is the 'blank alkali solution' (B).
3. Take three 25 ml volumetric flasks and mark them **a1**, **a2** and **a3**, respectively. Transfer to each flask: 1 ml of the filtrate, 2.7 ml of water, 2 ml of hydrochloric acid. Dilute each flask to the mark with methanol.
4. Take three 25 ml volumetric flasks and mark them **b1**, **b2** and **b3**, respectively. Transfer to each flask: 1 ml of the filtrate, 2.7 ml of water, 2 ml of sodium hydroxide solution. Dilute each flask to the mark with methanol.

Taking spectrometric measurements using a *double-beam spectrometer*:

1. Adjust the zero and the 100 % absorption with methanol solution.
2. Measure the blank absorbances at wavelengths of 248 nm and 296 nm by placing first the blank alkali solution (B) in the measuring cell and the blank acid solution (A) in the reference cell.
3. Measure the absorbances of each sample solution at wavelengths of 248 nm and 296 nm by placing the solution from flask **b1** in the measuring cell and the solution from flask **a1** in the reference cell. Then measure the absorbances with solutions from flasks **b2** and **a2**, and flasks **b3** and **a3**, respectively.

Calculation:

Calculate the total capsaicinoid content, w_{248}, as a percentage by mass, at a wavelength of 248 nm,using the following formula:

$$w_{248} = \frac{(A_S - A_b) \times d}{314 \times m}$$

where

A_s is the absorbance of the sample solution;
A_b is the absorbance of the blank solution;
d is the dilution factor;
m is the mass (g) of the test portion.

Carry out an additional dilution when the absorbance is greater than 0.8.
Calculate the total capsaicinoid content, w_{296}, as a percentage by mass, at a wavelength of 296 nm:

$$w_{296} = \frac{(A'_s - A'_b) \times d}{127 \times m}$$

where
A'_s is the absorbance of the sample solution;
A'_b is the absorbance of the blank solution;
d is the dilution factor;
m is the mass (g) of the test portion.

5.5.7 Determining the pungency of capsicums and their oleoresins: high-performance liquid chromatographly (HPLC) method

Purpose: To determine pungency levels in crushed red pepper, chilli pepper, jalapeno pepper and red pepper oleoresins.

Apparatus required:
Liquid chromatograph equipped with: (i) integrator; (ii) 20 μL sample loop injection valve; (iii) fluorescence detector and/or ultraviolet detector. Chromatographic column stainless steel, 150 × 4.6 mm packed with 5 μm LC-18. Glass beads.

Reagents:
Ethyl alcohol (EtOH), 95 % or denatured. Acetone, pure, mobile phase. Use LC grade solvents, or equivalent: (i) deionized H_2O, add 1 % HOAc (v/v); (ii) acetonitrile; (iii) mix 400 ml of (ii) with 600 ml of (i). (iv) De-gas with helium or by other suitable means. Standard: N-vanillyl-*n*-nonanamide, 99 + %. C18 Sep-Pak cartridge, 6 ml capacity.

Preparation of sample:
- **Ground or crushed peppers:** Weigh accurately about 25 g pepper into a 500 ml boiling flask. Pipette 200 ml EtOH into the flask and add several glass beads to aid boiling. Reflux gently for 5 hours. Allow to cool. Filter 3–4 ml through a 0.45 μm syringe filter into glass vial.
- **Oleoresins:** Accurately weigh 1–2 g oleoresin (increase sample size if total capsaicinoid concentration is below 1 %) into 50 ml volumetric flask, being sure not to allow any oleoresin to coat the sides of the flask. Add 5 ml of acetone to flask and swirl acetone until sample is completely dispersed. Filter through a 0.45 μm syringe filter into a glass vial.

Procedure:
1. Prepare all standard solutions with EtOH. Keep solutions out of direct sunlight.
   - Standard solution A – 0.15 mg/ml. Accurately weigh and transfer 75 mg of standard into a 500 ml volumetric flask, dissolve, dilute to volume and mix.

- Standard solution B – 0.015 mg/ml. Pipette 10 ml standard solution A into a 100 ml volumetric flask, dilute to volume and use with chilli peppers.
- Dilute standard solution C – 0.00075 mg/ml. Pipette 5 ml of working standard solution B into a 100 ml volumetric flask, dilute to volume and mix. (Use with samples, which contain capsicum heat levels below 5000.)

2. Chromatographic conditions: Mobile phase: 40 % acetonitrile and 60 % deionized H_2O with 1 % acetic acid (v/v). Flow rate: 1.5 ml/minute, isocratic. Column: LC-18 150 × 4.6 mm i.d., 5 μm particle size. Injection volume: 20 μL. Detection: excitation 280 nm; emission 325 nm for fluorescence or 280 nm for ultraviolet.

Using a sample loop injection valve, inject in duplicate 20 μL of the prepared sample solution onto the column. Inject the appropriate standard solution before first sample injection and after no more than six sample injections. Purge the column with 100 % acetonitrile for 30 min. at 1.5 ml/min after no more than 30 sample injections. Return to previous mobile phase for further determinations (Hoffman *et al*., 1983).

Calculation:
Scoville heat units (SHU) are the sum of SHU of the three major capsaicinoids. Calculate SHU as follows:

- Nordihydrocapsaicin, $SHU_N = (N/A) \times (C_S/ C_X) \times (H_N/R_N)$
- Capsaicin, $SHU_C = (C/A) \times (C_S/ C_X) \times (H_C/ R_C)$
- Dihydrocapsaicin, $SHU_D = (D/A) \times (C_S/C_X) \times (H_D/R_D)$
- Total $SHU_T = SHU_N + SHU_C + SHU_D$

where:
A is average peak area of standard;
N, C and D are average peak areas for respective capsaicinoids (nordihydrocapsaicin, capsaicin and dihydrocapsaicin) from duplicate injections;
C_S is concentration of standard (mg/ml);
C_X is concentration of sample in extract (mg of sample/ml);
H_N, H_C and H_D are heat factors for respective capsaicinoids;
R_N, R_C and R_D are response factors of respective capsaicinoids relative to standard.

Accepted heat factors and response factors and relative retention times are given in Table 5.1.

5.6 Antioxidant potential of plant extracts

For an aerobic organism, oxygen is a double-edged sword since although essential for aerobic life processes, 5 % or more of the inhaled oxygen is converted to reactive oxygen species (ROS) such as superoxide, hydrogen peroxide and the extremely reactive hydroxyl radical. Cellular antioxidant enzymes catalase, superoxide dismutase and various peroxidases and free-radical scavengers like glutathione, vitamin C and vitamin E normally protect the cells from the damaging effects of ROS but, when the dynamic equilibrium is upset, pathological conditions result from the oxidative damage to the cellular macromolecules such as lipids, proteins and nucleic

Table 5.1 Heat factors, response factors and retention times of capsaicinoids

	Heat factor	Response factor		Retention time
		UV	FLU	
Nordihydrocapsaicin (N)	$H_N = 9.3 \times 10^6$	$R_N = 0.98$	0.92	0.90
Capsaicin (C)	$H_C = 16.0 \times 10^6$	$R_C = 0.89$	0.88	1.00
Dihydrocapsaicin (D)	$H_D = 16.0 \times 10^6$	$R_D = 0.93$	0.93	1.00
N-vanillyl-n-nonanamide		$R = 1.00$	1.00	1.58

acids. These include cardiovascular dysfunction, neurodegenerative diseases, gastroduodenal pathogenesis, metabolic dysfunction of almost all vital organs, cancer, premature ageing, gallstones and such. It is in this context that antioxidant therapy becomes relevant. Polyphenols are widely distributed in the plant kingdom, and one of their key nutraceutical attributes is to lend protection against oxidative damage by scavenging free radicals. Protection against peroxidation of low-density lipoprotein (LDL) is likely to be beneficial to heart disease, whereas protection against oxidative DNA damage is likely to protect against cancer and genomic instability. Also, certain polyphenols may induce phase II enzymes such as glutathione transferase (GST) that will enhance the excretion of oxidizing species, or induce antioxidant enzymes such as metallothionein (a metal-binding protein with antioxidant property).

Polyphenols may also inhibit cytochrome P450s (CYPs) or enzymes such as cyclooxygenase or lipoxygenase that have oxidant activities. The ability of certain polyphenols to bind minerals may be beneficial in some cases, since copper and iron can be initiators of hydroxyl radical production by the Fenton and Haber-Weiss reactions. For example, tannic acid has been shown to inhibit hydroxyl radical formation from the Fenton reaction by complexing ferrous ions. Most antioxidant polyphenols function as terminators of free radicals and may also chelate metal ions that are capable of catalyzing lipid peroxidation. They interfere with the oxidation of lipids and other molecules by rapid donation of a hydrogen atom to radicals as follows:

$$RO^{\cdot} + PPH \rightarrow ROH + PP^{\cdot}$$

The phenoxy radical intermediates are relatively stable, and also act as terminators of the propagation route by reacting with other free radicals:

$$RO^{\cdot} + PP^{\cdot} \rightarrow ROPP$$

Protection against oxidative stress in intact cells or tissues is often measured by the presence of malondialdehyde, one of the end-points of the peroxidation of polyunsaturated fatty acids. Two principle mechanisms of action have been proposed for antioxidants. The first is a chain-breaking mechanism, by which the primary antioxidant donates an electron to the free radical present in the system (e.g., lipid radical). The second mechanism involves removal of ROS/RNS initiators (secondary antioxidants) by quenching chain-initiating catalysts.

The ability of antioxidants to chelate transition metal ions can be followed spectroscopically. High molecular weight proteins bind directly or indirectly to redox

active metals and thus inhibit the production of metal-catalyzed free radicals. Some low molecular weight compounds, such as polyphenols, in addition to their ability to donate hydrogen atom and thus to act as chain-breaking antioxidants, can also chelate transition metal ions and hence inhibit free radical formation.

Another simple, but very informative technique for quantifying antioxidant activity is the reaction of an antioxidant with 1,1-diphenyl-2-picryl-hydrazyl (DPPH) or with galvinoxyl. The principle of this method is that, in the presence of a molecule consisting of a stable free radical (DPPH), an antioxidant with the ability to donate a hydrogen atom will quench the stable free radical, a process which is associated with changes in absorption which can be followed spectroscopically. This simple method can be applied either when the antioxidant is in its pure form, or in a mixture (e.g., a natural extract). Using this method, it is possible to follow the kinetics of the reaction, the number of electrons an antioxidant molecule can donate, and also to estimate the structure of the oxidized antioxidant after it has donated hydrogen atom(s).

5.6.1 Sample preparation

Fresh plant samples (0.5–1.0 g) are homogenized in appropriate solvents (water, methanol, ethanol, petroleum ether, chloroform, etc.) to obtain extracts. The extracts are centrifuged at 10 000 rpm for 20 min. The volume of the supernatant is adjusted to yield a 0.01 g/ml solution, which is used for the following assays.

DPPH radical scavenging assay

Principle: 1,1-Diphenyl-2-picryl hydrazyl is a nitrogen centred free radical, the colour of which changes from violet to yellow on reduction by H^+ or e^- donation. Substances able to perform this reaction are antioxidants and therefore radical scavengers like ascorbic acid, BHA, BHT and gallic acid are references.

Reagents:

0.004 % DPPH in ethanol.

Procedure:

Ethanol is added to appropriate volumes of test solution to bring the total volume to 4.0 ml; 1 ml of 0.004 % DPPH in ethanol is added. A control of 4 ml ethanol and 1 ml 0.004 % DPPH is maintained; absolute blank consisted of only ethanol. The tubes are incubated in the dark for 30 min., and absorbance read at 517 nm in a spectrophotometer (Braca *et al.*, 2001).

Calculation:

$$\text{Percentage radical scavenging capacity} = \frac{\text{ABS}_{\text{control}} - \text{ABS}_{\text{sample}}}{\text{ABS}_{\text{control}}}$$

Total antioxidant capacity by phosphomolybdenum method

Principle: Antioxidants present in the extract react with the molybdenum in the reagent, and reduction of Mo(VI) to Mo(V) results in green colour.

Reagents:

- **Reagent solution:** 0.6 M H_2SO_4, 25 mM Na_2HPO_4, 4 mM ammonium molybdate.
- **Stock solution:** 10 mM ascorbic acid; working standard: 1 mM.

Procedure:

Ethanol is added to appropriate volumes of test solution to bring the total volume to 3.0 ml. The working curve is obtained by pipetting aliquots of the working standard in the concentration range of 200–600 µM and the final volume is made up to 3 ml with ethanol. A blank of 3 ml ethanol is maintained. 1 ml of reagent solution is added to all the tubes, capped well, boiled for 90 min. at 95 °C and cooled. The absorbance is read at 695 nm in a spectrophotometer (Prieto *et al.*, 1999).

Calculation:

Antioxidant capacity is expressed as ascorbic acid equivalents (µmol/g of the sample).

Ferric reducing power method

Principle: This method monitors the reductive ability of the antioxidants manifested by the transformation of Fe(III) to Fe(II). The presence of reductants (antioxidants) causes the reduction of Fe^{3+}/ferricyanide complex to Fe^{2+} forms. This Fe^{2+} can be monitored by measuring the complex at 700 nm in the spectrophotometer. Increase in absorbance indicates increased reducing power of the sample.

Reagents:

0.2 M phosphate buffer, pH 6.6, 1 % potassium ferricyanide [$K_3Fe(CN)_6$], 10 % trichloroacetic acid (TCA), 0.1 % ferric chloride ($FeCl_3$). Stock solution: 10 mM ascorbic acid; working standard: 1 mm.

Procedure:

An appropriate volume of the extract is made up to 1.0 ml with distilled water; 2.5 ml of 0.2 M phosphate buffer pH 6.6 and 2.5 ml of 1 % potassium ferricyanide [$K_3Fe(CN)_6$] are added. The tubes are incubated for 30 min. at 50 °C, after which 2.5 ml of 10 % TCA is added to the mixture. 2.5 ml aliquot is taken from the above solution and diluted with 2.5 ml distilled water. 0.1 % $FeCl_3$ (0.5 ml) is added and absorbance measured at 700 nm in a spectrophotometer. Ascorbic acid is used as the working standard (0.25–1 mM) and phosphate buffer as the blank. The Fe^{3+} to Fe^{2+} reducing power is expressed in terms of ascorbic acid equivalents (Oyaizu, 1986).

Calculation:

Fe (III) reducing activity is expressed as ascorbic acid equivalents (µmol ascorbic acid/g extract).

Thiobarbituric acid reactive species (TBARS) assay

Principle: TBARS assay quantifies oxidative stress by measuring the peroxidative damage to lipids that occurs with free radical generation. Free radical damage to lipids

results in the production of malondialdehyde (MDA), which reacts with TBA under conditions of high temperature and acidity generating a chromogen that can be measured either spectrophotometrically or spectrofluorometrically.

Reagents:
Egg yolk homogenate, as lipid-rich media, 0.07 mol/l 2,2′-azobis (2-amidinopropane) dihydrochloride, 20 % acetic acid, 0.8 % TBA in 1 % (w/v) SDS solution.

Procedure:
A modified TBARS assay is used to measure the potential antioxidant capacity using egg yolk homogenates as lipid-rich media. Briefly, 0.5 ml of 10 % (w/v) egg yolk homogenate and appropriate volumes of extracts are added to a test tube and made up to 1.0 ml with distilled water. 0.05 ml of 2,2′-azobis (2-amidinopropane) dihydrochloride solution (0.07 M) in water is added to induce lipid peroxidation. 1.5 ml of 20 % acetic acid (pH 3.5) and 1.5 ml 0.8 % (w/v) TBA in 1.1 % (w/v) SDS solution is added and the resulting mixture vortexed, and then heated at 95 °C for 60 min. After cooling, 5.0 ml of butan-1-ol is added to each tube, then extensively vortexed and centrifuged at 1200 g for 10 min. The absorbance of the organic upper layer is measured using a spectrophotometer at 532 nm. All the values are based on the percentage antioxidant index (AI%):

$$\text{AI\%} = (1 - A_T/A_C) \times 100$$

where A_C is the ABS of the fully oxidized control and A_T is the ABS of the test sample (Ruberto and Baratta, 2000).

Non-site-specific hydroxyl radical mediated 2-deoxy-D-ribose degradation
Principle: Site- and non-site-specific hydroxyl radical mediated deoxyribose degradation occurs by hydroxyl radicals generated by Fenton reaction. Activities of extracts are compared to mannitol, which is an effective hydroxyl radical scavenger.

Reagents:
5.6 mM 2-deoxy-D-ribose in KH_2PO_4-sodium hydroxide buffer (50 mM, pH 7.4), premixed 100 μM ferric chloride and 104 mM EDTA (1 : 1 v/v solution), 1 mM H_2O_2, 1 mM ascorbic acid, 2.8 % TCA, 1 % TBA, standard mannitol: 1 mM.

Procedure:
To appropriate volumes of the sample made up to 1 ml with distilled water is added 0.5 ml of 2-deoxy-D-ribose in KH_2PO_4-sodium hydroxide buffer, 0.2 ml of premixed 100 μM ferric chloride and 104 mM EDTA (1 : 1 v/v solution), 0.1 ml of 1 mM H_2O_2 and 0.1 ml of 1 mM ascorbic acid. The tubes were incubated at 50 °C for 30 min., and 1 ml each of 2.8 % TCA and 1 % TBA were added. The tubes were vortexed, heated in a water bath at 50 °C for 30 min. and the absorbance read at 532 nm in a spectrophotometer. Antioxidant activity of the extracts is expressed as mannitol equivalents per gram of sample (Halliwell *et al.*, 1987).

5.6.2 Estimation of phenols

Phenols and polyphenols are associated with antioxidant activity of plant extracts, so a quantification of the total phenols and profiling of the type of phenols present in an extract is relevant in any study involving measurement of antioxidant potential (Oyaizu, 1986).

Principle: Phenols react with the oxidizing agent phosphomolybdate in Folin–Ciocalteau reagent under alkaline conditions, resulting in the formation of a blue-coloured complex, the molybdenum blue, which is measured at 760 nm colorimetrically.

Reagents:

80 % ethanol, Folin–Ciocalteau reagent, Na_2CO_3, 20 %, standard (100 mg catechol in 100 ml water), dilute 10 times for a working standard.

Procedure:

An appropriate volume of extract is made up to 4 ml with water, and 0.250 ml of Folin–Ciocalteu reagent added. After standing the tubes for 1 min., 0.750 ml of 20 % Na_2CO_3 is added, mixed thoroughly, placed in a boiling water bath for exactly 1 min. and cooled, or incubated at room temperature, for 2 hours. The absorbance is read at 760 nm in a spectrophotometer. The working curve is obtained by running a working standard in the concentration range of 20–100 μM. A blank was maintained with 3 ml of water (Singleton *et al.*, 1999).

Calculation:

The amount of total phenols was expressed as g%.

Estimation of anthocyanin

Reagents:

Ethanolic HCl: 95 % ethanol in 1.5 N HCl (85:15 ratio).

Procedure:

Fresh sample extracted in ethanolic HCl and read the absorbance at 535 nm.

Calculation:

Total absorbance per 100 g of plant material is given by:

$$ABS_T = \frac{V_{EXT} \times V_T \times 100}{\text{extract used (ml)} \times W}$$

where:

ABS_T is total absorbance per 100 g of the plant material;
V_{EXT} is volume made up of the extracts (ml);
V_T is total volume used for colour measured (ml);
W is weight of sample taken (g).

The ε value for 1 % solution (i.e 10 mg/1 ml) at 535 nm is equal to 982.

Therefore the absorbance of a solution containing 1 mg /ml is equal to 98.2.

$$\text{total anthocyanin content in mg/100 g of material} = \frac{ABS_T/100 \text{ g of plant material}}{98.2}$$

Estimation of carotenoid
Reagents:
Acetone.

Procedure:
Acetone extract to be read at 480 mn and 510 nm.

Calculation:
Total carotenoid content in mg/1 g tissue is given by:

$$7.6\,(480\text{ nm}) - 1.49\,(510\text{ nm}) \times \frac{F_V}{\text{weight}} \times 1000$$

where F_V is the fluorescence parameter (maximum fluorescence minus minimum fluorescence – $F_M - F_0$).

5.7 Estimation of fibre

5.7.1 Fibre (crude) in animal feed and pet food (Ceramic fibre filter method)

Principle: Crude fibre is loss on ignition of dried residue remaining after digestion of sample with 1.25 % (w/v) H_2SO_4 and 1.25 % (w/v) NaOH solutions under specific conditions. Method is applicable to materials from which the fat can be and is extracted to obtain a workable residue, including grains, meals, flours, feeds, fibrous materials and pet foods.

Reagents:
Sulphuric acid solution – 0.128 ± 0.003M. 1.25 g H_2SO_4/ 100 ml.

$$ABS_T = \frac{V_{EXT} \times V_T \times 100}{\text{extract used (ml)} \times W}$$

Apparatus required:
Digestion apparatus with built-in heaters and filter system are to be adopted.

Procedure:
Pre-weighed crucible suitable for the extraction units is loaded with coarsely ground ginger sample. Extract with hot acid for 30 min., followed by washing with hot water to neutral pH. Extract the residue with hot alkali for 30 min. followed by washing with water to neutral pH. Evaporate the residue to dryness. Ash the residue in a muffle furnace, cool and weigh (AOAC, 2007).

Calculation:
Crude fibre in ground test portion, % (C) is given by

$$\frac{\text{Weight of crucible after digestion} - \text{Weight of crucible after ashing}}{\text{weight of test portion}} \times 100$$
$$= \frac{W_1 - W_2}{W} \times 100$$

where:
W_1 is weight of crucible after digestion;
W_2 is weight of crucible after ashing;
W is weight of test portion in gm;

5.8 References

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6

Ajowan

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Abstract: This chapter discusses ajowan (*Trachyspermum ammi* L.), opening with information on its chemical composition and production methods. Ajowan is largely grown for its fruits (seeds) which are valued for their peculiar aroma and rich medicinal properties. The aroma chemicals present in essential oil from fruits are detailed, the key constituents from the volatile oil being thymol and carvacrol. Several processed products from ajowan fruits such as essential oil, powder, oleoresin, thymol and thymene which have potential in the international market are discussed. The functional properties underlying the medicinal uses of ajowan such as its antimicrobial, insecticidal and parasiticidal activities, amongst others, are given. Toxicity and adulteration are also mentioned. The quality specifications for different ajowan products such as whole seed, powdered seed, volatile oil, oleoresins and thymol production are described.

Key words: ajowan, cultivation, chemical composition, main products, functional properties, medicinal uses, toxicity, adulteration, quality specifications.

6.1 Introduction

Ajowan [*Trachyspermum ammi* (L.) Sprague ex Turrill] of the carrot family (Apiaceae) is native to India (Clevely *et al*., 1997; Sayre, 2001; Lemoine, 2002). India is the leading producing and exporting country in the world, but other countries where it is grown include Pakistan, Afghanistan, Iran and Egypt. In the modern literature, ajowan is usually referred to as *Trachyspermum ammi* (L.). Synonyms for *Trachyspermum ammi* used in the literature are *Trachyspermum copticum* Linn, *Carum copticum* Benth and Hook, *Ammi copticum* Linn., *Ptychotis coptica* DC. and *Lingusticum ajowain*, Roxb. The correct generic position of this spice has been a matter of debate. Boissier considers it to belong to the genus *Ammi*, where Linnaeus originally put it, but it has been referred to as *Carum* and in the recent past it was placed in the section *Trachyspermum*, which includes about 14 species (Bentley and Trimen, 1999). On the basis of pollination behaviour, *Trachyspermum* is cross-pollinated crop and has a somatic chromosome number of 2n = 18.

6.1.1 Chemical composition

T. ammi seeds usually contain between 2.5 and 5 % essential oil, and 26 % fatty oils. However, the chemical composition of ajowan varies with variety, region and stage

of harvest. As an example, ajowan from Pakistan yielded 3.5 % and 5.2 % essential oil from large and small size fruits, respectively (Ashraf and Bhatty, 1975).

The principal constituents of the essential oil, which are responsible for typical ajowan flavour, are phenols: thymol (35–60 %) and carvacrol (11 %). The chemical structures of thymol and carvacrol are shown in Fig. 6.1. Thymol crystallizes easily from the oil on cooling. The remainder of oil is called thymene, which contains ρ-cymene (50–55 %), β-pinene (4–5 %), limonene with α and β terpinenes (30–35 %) (Raghvan, 2006).

Various authors have studied the chemical composition of ajowan; the results of a few of these studies are described here. Gas chromatography–mass spectroscopy (GC–MS) analysis of ajowan essential oil by Abdolahi *et al*., (2010) revealed that thymol (63 %) was the main component. Krishnamoorthy *et al.* (2000) reported that ajowan oil contained 27 compounds, of which thymol (61 %) was present in the largest amounts, with paracymene (15.6 %), terpinene (11.9 %), β-pinene (4–5 %), dipentene (4–6 %), camphene and myrecene in trace amounts. The main constituents of ajowan essential oil as reported by Urbaniak (2010) are phenols (thymol 40–48 %, carvacrol 5 %) and monoterpenes (γ-terpinene, 20–35 %), para-cymene, 20–25 %). The water-distilled oil from aerial parts and fruits of ajowan was analysed and found to contain thymol (42.7 % and 46.2 %), γ-terpinene (38.5 % and 38.9 %) and *p*-cymene (14.1 % and 13.9 %) as the main compounds (Masoudi *et al*., 2002). The phenolic components of the ajowan essential oil contained 87.75 % thymol and 11.17 % carvacrol as major constituents, and major non-phenolic components quantified were 60.78 % *p*-cymene and 22.26 % γ-terpinene (Bhattacharya *et al*., 1998). The physicochemical characteristics of ajowan volatile oil as determined through GC–MS are given in Table 6.1 (Bhattacharya *et al*., 1998; Nagalakshmi *et al*., 2000).

Variations in aroma compounds have been observed in seeds collected from different geographical regions and plant parts (flowers, leaves) of ajowan. In Algeria, isothymol (50 %) was found to be the dominant constituent before *p*-cymene, thymol, limonene and γ-terpinene. However, the name isothymol is not well defined and it might refer to both 2-isopropyl-4-methylphenol and 3-isopropyl-6-methylphenol (carvacrol). From South Indian ajowan fruits, almost pure thymol has been isolated (98 %), but the leaf oil was found to be composed of monoterpenoids and sesquiterpenoids, 43 % cadinene, 11 % longifolene, 5 % thymol, 3 % camphor and others (Pruthi, 2001). According to two studies, carvacrol (54.4 %),

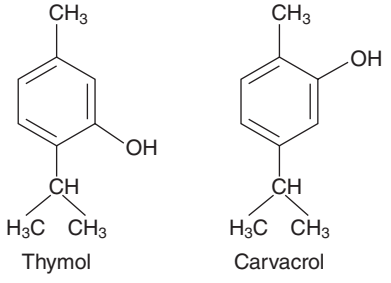

Fig. 6.1 Chemical structures of thymol and carvacrol.

Table 6.1 Composition of essential oil ajowan seeds

Components	Components of essential oil (%)	
	Bhattacharya *et al.* (1998)	Nagalakshmi *et al.* (2000)
Phenolic parts		
Safrole	0.10	–
Thymol	87.75	39.36
Carvacrol	11.17	–
Non-phenolic part		
α-Thujene	0.27	–
α-Pinene	0.28	1.48
β-Pinene	2.38	5.45
Myrcene	0.81	1.40
p-Cymene	60.78	19.47
Limonene	8.36	0.48
γ-Terpinene	22.26	30.97
Terpinolene	0.13	–
Linalool	0.27	0.07
Camphor	0.28	–
(*Z*) β Terpineol	0.19	–
(*E*) β-Terpineol	1.35	–
Borneol	0.49	–
Terpinen-4-ol	0.12	0.12
α-Terpineol	0.22	0.12
Carvone	0.15	–
Safrole	0.16	–

Table 6.2 Chemical composition of the essential oil from *Carum copticum*

Compounds	Retention index	Percentage
Thymol	1332.84	64.51
γ-Terpinene	1055.83	17.52
p-Cymene	1026.59	16.16
β-Pinene	975.57	0.39
Myrcene	986.80	0.33
α-Thujene	928.98	0.17
α-Pinene	938.19	0.06
Sabinene	972.16	0.02
Other compounds	–	0.84

Source: Shojaaddini *et al*. (2008).

γ-terpinene (28.3 %) and *p*-cymene (15.4 %) were the major components of African ajowan whereas ajowan seeds from South India have been reported to contain the highest quantity of thymol, 97.9 % (Minija and Thoppil, 2002). Essential oil from ajowan seeds from Tehran as analysed by GC and GC–MS revealed thymol (64.51 %), γ-terpinene (17.52 %) and *p*-cymene (16.16 %) as the main components among eight

constituents characterized in the oil representing 98.19 % of the total components detected (Shojaaddini *et al*., 2008) and given in Table 6.2.

On analysis of the water-soluble portion of methanol extract of the fruits of ajowan, Ishikawa *et al*. (2001) reported 25 compounds, including five new monoterpenoid glucosides, a new monoterpenoid, two new aromatic glucosides and two new glucides. The alcoholic extract was found to contain a highly hygroscopic saponin, with a haemolytic index of 500. Yellow crystalline (mp 91–94 °C) and steroidal substances (mp 140–150 °C) called stearoptenes have also been isolated from ajowan fruits (Pruthi, 2001). On analysis of the fixed oil from the seeds of *Carum copticum* Benth. of Indian origin, Farooq *et al*. (1953) reported petroselinic acid as the major component of the fatty acids whereas linolenic acid and any saturated acid other than palmitic were absent.

6.2 Production and trade

Ajowan is cultivated in the Mediterranean region, South-West Asian countries, Iran, Iraq, Afghanistan and Egypt; however, the major producer and exporter is India. In India, it is grown on a large scale in the states of Rajasthan, Gujarat and Andhra Pradesh and grows on a smaller scale in Uttar Pradesh, Bihar, Madhya Pradesh, Punjab, Tamil Nadu, West Bengal and Karnataka. In India during the year 2008–2009, about 16410 tonnes of ajowan seed was produced from an area of 19590 ha whereas, 2200 tonnes of ajowan seed worth Rs 44.0 million was exported. India exports ajowan seed to around 46 countries. The major importing countries are Pakistan, Saudi Arabia, the USA, the UAE, Malaysia, Indonesia, Nepal, South Africa, Kenya, Bangladesh, Canada and the UK. A large quantity of ajowan seed from India, Egypt, Persia and Afghanistan has been reported to be exported to Germany for distillation of the essential oil and extraction of thymol (NHB, 2010).

6.2.1 Cultivation

Ajowan can be grown on any soil type ranging from loamy to sandy loam and even in black soils. In India, Afghanistan and Egypt, it is grown under both rain-fed and irrigated cultivation systems. The seeds are sown, broadcasted or drilled in rows 45 cm apart during September–October at a depth of 1 cm. The seed rate is 4 kg per hectare. The plant to plant spacing should be maintained at 20–30 cm. The ripe fruits germinate relatively quickly and the germination time is 12 days. Since Ajowan is slow growing during the initial stage after emergence, therefore it is necessary to keep the field free from weeds. Ajowan is not greatly affected by diseases and pests.

Farmers mostly use regional cultivars. Some of the regional cultivars developed through selection in India are GA-1 for Gujarat; BEN-1 for Karnataka; RPA180 for Bihar; Lam Sel-1 and Lam Sel-2 for Andhra Pradesh. The varieties NRCSS AA 1 and NRCSS AA 2 have been developed. They are high seed-yielding cultivars suitable for cultivation under semi-arid conditions. The variety NRCSS AA 1, produces 1420 kg ha^{-1} under irrigated conditions with essential oil levels of up to 3.5 %, whereas, the variety NRCSS AA 2 is suitable for cultivation under rain-fed farming

system, has a seed yield ranging from 500–600 kg ha^{-1} and the seeds have an essential oil yield of 3 % (Malhotra and Vashishtha, 2008).

6.2.2 Organic farming

The ajowan seed produced from India and Afghanistan is mostly from arid and semi-arid regions, which are by default organic, since there are minimal or no chemical inputs to the system. Such products are termed and sold as near organic in the market. General and specific guidelines for organic production of seed spices including ajowan have been detailed by Malhotra and Vashishtha (2008). The demand for organic spices is steadily increasing. The European countries, USA, Canada and Japan are the largest markets. Emerging markets for organic spices are Australia and New Zealand. No reliable published data on ajowan organic seed production and export is available, but the future demand for organic spices appears to be bright.

6.2.3 Harvesting and yield

In India, the small white flowers bloom in November and December in the plains and mid-summer in hills. The harvesting is usually done from February to May. The flower production ceases when the seed starts maturing and becomes greyish-brown in colour. The yield is 400–600 kg/ha under a rain-fed farming system and 1200–2000 kg/ha under irrigated conditions (Malhotra, 2006).

6.2.4 Post-harvest processing

The harvested ajowan crop is first transported to a clean threshing floor, where it is dried hygienically in a thin layer for 1 or 2 days before threshing is carried out to separate the seeds. The seed is then dried. Seed dried in the shade has a greater oil content than the sun-dried seed. The drying of ajowan seed in India is often carried out in zero energy solar drier tunnels to avoid entry of dust and foreign matter. The seed is cleaned in a screening mill and then processed through a gravity separator. Seeds that have been well dried (8–9 % moisture), cleaned and graded by sieving are stored in polyethylene-lined gunny bags in a cool dry place (Malhotra, 2006).

Ajowan powder is produced by grinding dried seeds. Loss of volatile oils (i.e. flavour loss) can be minimized by providing suitable cooling arrangements in the milling zone. Pre-cooling of the spices also can minimize flavour loss. Since the application of liquid nitrogen for quick freezing has become more common, the advantages of freeze grinding of spices have become clear (Malhotra, 2010). The finer powder product is mostly used for seasoning of foods, whereas coarse product is used for the purpose of extraction of oils, oleoresin and other extractives (Malhotra, 2010).

If oil is to be extracted from the seeds, it is best to do this when they are fresh. Both essential oil (volatile oil) and non-volatile fatty oils can be extracted. Ajowan

essential oil is often obtained by steam distillation or hydrodistillation of the seeds. However, oil of the highest quality can be obtained by using the supercritical fluid extraction method. Integrated methods by which to recover both types of oil from the crushed seed have been developed by Ramachandriah *et al*. (1988). The yield of essential oil and fatty oils obtained through different methods varies. Processing conditions in general, such as temperature, pH and heating time, have a considerable effect on the overall aroma of processed essential oil of ajowan.

As reviewed by Bentley and Trimen (1999) the thymol fraction ($C_{10}H_{14}O$) crystallizes partially from the essential oil. Thymol is extracted by treating the essential oil with a warm solution of sodium hydroxide. This alkali dissolves the thymol to form sodium thymol and, on dilution with water, the undissolved oil (terpenes, etc.) rises to the surface. Thymol can then be liberated by means of hydrochloric acid and may be finally recrystallized from alcohol. The remainder of the oil consists of cymene ($C_{10}H_{14}$) boiling at 175 °C, and a terpene boiling at 172 °C, with traces of pinene and dipentene, the mixture being known commercially as 'thymene' (Pruthi, 2001). Ajowan was used extensively for the isolation of thymol in the past, but this has largely been replaced by synthetic thymol.

Ajowan oleoresin is prepared by extraction of crushed dried seeds with suitable volatile oil solvents like food-grade hexane ethanol, ethyl acetate or ethylene dichloride, filtration and desolventization under vacuum. The organic solvent should be recovered completely from oleoresin as per the ISO maximum permissible limits.

6.3 Main uses in food and cosmetics

6.3.1 Whole seed and powder

Ajowan seeds have an aromatic smell and a warm pungent taste. The whole seed is the basic material for the preparation of various value-added items, viz., oil, oleoresin, thymol, thymol crystals, dethymolized oil (thymene) and fatty oils for flavouring and aromatic purposes in foods, beverages and perfumery and for medicinal purposes in the pharmaceutical industry. The seeds are used both as spices and as a condiment in many countries. Ajowan seeds are used in India as a traditional spice in many foods including curries. A small amount of ajowan whole seed will completely dominate the flavour of a dish. In most of the Indian dishes, ajowan is either dry-roasted or fried in butter or oil in various preparations such as potatoes or fish. (The aroma compounds in spices are lipophilic and dissolve much better in fat than in water. Therefore, frying in butter not only enhances the fragrance due to high temperature, but also extracts the flavour to the fat and disperses it in the food.) Processed in this way, it is known to develop a subtle and peculiar aroma. In South Indian cuisine (which is predominantly vegetarian) *tadka* (frying in butter or oil) preparations are not only applied to dried legumes (lentils, beans) but also to green vegetables, boiled rice and ajowan *paratha* (potato stuffed bread). Ajowan raw is particularly popular in savoury foods commonly used by Indians, Arabians, Iranians, Pakistanis and Africans, in various kinds of preparations/recipes such as savoury pastries, snacks (including

Bombay mix) and breads (especially *paratha*), bean and pulse recipes, all of which illustrates ajowan's affinity for starchy foods and meats where it is considered to make easier to digest.

Ajowan seed is powdered and blended with several spices to make certain curry powders. Two commercial blends are given below:

1. **Chat masala:** Chat masala is a mixture used to add taste in Indian salad snacks consisting of fruits banana, papaya, sapota or apples. This mixture consists of whole spices and salt ground without dry frying or roasting and thoroughly mixed (Clevely *et al*., 1997).

   Makes 2.5 tbsp
   1 tsp black pepper corns
   1 tsp cumin seeds
   1 tsp ajowan seeds
   1 tsp pomegranate seeds
   1 tsp mixed black salt & sea salt to taste
   ¼ tsp asafoetida
   1 tsp mango powder
   ½ tsp cayenne pepper or to taste
   ½ tsp garam masala (optional)
2. **Berbera:**
   This is an Ethiopian blend of spices added to many local dishes, from baked fish dishes to chicken stews (Clevely *et al*., 1997).

   Makes 50 g
   10 dried red chillies
   8 white cardamoms
   1 tsp cumin seeds
   1 tsp coriander seeds
   1 tsp fenugreek seeds
   8 cloves
   1 tsp all spice berries
   2 tsp black pepper corns
   1 tsp ajowan seeds
   1 tsp ground ginger
   ½ tsp ground nutmeg
   2 tbsp salt

6.3.2 Ajowan essential oil

Ajowan essential oil is a rich source of bioactive compounds and thus is used as a fragrance and flavouring agent for food and beverages. Ajowan essential oil has been identified as natural food flavouring component with potential for use either individually or in admixture in beverages, bakery, ice creams and other food preparations. Ajowan essential oil is also used in foods for its antimicrobial properties. Ajowan extract has been proven to be a novel natural antioxidant for stabilization of edible oils (*Terminalia bellirica roxburghii* and *Linum usitatissimum*) which are

mostly used in foods (Bera *et al*., 2004). The oil is occasionally used in the production of soap.

6.3.3 Ajowan oleoresin and fatty oils

Ajowan oleoresin prepared from seeds gives a warm, aromatic and pleasing flavour to food products. It is used in processed foods, snacks, sauces and various vegetable preparations. Fatty oils produced from ajowan seed have their use in various pharmaceutical and cosmetic preparations. The fatty oils are mainly used in the soap industry for flavouring and as a deodorant. They are also used for perfuming disinfectant soaps and as an insecticide.

6.3.4 Thymol and thymene

Thymol is a powerful antiseptic and an ingredient in a number of skin ointments/powders, deodorants, mouthwashes, toothpastes and gargles. 'Thymene' finds application as a perfume for soap. Thymene is sold as ajowan-ka-phool (crystals) or sat-ajowan (water of ajowan) in the Indian market and is valued as a medicine.

6.4 Functional properties

The nutritional composition of ajowan seeds according to Agarwal *et al*. (2000) is given in Table 6.3. Ajowan has been a highly esteemed medicinal herb since early times. Traditionally, the seed is used in India as a folk remedy for arthritis, asthma, coughs, diarrohea, indigestion, intestinal gas, influenza and rheumatism

Table 6.3 Chemical composition of ajowan ground spice per 100 g

Composition	Content
Carbohydrate (g)	24.6
Protein (g)	17.1
Fibre (g)	21.2
Water (g)	7.4
Food energy (calorie)	363
Minerals (g)	7.9
Ca (g)	1.525
P (g)	0.443
Na (mg)	56
K (mg)	1.38
Fe (mg)	27.7
Thiamine (mg)	0.21
Riboflavin (mg)	0.28
Niacin (mg)	2.1

Source: Agarwal *et al*. (2000).

(Sayre, 2001). A paste of the crushed fruits is applied externally for relieving colic pains and a hot and dry fomentation of the fruits applied on the chest is used as a common remedy for asthma (Anon., 1995). The seeds of the plant are also used for curing amoebiasis, dyspepsia and febrile conditions. It is much valued for its antispasmodic and antiseptic properties. According to Krishnamoorthy *et al*. (2000), the aqueous portion left after the separation of essential oil from ajowan is known as omum-water (ajowan water) which is used against flatulence and in gripe water preparation for children. In the Unani system, ajowan is used as an enhancer of body's resistance. About 30 ajowan-based ayurvedic formulations have been reported which are used for therapeutic purposes such as analgesic, digestive, appetiser, micturitive and expectorant (ayurvedaconsultants.com). In India, westerners generally use it for coughs and throat issues, and it is an ingredient in mouthwashes and toothpastes because of its antiseptic properties (Raghavan, 2006). Some traditional and more recent preparations including ajowan and their applications as medicine are summarized in Table 6.4.

Modern research has been carried out to try to validate these traditional medicinal uses. However, many have not yet been fully scientifically analysed. Some recent studies are outlined in the sections below.

6.4.1 Antimicrobial properties

Many studies have been conducted on the antibacterial and antifungal properties of ajowan and their impact on food safety and shelf-life, human health and diseases of plants in particular. The antimicrobial activities of the essential oil distilled from ajowan seed was tested against a range of foodborne microorganisms such as *Lactobacillus acidophilus*, *Bacillus cereus*, *Saccharomyces cerevisiae*, *Mycoderma* sp. and *Aspergillus niger*, and various degrees of inhibition have been reported (Meena and Sethi, 1994). The susceptibility followed the order *B. cereus*, *L. acidophilus*, *S. cerevisiae*, *A. niger* and *Mycoderma* sp. Greater antimicrobial activity was observed in oil of ajowan both at ambient temperature and 37 °C. Ajowan essential oil exhibited a remarkable antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Shigella dysenteriae* and *Vibrio cholera* (Syed *et al*., 1986; Anon., 1995). The inhibitory effect of ajowan (300 ppm) spray on the growth of *A. parasiticus* spores on pear fruits during storage was observed (Maskouki and Mortazavi, 2004). Bansod and Rai (2008) demonstrated that mixed oils (cumin, garlic, tulsi, ajowan, fennel and cardamom) showed maximum activity to prevent mycotic infections by human pathogenic *A. niger* and *A. fumigatus*.

The application of ajowan ethanolic extract (AEE) in food samples resulted in considerable inhibition of the growth of *A. ochraceus* in foods such as maize and poultry feed at 125 mg/g and no detectable amount of ochratoxin A was found at a high moisture level of 20 % even after 7 days (Murthy *et al*., 2009). Hajare *et al*. (2005) reported the aflatoxin-inactivating property of ajowan. An aqueous extract of ajowan seeds caused an 80 % reduction in total aflatoxin content in the test samples. This study emphasizes the potential of ajowan in aflatoxin removal from contaminated food commodities.

Thymol inhibited the ability of *E. coli* and *S. aureus* to adhere to human cells. Thymol also has an antimicrobial mechanism whereby it disrupts the bacterial cell

Table 6.4 Key preparations from ajowan for application as medicine

Preparation name	Ingredients in the preparation	Dose recommended	Functional properties	Reference
1. Chitrakadi vati	Chitrak, 5 salts, trikatu (black pepper, pippali, dry ginger), **ajowan**, chavya, asafoetida	2–4 tablets, twice a day after meals	Stomachic, antacid, carminative	Dhiman (2006)
2. Hingashtak churna	Asafoetida, trikatu, rock salt, cumin, black cumin, **ajowan**	1–4 or 2–8 tablets, 2–3 times a day	Carminative, stimulant, antispasmodic	
3. Trikatu plus	Dry ginger, and black pepper, pippali, coriander, nutmeg, **ajowan**	1–4 g, three times a day before meals	Stimulant, expectorant, carminative	
4. Healthy Alternatives Plus	1. Slippery elm 200 mg 2. Marshmallow 200 mg 3. Ginger root 40 mg 4. Green tea 100 mg 5. DGL licorice 50 mg 6. Quercetin 40 mg **7. Ajowan 2 mg** 8. Gamma oryzanol 130 mg 9. L-Acidophilus 25 mil viable organisms 10. Amylase 3000 DU 11. Glucoamylase 7 AG 12. Lipase 60 LU 13. Cellulase 400 cu 14. Invertase 0.2 IAU 15. Lactase 900 LAC U	5 g per day	Digestion and stomach upset support	Anon. (2012b)
5. Folk Indian remedies with ajowan seed and powder	**Ajowan** seed with little rock salt mixture	A teaspoonful daily after meals	Improves indigestion and irregular diet	Nadkarni (2001)
	Compound powder of **ajowan** seed, rock salt, *sonchal* salt, *yavakshdra*, asafoetida, *myrobalan* equal part, powder the ingredients and mix	A teaspoonful daily after meals for a week	Relieves colic or pain in bowel.	
	Ajowan taken daily with treacle	10–20 grains to be taken with wine	Urticaria	

wall and causes the contents, or cytoplasm, to leak out (Thangam and Dhananjayan, 2003). An Ayurvedic formulation containing dried fruits of amla (*Emblica officinalis*), harda (*Terminalia chebula*), marich (*Piper nigrum*) and ajowan (*Carum copticum*) along with minerals prepared in cow buttermilk exhibited antibacterial activity against all strains tested: *S. aureus*, *S. typhi*, *B. subtilis*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Micrococcus luteus*, *B. cereus*, *E. coli*, *Shigella flexneri* and *Clostridium perfringens* (Bhardwaj *et al*., 2006). Extracts prepared in different solvents exhibited variable activity against *E.coli*, *P. aeruginosa*, *S. typhi* and *S. aureus* (Ahmad *et al*.,1998; Patel *et al*., 2008), supporting their centuries old usage in the treatment of gastrointestinal disorders (Kaur and Arora, 2009).

The oil was stable up to 150 °C and thymol was identified as the fungitoxic chemical (Singh *et al*., 1986). In addition, methanol extracts of *T. ammi* showed significant *in vitro* inhibitory effect on hepatitis C virus (HCV) protease at a concentration 100 μg/ml (Hussein *et al*., 2000). Myers and Thienes (1925) also reported that thymol destroyed a pathogenic yeast-like organism responsible for an occupational dermatosis in persons packing fruit for the canning industry, known as fruit poisoning.

Ajowan seed extract at 1:20 dilution was reported to possess fungicidal action against *Rhizoctonia solani*, a causative agent of sheath blight of rice (Ansari, 1995).

Antifungal activity of ajowan essential oil may be the best alternative to synthetic chemicals for control of post-harvest phytopathogenic fungi (*Alternaria alternata* and *Penicillium digitatum*) in tomato and other vegetables and fruits (Abdolahi *et al*., 2010). Grey mould decay of tomato (caused by *Botrytis cinerea*) was effectively controlled when ajowan essential oil was used as biofumigant (Siripornvisal, 2010). Yang and Clausen (2007) reported the ability of ajowan to inhibit growth of *A. niger*, *Trichoderma viride* and *P. chrysogenum* on southern yellow pine stakes. This finding offers scope for further study into the potential application of natural plant extracts to prevent mould infestation on wood which can be attributed to the presence within them of monoterpene phenols. Thymol has also been used in book binding, to kill spores in books that need rebinding after mould damage (Anon., 2008).

6.4.2 Insecticidal and parasiticidal effects

Ajowan, due to the presence of thymol and other compounds, has been reported to have insecticidal properties against mites, termites, moths and mosquitoes. It is also used to treat hookworms and other parasites. The following authors have reported the insecticidal and parasiticidal effects of ajowan: insecticidal (Chaubey, 2008), molluscicidal (Singh *et al*., 1997, 1999; Singh and Singh, 2000); mosquito repellent (Pandey *et al*., 2009); and nematicidal activities (Park *et al*., 2007).

Thymol is an effective natural way to fight a type of mite called Varroa that attacks honeybees. Thymol is lethal to the mites, but doesn't affect the bees or humans. Studies have found that residues are left in the beeswax from this treatment but levels in the honey itself are much lower. Also, this compound tends to break down very quickly, so the residues do not accumulate over time. The only concern is that the thymol residues change the taste of the honey (Kevan *et al*., 1999).

Seo *et al*., (2009) observed strong insecticidal activity in ajowan against Japanese termites. The fumigant activity of the ajowan essential oil was also assessed against eggs, larvae, pupae and adults of Indian meal moth (*Plodia interpunctella*) in storage (Shojaaddini *et al*., 2008). Hindustan. *et al*. (2010) formulated a poly-herbal environment-friendly mosquito repellent containing ajowan seeds, ground garlic bulb, basil leaves, neem leaves, lemon peel, mentha leaves, chrysanthemum flower petals, cinnamon oil and castor oil, which was effective, cheaper than conventional repellents and non-poisonous. Thymol has been registered for use as a pesticide in the USA since 1964, to be used for repelling domestic animals.

6.4.3 Other functional properties

As mentioned above, ajowan is described as an Ayurvedic medicine for its antispasmodic, stimulant, tonic and carminative properties. Hence, it has been a subject of research by many scientists (Mehta *et al*., 1994; Nagalakshmi *et al*., 2000; Ishikawa *et al*., 2001). Research has suggested that *T. ammi* possesses anti-aggregatory (Srivastava, 1988), antispasmodic (Mahmoud, 1994; Nagalakshmi *et al*., 2000), anthelmintic (Lateef *et al*., 2006), antihyperlipidaemic (Javed *et al*., 2006), antifilarial (Mathew *et al*., 2008) and kidney stone inhibitory (Kaur *et al*., 2009) effects. Ajowan also has significant hypocholesterolaemic effects (Ganachari *et al*., 2010) and anti-hypertensive activity (Taranalli *et al*., 2005). In animals, the ethanolic extract of ajowan fruit at doses of 100 mg/kg and 200 mg/kg showed significant anti-ulcer activity (Ramaswamy *et al*., 2010), probably as a result of antisecretory and cyto-protective action. The methanolic extract of ajowan exhibits good antioxidative activity and is recommended as a potential source of natural antioxidants (Mehta *et al*., 1994). The total alcoholic extract (TAE) and total aqueous extract (TAQ) from ajowan seeds exhibited significant anti-inflammatory potential (Thangam and Dhananjayan, 2003).

6.4.4 Toxicity

The amounts of ajowan normally used in food are non-toxic and do not pose a threat for consumption or to field workers handling the plants. Large amounts of ajowan taken orally can cause headache, nausea and vomiting, however, ajowan essential oil can be mildly to severely toxic if used without dilution. Ajowan essential oil should not be used in aromatherapy, ingested or come in contact with the skin. The essential oil of ajowan, being slightly dermocaustic, should be diluted to 20–25 % in a vegetable oil base, and not used on infants (Urbaniak, 2010). After dilution of ajowan oil to concentration of 10 % it can be used in massage oil (Guba, 2000).

Thymol was listed by the Food and Drug Administration (FDA) as a food additive, on the GRAS (generally recognized as safe) list. However, some consider that thymol is harmful if swallowed, inhaled or absorbed through the skin and thus it is suggested to use safety glasses, adequate ventilation, avoid contact with skin or other tissue and avoid breathing fumes. Chronic paronychia has been reported

to be treated by use of thymol-containing solutions (Fisher, 1989; Lorenzi *et al*., 1995). Thymol-containing toothpastes are also in use for effective mouth hygiene (Beinhauer, 1940; Sainio and Kanerva, 1995). Larsen (1977) observed positive patch test reaction to thymol in a study of 20 patients with perfume dermatitis. However, the contact dermatitis patients patch tested by Reichert-Penetrat *et al*. (2001), did not react to thymol and none of the patients investigated by Santucci *et al*. (1987) in Italy reacted to thymol. Patch testing has normally been performed with thymol 1 % in petroleum as per Botanical Dermatology Database (Anon., 2012a).

6.5 Quality issues

6.5.1 Specification for whole seed and powder

In the first instance, the quality of ajowan seed depends mainly on its external appearance. Qualities such as colour, uniformity of size, shape and texture are perceived visually. Ajowan fruits should be ovoid in shape and greyish brown in colour, measuring 1.7–3.0 mm in length × 1.5–2.4 mm in width and 0.5–1.4 mm in height. In addition, each mericarp should have five ridges. Ajowan powder is yellowish brown. The odour of ajowan is also important. It should be similar to that of thyme (Chopra, 1998). Agmark of India provides three grades of ajowan seed on the basis of size and shape, viz. Grade-I, Grade-II and Grade-III. These are given in Tables 6.5 and 6.6. In addition, Agmark specifies some general requirements for ajowan:

- seed shall be the dried ripe fruits of plant botanically known as *Trachyspermum ammi* (Linn);
- seed shall have the characteristic size, shape, colour, taste and aroma normal to the variety;
- seed shall be free from visible mould or insect, living or dead
- seed shall be from musty odour

For powder, the specification for whole seed should be strictly followed in addition to general seed powder quality specifications.

Ajowan has not received a place in spice lists of the American Spice Trade Association (ASTA) or European Spice Association (ESA), nor is there an ISO specification for ajowan, presumably because the spice has been considered of little importance because of the availability of *Thymus vulgaris* as an alternative source of thymol. The minimum specific quality indices for ajowan seed as per Pruthi (2001) are:

- seed moisture: not more than 12 % by weight
- total ash: not more than 7 % by weight
- ash insoluble in dilute HCl: not more than 1.5 % by weight
- organic extraneous matter: not more than 3 % by weight
- inorganic extraneous matter: not more than 2 % by weight
- volatile oil: not less than 1 %(v/w)
- insect damaged matter: not more than 5 % by weight

Table 6.5 Agmark grade designations and definitions of quality of ajowan (whole) seeds

Grade designation	Definition of Quality					General requirements
	Special requirements					
	Moisture percent by mass (Maximum)	Organic extraneous matter percent by mass (Maximum)	Inorganic extraneous matter percent by mass (Maximum)	Shrivelled, immature, weevil led, damaged and discoloured seeds, percent by mass (Maximum)	Volatile oil ml/100 gms (Minimum)	
1	2	3	4	5	6	7
Grade-I	10.0	1.0	0.25	1.0	3.5	The Ajowan seed shall: (1) be the dried ripe fruits of the plant *Trachyspermum ammi* (L): (2) taste and smell of ajowan seeds shall be fresh and normally associated with the produce. It shall not give rancid taste and musty odour; (3) be free from visible mould, live insects, any harmful foreign matter, insect infestation, rodent contamination and added colouring matter; (4) comply with the restrictions in regard to aflatoxin content, metallic contaminants, insecticide residue, poisonous metals, crop contaminants, and naturally occurring toxic substances as prescribed under the prevention of food adulteration rules 1955.
Grade-II	10.0	1.5	0.5	1.5	2.5	
Grade-III	11.0	2.0	1.0	2.0	1.5	

Explanation: 1) **Organic extraneous matter**: includes leave, stem, chaff, other seeds or any other foreign matter.
2) **Inorganic extraneous matter**: includes – sand, earth, dust, stones or any other inorganic matter.
3) **Shrivelled and immature seeds**: that have not properly developed.
4) **Weevilled seeds**: seeds that are partially or wholly bored or eaten away by weevil or other insects.
5) **Damaged and discoloured seeds**: include seeds that are cut, broken, damaged and discoloured; damaged and discoloration materially affecting the quality.

Source: Anon (1997).

Table 6.6 Agmark grade designations and definitions of quality of ajowan powder

Grade designation	Moisture percent by mass (Maximum)	Special requirements					General requirements
		Total ash on dry weight basis, percent by mass (Maximum)	Acid insoluble ash on dry weight basis, percent by mass (Minimum)	Nonvolatile ether-extract on dry weight basis, percent by mass (Maximum)	Crude fibre on dry weight basis percent by mass (Minimum)	Volatile oil ml/100 gm (Maximum)	
1	2	3	4	5	6	7	8
Grade-I	10.0	7.0	0.5	20.0	14.0	3.5	(1) Ajowan powder shall be the material obtained by grinding the dry, clean, ripe, fruits of the plant *Trachy spermum ammi* (L); (2) The taste and smell of the powder shall be fresh and normally associated with the product. It shall not give rancid taste and musty odour; (3) The produce shall be free from dirt, mould and insect infestation; (4) It shall be free from added colouring matter, preservatives and foreign starch; (5) It shall be free from coarse particles and ground to such a fineness that the whole of it passes through 500 micron sieve; (6) It shall comply with the restrictions in regard to aflatoxin content, metallic contaminants, pesticide residue, poisonous metals, crop contaminants and naturally occurring toxic substances as specified under the prevention of Food Adulteration Rules, 1955.
Grade-II	10.0	8.0	1.0	15.0	18.0	2.0	
Grade-III	11.0	10.0	1.5	10.0	20.0	1.0	

Note: principal rules were published in the Gazette of India, part II, Section 3, sub-Section (i) dated 8-11-97 vide GSR 372 date 26-9-97.
Source: Anon. (1997).

6.5.2 Volatile oil and oleoresin

The variations in chemical composition of ajowan essential oil have been outlined in Section 6.1.2. The volatile oil content of ajowan seed averages 2–5 % and the oil consists primarily of 35–60 % thymol, *p*-cymene (10–16 %), α-terpinene (10–12 %), β-pinene (4–5 %) and dipenene(4–6 %). Ajowan essential oil has a warm, spicy and slightly fatty aroma, which is persistent and has burning sensation. It is a colourless to brownish yellow liquid with the characteristic odour of thymol. The physical properties of ajowan essential oil (from Singhal *et al.*, 1997) are:

- specific gravity: 0.910–0.930
- refractive index: 1.498–1.504
- optical rotation: up to 5°
- solubility: 1–2 or more vols of 80 % alcohol
- phenols: 45–57 %

Ajowan oleoresin represents the overall flavour profile of the spice. It consists of the volatile essential oil and non-volatile resinous fraction comprising taste components. Ajowan oleoresin should be prepared with recommended organic solvents followed by the subsequent removal of the solvent as per specifications of importing countries. Oleoresin of ajowan is a pale green oily liquid with characteristic aroma and sharp taste attributable to the essential oil. The non-volatile fraction of the oleoresin contains essentially the fixed oils of the seed.

6.5.3 Adulteration

Ajowan is available in both whole or ground form and is thus subject to adulteration by addition of exhausted or spent seed (from which oil has been extracted), excess stems, chaff, earth or dust, etc. The oil may also be adulterated with ajowan chaff oil. The essential oil content of ajowan seed is 2–5 % and its thymol content should range from 35–60 %. If chaff oil is added to it then the thymol content could be reduced to below 35 %. The oleoresin may be adulterated by addition of synthetic saturated acids. These adulterants can be detected using GC or thin-layer chromatography (TLC) coupled with HPLC. Adulteration at any level can be detected by checking the product against established specifications for whole seed, powdered seed, volatile oil and oleoresin.

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7

Aniseed

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Abstract: This chapter looks at anise (*Pimpinella anisum* L.), an annual plant cultivated for its seeds (fruits) and oil. The chapter opens with a description of the plant followed by an examination of its chemical structure. Cultivation area, crop husbandry, including soil conditions, fertilization and harvesting, and post-harvest processing, including storage, irradiation and heat processing, are discussed together with the application of anise as a pest control agent. The chapter covers the main uses of aniseed in food processing and the plant's functional properties and medicinal applications, before concluding with a look at toxicity and allergy and some quality and regulatory issues.

Key words: anise, *Pimpinella anisum*, volatile oil, chemical composition, anethole, functional properties, toxicity, quality.

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7.1 Introduction

Source: *Pimpinella anisum* L. (Syn. *Anisum vulgare* Gaertn.; *Anisum officinarum* Mönch; *Apium anisum* (L.) Crantz; *Carum anisum* (L.) Baill.; *Selinum anisum* (L.) E.H.L. Krause; *Pimpinella anisum* (var.) *cultum* Alef; *Sison anisum* Spreng.; *Tragium anisum* Link).[1,2]
Family: *Apiaceae* (= *Umbelliferae*)
Synonyms: Aniseed, Anis seed, Anis, Anise, Sweet cumin
Parts used: Seeds (fruits), oil
Classification:

Division:	Spermatophyta
Subdivision:	Angiospermae
Class:	Magnoliospida
Subclass:	Rosidae
Order:	Apiales
Family:	Apiaceae
Genus:	Pimpinella[3]

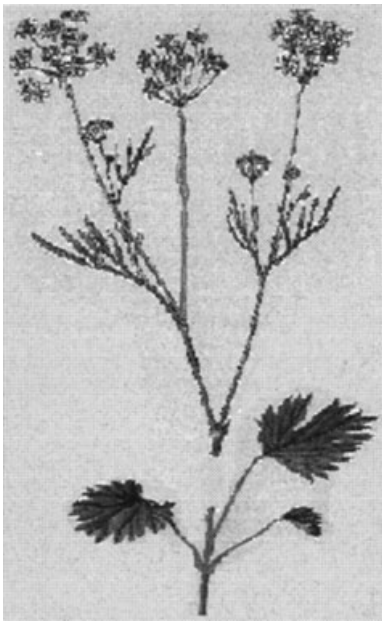

Fig. 7.1 *Pimpinella anisum* L.

Anise is an annual plant that reaches an average height of 30–50 cm. The plant is completely covered with fine hairs. The root is thin and spindle-shaped, the stem up, stalk- round, grooved and branched upward (see Fig. 7.1). In midsummer the thin stems are topped with umbrella-shaped clusters of tiny white flowers, which are heavy enough to make the stems flop. They turn into seedlike fruits. Anise is a cross-pollinating species and is genetically heterogeneous. The fruit is an ovoid-pear-shaped schizokarp somewhat compressed at the side. The two-part fruits separate heavily. The carpophore is almost two-piece up to the base. Commercially available aniseed usually contains the whole fruits and occasionally parts of the fruitstalk (see Fig. 7.2). The fruits with the style-foot are 3–5 mm long, 1.5–2.5 mm wide and 2–4 mm thick. Vittae (oil ducts) are almost always present embedded in the fruit wall on the dorsal surface, sometimes in or directly beneath the ridges. The fruits are downy. Their colour is greyish-green to greyish-brown.[4,5]

7.1.1 Chemical structure

Anise contains:

- 1–4 % volatile oil;
- coumarins: bergapten, umbelliprenine, umbelliferone, scopoletin;
- ca. 8–16 % lipids, including fatty acids: 50–70 % petroselinic acid (C18:1), 22–28 % oleic acid (C18:1), 5–9 % linoleic acid (C18:2) and 5–10 % saturated fatty acids mostly palmitic acid (C16:0);
- β-amyrin, and stigmasterol and its salts (palmitate and stearate);
- flavonoid glycosides: quercetin-3-glucuronide, rutin, luteolin-7-glucoside, isoorientin, isovitexin, apigenin-7-glucoside (apigetrin) etc;

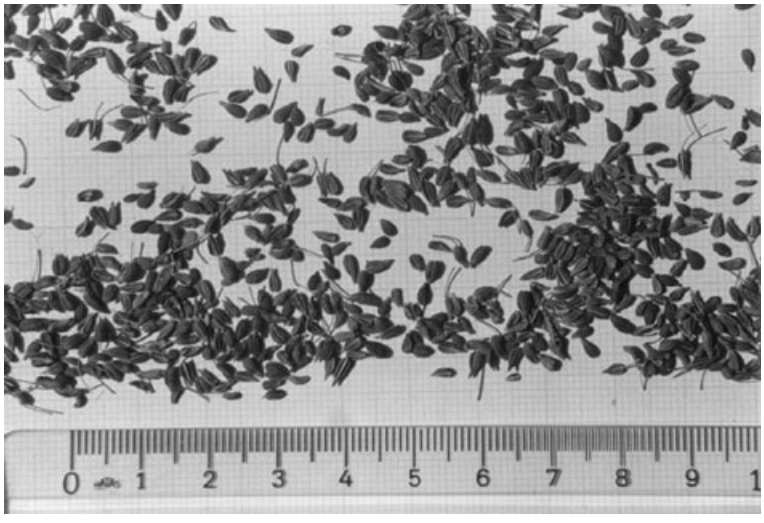

Fig. 7.2 Dried aniseed.

- myristicin;
- ca. 18 % protein;
- ca. 50 % carbohydrate and others.

Fatty acids can be obtained by extraction, as in the case of caraway, in the remainders of oil extraction via steam distillation. Lauric acid, which is most important to oleochemistry, is obtained from petroselinic acid which is found in high quantities (50–70 %) in anise. Fatty oil shows excellent future potential. Successful production of anise seed for economical oil production would probably occur if the seed yields could be improved significantly, and high content of oil and essential oils and large quantity of petroselinic acids could be reached.[6,7]

The major constituent in volatile oil of aniseed is *trans* (E)-anethole (75–90 %[7]; 80–90 %[8]; 86 %[9]; 96–98 %[10]; 86–89 %[11]; 89–92 %[12]). Methylchavicol (estragole) (4.95 %[9]; 1.7–3.7 %[10]; 3.6–5.5 %[11]; 1.0–2.4 %[12]), anise ketone (para-methoxyphenylacetone) (0.78 %[9]; 0.5–0.9 %[11]) and β-caryophyllene are also present, but in lesser relative amounts. Other components in minor concentrations include anisaldehyde, anisic acid (oxidation products of anethole), linalool, limonene, α-pinene, acetaldehyde, *p*-cresol, creosol, hydroquinine, β-farnasene, γ-himachalene and *ar*-curcumene.[7]

7.2 Production and cultivation

7.2.1 Cultivation

Anise is cultivated in Turkey, Egypt, Spain, Russia, Italy, India, Greece, Northern Africa, Argentina, Malta, Romania and Syria. Anise is primarily exported from Turkey, and also from Egypt and Spain in particular. From an industrial standpoint,

the quality differences between anise seed from different origins are not significant and therefore specifications need not limit the spice to a specific country of origin.[13,14,15]

P. anisum requires a warm and long frost-free growing season of 120 days. The plant needs a hot summer to thrive and for seeds to ripen. The reported life zone for anise production is 8 to 23 °C with 0.4 to 1.7 metres of precipitation on a soil pH of 6.3 to 7.3. Anise develops best in deep, rich, well-drained, sandy and calcerous soils. Cold, loamy and moist soils are unsuitable for the cultivation of anise. During germination anise tolerates salinity up to 160 μm NaCl. The thousand seeds weight of the part-fruits amounts to 1.5 to 3.0 g and should have a minimum purity of 90 % and a minimum germination of 70 %.

Ripe-fruits seeds germinate relatively quickly. The germination time is 14 days. Only seeds from the previous year's harvest germinate well. Long storage quickly reduces germination vigour: seeds stored for five years will no longer germinate. Planting begins when the soil in the beds is warmed. Optimum soil temperature for germination is 18–21 °C. It is essential to prepare good seedbeds and to create a good contact between the planted seed and the soil because the seeds are small and have low germination percentage (70 %). The planting is carried out in spring or autumn depending on the areas it is cultivated. The seeds with a seeding rate of 20–25 kg/ha are sown in rows 20–30 cm apart, at a depth of 1 cm. The plant develops slowly after germination and for the following few weeks it is necessary to control weeds closely. It is recommended to apply fertilizers at a rate of 80–100 kg K_2O and 50–75 kg P_2O_5 per hectare. With nitrogen, it is important to be careful, since excessive nitrogen fertilization results in luxuriant vegetative growth with reduced yields, and increased vulnerability to lodging. 50–100 kg/ ha N is normally enough. The small white flowers bloom in midsummer, and seed maturity usually occurs one month after pollination, when the oil content in the dried fruits is about 2.5 %. Anise seeds are harvested between from the end of July to the beginning of September, depending on the cultivation areas. Yields of seed up to 500–1000 kg/ha have been achieved. *P. anisum* is recommended in companion planting to repel aphids and cabbage worms. The flowers attract parasitic wasps.[5,6,16,17,18] Constituents in plant volatile oils are known to be useful in pest control. Various authors have reported that vapours of essential oils extracted from anise were found to be toxic to two greenhouse pests, viz. the carmine spider mite, *Tetranychus cinnabarinus* and cotton aphid, *Aphis gossypii* Glov.[19] Sarac and Tunc[20] indicated that the essential oil of anise had a high residual toxicity to adults of *Tribolium confusum*, and was the most repellent to *Sitophilus oryzae* adults in food preference tests.

7.2.2 The production of anise oil

The world production of anise oil amounts to 40–50 tons per annum. The most significant importing countries of anise oil are the USA and France. Russia, Spain and Poland are among the largest producers of anise oil. There is no distillation of anise oil and no production of anethole in many of the countries which cultivate the crop.[21,22,23]

Anise oil is steam distilled from the crushed seeds of the plant *Pimpinella anisum*. The process of steam distillation is the most widely accepted process for the

production of essential oils on a large scale. A still is charged with plant material to be processed. Steam is introduced at the base of the still and the crushed anise seeds' volatile elements evaporate with the steam. A condensation process turns this vapour-mix into a liquid form of water and essential oil. The essential oil floats on top of the water and is separated off. The essential oil of aniseed is a colourless to faintly yellow oil which solidifies upon cooling to about 15–19 °C due to the crystallization of anethole.

Oleoresin anise is a yellowish-green to orange-brown fluid oleoresin. Volatile oil content of oleoresin anise is 15–18 %. The presence of a large quantity of fixed oil in this product limits its shelf-life and the addition of a permitted antioxidant is advised.[24] Anise and anise oil are widely used as flavouring ingredients in all major categories of foods, including alcoholic and non-alcoholic beverages, frozen dairy desserts, sweets, baked goods, gelatines and puddings, and meat and meat products. The highest average maximum use levels for anise oil are about 0.06 % (570 ppm) in alcoholic beverages and 0.07 % (681 ppm) in sweets.[7] Suggested use rate of oleoresin anise is 7.5 to 9 %.[24] In Turkey the different types of aniseed spirits are distinguished by their anise seed content: Yeni raki (80 g/L aniseed), Kulup raki (100 g/L aniseed) and Altinbas raki (120 g/L aniseed).[25]

7.2.3 Stability during storage, irradiation and heat processing

Anise has to be stored away from daylight and kept in a dry place in cool conditions (DAB 10 Eur, ÖAB 90, Helv VII). The average loss of the content of the volatile oil has been calculated at 1 % of the original content per month. The content of *trans*-anethole decreases from 89 % to 73 % during a storage of six weeks with the influence of sunlight, while the content of *cis*-anethole increases from 0.8 to 4.5 % and the content of anisaldehyde from 0.8 to 7.0 %. At the same time additional decomposition products are formed. Investigations on airsealed, grinded aniseed clearly show changes of odour within the first 12 months if the temperature of storage exceeds 5 °C. Because of the sensitivity to light and oxidation it is recommended that the volatile oil of anise is stored in well filled and well closed containers (glass or tin, but not plastic) protected against daylight (DAB 10, BP 88, PFX, ÖAB 90, HELV VII). Moreover, PFX demands a storage temperature below 10 °C and BP 88 a storage temperature below 25 °C. With the influence of daylight, *trans*-anethole is transformed into its more toxic isomer *cis*-anethole.[26]

It is reported that there is an increase in anise ketone, anisaldehyde and anisic acid[9] and decrease in *trans*-anethole[27] of anise oil during long-term storage. Moisture content of the seeds or humidity of the storage atmosphere is the most important parameter to be considered in preserving the desired properties of anise. At high moisture levels deteriorative reactions and off-flavours are inevitable in addition to the increased rate of loss of volatile oil by diffusion. Oxidation reactions are responsible for the loss of oil during storage by converting the components mostly to acids and aldehydes. Also, daylight catalyzes oxidative reactions and increases the rate of deterioration. Extreme variations in the moisture content of the storage atmosphere favour oil evaporation and particularly oxidation.[28] The dimers of anethole (dianethole) and anisaldehyde (dianisoin) are mentioned repeatedly in the literature[14,29,30] and are supposedly responsible for the oestrogenic activity in old drugs and in

stored oils under exposure to sunlight, and air could not be found after thorough investigation.[31]

One interesting item to note in this spice is that when the ground product is irradiated, a slightly putrid off odour and flavour results. This contradicts most research that irradiation does not change the chemical properties of a spice when treated. It is possible that it does, in limited cases, change the flavour balance of essential oils.[15] Similarly numerous authors report that volatile oil of anise, extracted after irradiation with 1.5 and 10 kGy γ-rays, contained the most oxygenated compounds, and irradiation caused a general increase in oxygenated compounds at 1 kGy.[32] Farag-Zaied *et al*.[33] indicated that γ-irradiation was effective in decontamination, especially at 10 kGy, but caused losses in the major components of flavour such as anethole, methylchavicol and anisealdehyde in anise.

Thermal treatment at 70 °C for 15 minutes reduced the microbial count and pathogenic microbes, improving the anethole in anise, and washing the spice removed some of the microbes but improved markedly the anise flavour. Thermal and washing treatments may be of value as simple natural techniques to produce spices with a good flavour and with an acceptable level of contamination.

Bendini *et al*.[34] detected linear, unsaturated hydrocarbons in aniseed samples treated with γ-rays or microwaves. The microwave treatment of aniseeds did not modify the hydrocarbon profile with respect to the untreated samples. In contrast, γ-irradiation gave rise to a series of unsaturated hydrocarbons of which C16:2, C16:1, C17:2 and C17:1 were determined. In most cases, when these products were quantified, their amounts increased with the dose of radiation. C17:1 could be considered as the marker of the γ-irradiation treatment. The essential oil of anise extracted from γ-irradiated and microwaved fruits exhibit antioxidant properties. γ-irradiation and microwave treatments have no effect on the antioxidant properties of essential oil. Essential oil extracted from the γ-irradiated fruits are more effective as antioxidants than those produced from microwaved fruits.[35,36]

7.3 Main uses in food processing

Aniseed's long popularity throughout so many lands stems from its many uses: flavourant, culinary, household, cosmetic and medicinal. While the entire plant is fragrant, it is the fruit of anise, commercially called aniseed, that has been highly valued since antiquity. Aniseed is one of the oldest spices used widely for flavouring curries, breads, soups, baked goods such as German springerle, and Italian biscotti, sweets (e.g. licorice candies, especially aniseed-balls), dried figs, desserts, cream cheese, pickles, coleslaw, egg dishes, non-alcoholic beverage. It is a favourite flavouring for alcoholic drinks in the Mediterranean region, such as French Pastis, Pernod, Anisette, and Ricard, Greek Ouzo, Turkish Raki and Arabian Arak, and also South American Aguardiente, Russian Allasch, Puerto Rican Tres Castillos. Aniseed oil is a component in German Boonekamp, Benediktener, Goldwasser and Spanish Pacharan and Ojen. Anisette combines anise, coriander and fennel seeds in sweet vodka. Anise and anise oils are used in Italian sausage, pepperoni, pizza topping and other processed meat items. Anise is an essential component of Italian anise cake and cookies. All parts of the plant can be used in the kitchen. The flowers and

the leaves can be added to fruit salads. Freshly-chopped leaves also enhance dips, cheese spreads, vegetables, or green salads. Mixed into stews and soups, the stem and roots of anise give just a hint of licorice.[6,15,16,18,26]

The essential oil is valuable in perfumery, in dentrifices as an antiseptic, toothpaste, mouthwashes, soaps, detergents, lotions and skin creams, in tobacco manufacture, with maximum use levels of 0.25 % oil in perfumes. It is also used to mask undesirable odours in drug and cosmetic products. The oil is used for production of anethole and sometimes as sensitizer for bleaching colours in photography.[7,16,23]

7.4 Functional properties

The pharmaceutical data mentioned in the literature mainly refer to anise oil and anethole. Anethole is structurally related to the catecholamines adrenaline, noradrenaline and dopamine.[37] Anise oil and anethole have a number of functional properties:

- antibacterial
- antifungal
- antioxidant
- stimulant, carminative and expectorant.

The antibacterial activities of the essential oil distilled from *Pimpinella anisum* against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli* and *Corynebacterium ovis* were evaluated. Against *S. pyogenes*, aniseed oil was equally effective in the pure state and at dilution up to 1:1000. Against *C. ovis*, aniseed oil was equally effective at dilutions up to 1:100 and at higher dilutions.[38] The inhibitory properties of anise essential oil, alone or in combination with either benzoic acid or methyl-paraben, against *Listeria monocytogenes* and *Salmonella enteriditis* were investigated. *S. enteriditis* was particularly sensitive to inhibition by combinations of anise essential oil with methyl- paraben. *L. monocytogenes* was less sensitive but exhibited significant reductions in growth in response to combinations of essential oil with methyl-paraben.[39]

Kubo[40] reported that anethole, a naturally occurring phenylpropanoid extracted from aniseed, exhibited a broad antimicrobial spectrum and the antifungal activity (against *Candida albicans*) of two sesquiterpene dialdehydes, polygodial and warburganal (extracted from *Polygonum hydropiper*), was increased 32 fold when combined with low concentrations of anethole. In a study of the volatile oil from aniseed, significant antifungal activity against members of the genera *Alternaria, Aspergillus, Cladosporium, Fusarium* and *Penicillium* was recorded at concentrations of 500 ppm, the active constituent having been identified as anethole.[41] Anethole also inhibits growth of mycotoxin producing *Aspergillus* species in culture. Anethole has been reported to be mutagenic in Ames *Salmonella* reversion assay. Anethole, anisaldehyde and myristicin (in aniseed), along with d-carvone (present in *P. anisum* plant), have been found to have mild insecticidal properties.[7] Pharmacological studies were carried out in rats and mice, and anise oil showed significant antipyretic activities

in rats.[42] Curtis[43] reports that synthetic versions of compounds in herbs and spices such as *trans*-anethole have inhibitory and lethal activity against food spoilage yeast *Debaromyces hansenii*.

There is some evidence of anise oil's effectiveness as an antioxidant. Gurdip *et al*.[44] investigated the antioxidant activity of essential oil from spice materials on stored sunflower oil and found that anise oil possessed excellent antioxidant effects, better than those of synthetic antioxidant, butylated hydroxytoluene.

Anise oil is reported to be carminative and expectorant. The reputed lactogogic action of anise has been attributed to anethole, which exerts a competitive antagonism at dopamine receptor sites (dopamine inhibits prolactin secretion), and to the action of polymerized anethole, which is structurally related to the oestrogenic compounds stilbene and stilboestrol. Anethole is also structurally related to the hallucinogenic compound myristicin. Bergapten, in combination with ultraviolet light, has been used in the treatment of psoriasis.[37] Anise oil is used as carminative, stimulant, mild spasmolytic, weak antibacterial, and expectorant in cough mixtures and lozenges, among other preparations. It can be used internally for dyspeptic complaints and externally as an inhalant for congestion of the respiratory tract. The whole, crushed, or ground crude drug can be used for infusion, and other galenical preparations; e.g. several instant teas as powders containing aqueous extracts of aniseed, or as tea paste, some preparations with microencapsulated anise oil. Anise seed and anise oil are subjects of German official monographs; 3.0 g of seed or 0.3 g of essential oil (mean daily dose) allowed as a bronchial expectorant for upper respiratory tract congestion and as gastrointestinal spasmolytic.[7,31]

Anise may have other potential health benefits. The effect of the beverage extracts anise on absorption of iron was tested in tied-off intestinal segments of rats. Results showed that the beverage of anise promoted Fe absorption.[45] Preparations containing 5–10 % essential oil are used externally.[7,31] The oil added to an ointment helps in cases of aches of muscles and neuralgia.[6] Olfactory masking with aniseed oil decreased aggression and prevented the decrease in milk production in dairy cattles.[46] It is reported that anethole stimulates hepatic regeneration in rats, and also shows spasmolytic activity. Chemically it is used as a precursor in the manufacture of anisaldehyde. Occurring in the essential oil of *P. anisum*, *p*-anisaldehyde has fungistatic activity; *p*- cresol is a disinfectant agent and cresols are used in veterinary practice as local antiseptics, parasiticides and disinfectants; hydroquinone has antibacterial, antitumour, antimitotic and hypertensive activities. It is cytotoxic to rat hepatoma cells. Uses include a depigmentor, an antioxidant and a photographic reducer and developer.[47]

In traditional medicine anise is reportedly used as aromatic carminative, stimulant and expectorant; also as oestrogenic agents to increase milk secretion, promote menstruation, facilitate birth, increase libido, and alleviate symptoms of male climacteric.[7] Aniseed is traditionally regarded as an aphrodisiac. Externally, the oil may be used as an ointment base for the treatment of scabies. The oil by itself will help in the control of lice and as a chest rub for bronchial complaints. The oil is often mixed with oil of *Sassafras albidum* for skin parasites and with that of *Eucalyptus globulus* as a chest rub.[18]

7.4.1 Toxicity and allergy

Aniseed contains anethole and estragole which are structurally related to safrole, a known hepatotoxin and carcinogen. Although both anethole and estragole have been shown to cause hepatotoxicity in rodents, aniseed is not thought to represent a risk to human health when it is consumed in amounts normally encountered in foods.[37] Anise and oil of anise are generally regarded as safe for human consumption.

The toxicity and cancerogenity of anethole are controversial. Anethole has two isomers (*trans* and *cis*), the *cis* (Z) isomer being 15–38 times more toxic to animals than the *trans* (E) isomer.[7] The major component of the natural volatile oil of anise (80–96 %) is *trans*-anethole, which is most likely non-cancerogenic. *Trans*-anethole will be accompanied by *cis*-anethole (maximum 0.3–0.4 %), which is not caused by distillation, but exists naturally in anise seeds. In case of storage without protection of daylight the forming of *cis*-anethole is possible. Synthetic *trans*-anethole contains higher quantities of toxic *cis*-anethole compared to natural *trans*-anethole and therefore it is not used in food processing. Cases of intoxication with the volatile oil of anise are not known.[26] Current United States Pharmacopeia (USP) and Food Chemical Codex (FCC) specifications for anethole do not require differentiation between the isomers.[7]

Aniseed may cause an allergic reaction. It is recommended that the use of aniseed oil should be avoided in dermatitis, or any inflammatory or allergic skin conditions.[37] Patients with an allergy to pollen are often suffering from 'spice-allergy' like celery, carrot, etc. Skin-prick tests with anise extracts in several cases result in positive allergic reactions.[26] Freeman[48] reports an atopic man who experienced cutaneous allergy and periorbital edema after preparing and eating fresh dill. The patient reported here demonstrated reactive skin tests and positive radio allergo sorbent test (RAST) to other members of the *Umbelliferae* including aniseed in addition to dill. Similarly Fraj *et al*.[49] describe a case of occupational asthma induced from aniseed dust sensitization. A skin- prick test carried out with 13 spices showed positive reactions only to aniseed extract.

When consumed in sufficient quantities, anise oil may induce nausea, vomiting, seizures and pulmonary edema. Contact of the concentrated oil with skin can cause irritations.[16] Anethole has been reported to be the cause of dermatitis (erythema, scaling and vesiculation) in some people.[7] Compared with star anise however, the sensitization effect of anise oil is lower.[26]

7.5 Quality and regulatory issues

The recommended moisture limits from the American Spice Trade Association (ASTA) is 10 % in whole and in ground anise. Ash and acid insoluble ash should be no greater than 6.0 % and 1.0 %, respectively.[15] According to BHP 1983:[31] foreign organic matter, not more than 2 %; other fruits and seeds, not more than 2 %; total ash, not more than 10 %; acid-insoluble ash, not more than 2.5 %. The minimum content of volatile oil of anise is 2 % (BHP 1983; Ph. Eur, 2).[31] Anise oil is a colourless to pale yellow, strongly refractive liquid, having the characteristic odour and taste of anise. It should contain 84–93 % *trans*-anethole (major component and

typical carrier of odour and flavour) and 0.5–6.0 % methylchavicol (=estragole, which smells like anise but does not have its sweet taste) (HPLC profile Ph. Eur.).[14]

Anise oil is frequently adulterated with the lower priced star anise oil, which, according to several Pharmacopoeiae, is also considered 'anise oil'. Star anise (*Illicium verum* Hook f.) is the dried fruit of a tall evergreen tree, which is native to southern China and northern Vietnam. The profile of star anise oil is similar to that the *Pimpinella* oil and the two are equally acceptable and interchangeable in use. But, strictly from the flavouring viewpoint, anise oil (*P. anisum*) is undoubtedly superior to star anise oil (*I. verum),* the latter having a somewhat harsher odour. Pharmacopoeiae therefore demand the specification of the plant of origin out of which the anise oil was extracted (whether from aniseed, *P. anisum* or star anise, *I. verum*, which can be determined). This is obviously for the sake of consumer protection, since star anise oil is substantially cheaper than the oil extracted from anise. Characteristic of genuine aniseed oil is the presence of up to 5 % of the 2-methylbutyryl ester of 4-methoxy-2-(1-propenyl)-phenol (= pseudoisoeugenyl 2-methylbutyrate). On the other hand, fruit oil of *I. verum* is characterized by the presence of Foeniculin. The provenance of an oil can be determined by detection of each of these two substances. Star anise oil further differs from *P. anisum* oil by its content of several terpene hydrocarbons (THC) as well as its content of 1,4-cineol. This may explain why star anise oil does not reach the flavour quality of aniseed oil.[14,26,31,50] Other adulterants are synthetic anethole and fennel oil. The latter can be detected by a change in the optical rotation. Much cheaper synthetic anetholes are also available but some carry a risk of toxicity, which precludes their use in food and drinks.[50,51] A further criterion of quality is its solidification point which sinks with decreasing content of anethol. The solidification point of officinal anise oil lies between +15 °C and +19 °C (Ph. Eur.). Pure anethole becomes fluid above +23 °C and solidifies at +21 °C. All Pharmacopoeiae recommend checking physical properties like specific gravity, refractive index, optical rotation and temperature of solidification in order to get hints about the purity of anise oil. Table 7.1 lists physical properties according to different sources. The specifications of the limits as mentioned in the Pharmacopoeiae vary slightly. Anise oil has to be dissolvable in 1.5 to 3.0 times its volume of EtOH 90 % (DAB 10, NFXVII, ÖAB 90, Helv VII). This test is useful to exclude adulterations by fats, oils and mineral oils.[26]

Italian anis may be confused (in former times more often, nowadays very rarely) occasionally with poisonous fruits of *Conicum maculatum* L. (hemlock). Morphologically, hemlock fruit can be recognized by the undulate (especially in the upper part of the fruit) ridges. Crushed fruits that are moistened with a potassium hydroxide solution should not smell like mouse urine (coniine). Adulteration with parsley or dill fruits can be detected readily by their smaller size and missing hairs. Nearly all anise fruits currently traded are impurified with up to 1 % coriander fruits.[14,31] Adulteration of powdered aniseed or anise oil can be rapidly and reliably determined by direct mass spectroscopy via the 'marker' compound pseudoisoeugenyl 2-methyl-butyrate which only occurs in genuine 'anise oil'; as little as 0.2–1.4 % can be detected in the presence of 94 % anethole, without the necessity of its having to be separated or the sample specially prepared.[31]

In the USA, aniseed is listed as GRAS (Generally Regarded As Safe; §182.10 and §182.20). Aniseed is used extensively as a spice and is listed by the Council of

Table 7.1 Physical properties of anise volatile oil according to different sources

Properties	Turkish anise[11] volatile oil	Food chemical[52] codex specification	Pharmacopoeiae[26]	ISO[53]
Specific gravity (20 °C)	0,990	0,978–0,988	0,979–0,994 (DAB10)	0.980–0990
Refractive index (20 °C)	1,558	1,553–1,560	1,553–1,561 (DAB10)	1.552–1.559
Solidification point	19 °C	>15 °C	>15 °C (BP 88)	+15 °C to +19.5
Optical rotation (20 °C)	–	–2 ° to +1 °	–2 ° to +1 ° (BP 88)	–2 ° to +5 °

Europe as a natural source of food flavouring (category N2). Anise seed and anise oil are subject to different pharmacopoeial Monographs: Aust., Br., Cz., Egypt., Eur., Fr., Ger., Gr., Hung., It., Neth., Rom., Rus., and Swiss.[37] Aniseed is covered by the following: Anise DAB 10 (Eur), ÖAB90, Helv VII, Pimpinella BHP83, Aniseed Mar29. Anise oil is covered by: DAB 10, BP88, NFXVI, Essentia anisi Hisp IX, Huile essentielle d'anis PFX, Anisi aetheroleum ÖAB90, Helv VII, Anise Oil BPC79, Mar 29 (All pharmacopoeias mentioned under Monographs except Hisp IX additionally allow *Illicium verum* Hook as plant of origin). Homeopathic guidance includes: *Pimpinella anisum*, ethanol. Decoctum hom. HAB1, Anisum hom. HAB 34, Anisum hom. HPUS88.[26]

7.6 References

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8

Asafoetida

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Abstract: This chapter reviews the origin, description and patterns of production and trade of the spice asafoetida (*Ferula assafoetida* L.). Different forms and varieties of asafoetida and their division into Hing and Hingra are presented along with the exudates – galbanum, sagapenum and sumbul – produced and marketed. The chemical composition of asafoetida – volatile oil, gum and resin – is discussed. Methods of cultivation, processing and storage are described, together with the main products and their culinary and medicinal applications. Issues of quality and adulteration are also covered.

Key words: asafoetida, Hing, Hingra, *Ferula foetida*, *Ferula assafoetida*, silphium, galbanum, sagapenum, sumbul, volatile oil, tincture of gum resin and compounded asafoetida.

8.1 Introduction

Asafoetida or asafetida is the dried latex or oleogum resin exuded from the taproots of perennial herbs belonging to a few species of the genus *Ferula* of the family Apiaceae (Umbelliferacaea) (Pruthi, 1976). A native of Iran, *Ferula assafoetida* L. is the most important among them (Andi *et al*., 1997). The trade name, asafoetida, is based on the scientific name of one of the important species, *Ferula assafoetida* (Duke, 2003). It is derived from the Persian word for resin, 'asa', and the Latin word, *foetida*, which refers to its distinct sulphurous aroma – stronger than that of garlic (Anon., 2012a). Asafoetida is known by different names such as devil's dung, stinking gum, asant and hing.

Silphium, the predecessor of asafoetida (also known as silphion or lasar), was an ancient spice from the region of Cyrene (now in modern Libya). It was in great demand for cooking and medicine in ancient Greece and Rome, but true silphium became extinct by the end of the first century AD. By the time of the invasion of Asia by Alexander the Great in 334 BC, asafoetida had emerged as a substitute for silphium. The ancient Sanskrit text 'Kashyapa Samhita' (circa 200 BC) mentions the import of asafoetida from Afghanistan. It became very popular amongst the ancient Romans as a flavouring agent, and continued to be an essential ingredient in Indian cooking as well as in the preparation of traditional medicines. (Ramachandran, 2004).

There are now about 170 known species of the genus *Ferula* found mainly in three geographical areas, Central Asia, Europe and North Africa (Kurzyna-Młynik *et al*., 2008). Central Asia is the main source of asafoetida with Afghanistan and Iran as the major producers. The commercially important species of *Ferula* have been reviewed by Raghavan *et al.* (1974). They are summarized in Table 8.1. While true asafoetida is obtained from *F. assafoetida*, similar products are collected from *F. narthex* as well as a few other species. *F. narthex* is found in the dry valleys of the Ladakh region in Kashmir in India at an altitude of 4000 m. The oleogum resin of *F. narthex* is used as a substitute for asafoetida in India (Andi *et al*., 1997).

Table 8.1 Commercial species of *Ferula*

Species	Where found	Remarks
F. alliacea Boiss.	Iran	Gum resin is used as an intestinal antiseptic and carminative, and also for hysteria and epilepsy. Used as asafoetida.
F. assafoetida Linn.	Iran and Afghanistan	Gum resin is amorphous and reddish brown. Yields asafoetida for flavouring curries and sauces, and also for medicinal uses as expectorant, laxative and antispasmodic.
F. communis Linn.	N. Africa	Source of a gum resin known as 'Ammoniac of Morocco'. Used for medicinal purpose in Europe.
F. foetida (Bunge) Reget	S. Turkey, Iran and Afghanistan	Used as asafoetida.
F. ferulago Linn.	S. European countries	Used for medicinal purposes in Europe.
F. galbaniflua Boiss. and Bulise.	N.W. Africa, Iran and Turkey	Source of galbanum.
F. hermonis Boiss.	Lebanon	Used for medicinal purposes in Lebanon and neighbouring countries and as a sex stimulant.
F. jaeschkeana Vatke.	Kashmir and Turkey	Gum resin traditionally used as contraceptive.
F. marmarica Asch. and Taub.	N. Africa	Source of gum resin 'amariac of Cyrenaica'.
F. narthex Boiss.	West Tibet and Ladakh region of Kashmir	Used as asafoetida because of similarity in flavour.
F. orientalis Linn.	Iran and Afghanistan	Used for medicinal purposes in the Middle East.
F. persica Willd.	Iran	A source of sagapenum and sold as 'tears' or 'masses'.
F. rubricaulis Boiss.	Iran	Used for medicinal purposes in Iran, probably a source of galbanum.
F. sumbul f.	Mountains south east of Samarkand	Root has musk-like aroma. It is used as a stimulant and as a tonic for nervous disorders.
F. szowilziana D.C.	Central Asia	Source of sagapen gum resin and has the scent of galbanum.
F. *tingitana* Linn. (Syn. *F. sancta* Boiss.)	Syria and N. Africa	Sources of North African or Moroccan ammoniac.

Source: Raghavan *et al.* (1974).

8.1.1 Patterns of trade

Much of the production in Afghanistan, the main producer of asafoetida, is collected from Herat province near the Iranian border. In Iran, it is collected from adjoining areas like Mashhad and Kerman. There is no reliable information on the extent of area under cultivation or the amount of asafoetida produced. This is because production and trade are not centrally organized in either country. However, there is some information to indicate that annual production in 1990s in Afghanistan was about 500–600 Mt (Rosetti, 2009, 2010). Present production in Afghanistan is believed to be higher than in Iran, in spite of the ongoing upheaval there.

In a good year, with the required amount of rain and snow, total production in Iran may be 110 Mt, with 40 Mt being of the bitter variety and the rest the sweet variety. Whilst the bitter variety is produced in south and east Iran, the sweet variety comes from central and northeast Iran. During 2010, production has been reported to be only 30 Mt bitter and 50 Mt sweet varieties (Gashgayi, 2010). While the extent of area under asafoetida cultivation may not change significantly in different years, production can go up and down in both countries as demand and price determine the amount of gum resin extracted.

Information relating to world trade of asafoetida is also scarce as records are not systematically collected by producing or importing countries. India is the major buyer of asafoetida, where it is used in the preparation of some common vegetarian dishes, particularly in South India, and in the formulation of traditional medicines all over the country. Although import is mainly from Afghanistan and Iran, being the major producing countries, in certain years, very small quantities are imported from Uzbekistan, Kazakistan, Tajikistan, Kyrghyztan and Pakistan. Generally, the imported quantity from Afghanistan is almost three times that of Iran. During 2007–8 when total import was at the peak level of 927.2 Mt, Afghanistan contributed almost six times the amount imported from Iran. Imports of asafoetida into India from Afghanistan and Iran during 2005–10 are given in Table 8.2.

The import of asafoetida has been erratic. There was a steady increase in imports from 506.5 Mt in 2005–6 to 927.2 Mt in 2007–8, followed by an equivalent decline in the next two years to 651.7 Mt in 2008–9 and to 534.7 in 2009–10. However, the average import volume has been 661.2 Mt which is 27.7 Mt more than the average during the preceding five years. The average value of imported asafoetida per kg also increased considerably. The price was US $42.18 per kg in 2009–10. During the

Table 8.2 Import of asafoetida into India during 2005–10

Year	Quantity (Mt)	Value ('000US$)
2005–6	506.5	7792.6
2006–7	686.0	16522.5
2007–8	927.2	27640.5
2008–9	651.7	23968.7
2009–10(P)	534.4	22538.7

P = provisional.
Source: Spice Board, Cochin, Kerala, India.

Table 8.3 Export of asafoetida from India during 2005–10

Year	Quantity (Mt)	Value ('000 US$)
2005–6	723.2	2003.95
2006–7	491.1	2035.13
2007–8	375.6	2439.87
2008–9	311.9	2973.50
2009–10 (P)	349.2	3332.02

P = provisional.
Source: Spice Board, Cochin, Kerala, India.

last nine years, the price increase has been astonishing at 943.53 % more than the price in 2000–1.

A major part of the imported asafoetida is processed in India and a portion is re-exported. Unprocessed material is also re-exported, depending on the profit margin, but separate export figures are not available for unprocessed and processed asafoetida. Processed asafoetida is mainly compounded asafoetida. The quantity and value of asafoetida, including compounded asafoetida, exported from India during the 5-year period 2005–10, are shown in Table 8.3.

Exports have also not followed a steady pattern in terms of quantity. The quantity exported during 2005–6 was the highest at 723.2 Mt. Thereafter, exports declined steadily and reached 349.3 Mt in 2009–10. Compared to the average import price of US$42.18 per kg in 2009–10, export prices have been only US$9.54 per kg as compounding has made the product much cheaper.

India exports asafoetida to about 25 countries of which the UAE is the largest importer. The quantity imported from India reached a record high at 235.5 Mt during 2005–6 and thereafter declined to 54.5 Mt in 2008–9, although it marginally improved to 64.7 Mt in the next year. Other destinations are the USA, the UK, Singapore, Thailand and Malaysia, with quantities varying from 9.2 Mt to 106.0 Mt.

8.1.2 Description

Asafoetida plant grows to about 2 m high and has a perennial fusiform root that is either simple like a parsnip or has one or more forks. Bark of the root is wrinkled and blackish and the internal structure is fleshy and white, containing a large amount of thick, milky, fetid, alliaceous juice. Leaves are few in number, radical and appear in autumn. They grow to about 45 cm in length during winter and wither away by the end of spring. Leaves are shiny and are pinnated with pinnatifid segments characterized by oblong and obtuse lobes. Petioles are terete and channelled only at the base. Stem is herbaceous with a circumference of about 15 cm at the base, solid, smooth and clothed with membranous sheaths. Flowers are pale yellow, succeeded by flat, thin, reddish-brown fruit. Fruit is like that of parsnip, but larger and darker, slightly hairy and rough. Stalk of the inflorescence is big and leafless. Fruits are 0.8 cm long and 0.6 cm broad with tender hairs. White exudate of the fruits is fragrant, pure and crystalline. Commercial asafoetida is extracted from roots. Carrot-like taproots attain a diameter of 12–15 cm at the

crown after 4–5 years of growth. At this stage, the plant is ready for the commercial extraction of asafoetida. Cases in which seedlings of *Gardenia gummifera* have been sold as asafoetida plants have been reported. *G. gummifera* produces an exudation from its leaf axils which smells similar to asafoetida. It is, however, quite different from asafoetida (George, 1995).

8.1.3 Varieties of asafoetida

There are many varieties of asafoetida. They come under different classifications and are priced differently. The two major varieties are Hing and Hingra. While Hingra is obtained from *F. foetida*, Hing is from *F. assafoetida* (NIIR Board, 2010). Hingra is inferior to Hing which is richer in odour and thus more desirable. Hing and Hingra differ in solubility. The paler Hing is water-soluble whilst the darker Hingra is oil-soluble (Anon., 2006).

Hing is classified into Irani Hing and Pathani Hing according to the country of origin, the former being produced in Iran and the latter in Afghanistan. Irani Hing sometimes has woody residues, but Pathani Hing is comparatively free from them. Hadda is the expensive variety of Pathani Hing and has the strongest odour. Irani Hing has two types, based on sweetness or bitterness. Sweet Irani Hing is collected from horizontal cutting of the stem and is brown in colour. Bitter Irani Hing is gathered by making incisions in the roots and is more or less transparent (NIIR Board, 2010). Sweet Irani Hing may include pieces of stem while the other is devoid of them.

Today, there are many kinds of exudates in the market, all called asafoetida in general but sourced from different species using different collection procedures. Three of the most important are:

- galbanum
- sagapenum
- sumbul.

Galbanum is the gum resin exuded from the lower stems of the species, *F. galbaniflua* Boiss and Bulise, a stout perennial herb of North Western Asia. Gum resin occurs in the form of distinct, almost translucent and irregular 'tears' (the purest form of gum resin with a round and flattened shape) or yellow–brown 'masses' (tears agglutinated into a more or less uniform mass) which become soft and sticky at a temperature of 35–37.7 °C. Sagapenum is similar to asafoetida, but occurs as the hardened exudation of another species, *F. persica* Wild or *F. snowilziana* D.C. It is exported to India from Saudi Arabia and Iran and marketed largely in Mumbai.

Sumbul is the gum resin of *F. sumbal*. This gum resin (ammoniac) exudes from stems and roots through fissures developed (due to varying temperature) or animal and insect punctures. It is available in 'tears' or cakes (Culbreth, 1917). It is produced in Iran and the main market is, again, India.

There is another gum resin, Ushak, which is similar to asafoetida but obtained from a different genus of the family umbelliferae, *Doremia ammoniacum* or *D. anureum*. The plant was often found where there was a temple of Ammon, a God of ancient Egypt, Greece and Rome. Dioscoides, the Greek herbalist, first described this plant scientifically and named it ammoniacoon. The present botanical name,

Doremia ammoniacum is derived accordingly. It is sold particularly in Mumbai in India as Bombay Sumbul. Unlike the true asafoetida plant, *D. ammoniacum* is a shrub and the gum resin is found on its flowering and fruiting branches and not on the taproot underground.

8.2 Chemical composition

The constituents to which asafoetida owes its characteristic odour reside in the oil. There are two groups of compounds in the oil. One group belongs to the ferulic esters whilst the other, more important-group is a volatile fraction consisting of different sulphur compounds, some of which are similar to those found in garlic and onion. It is important to note that asafoetida, galbanum, sagapenum and sumbul differ significantly in their phytochemistry, polysulfanes, complex acetylenes, phenylpropanoids and sesquiterpene derivatives, etc. (Anon., 2007).

Asafoetida has volatile oil, gum and resin as important constituents, as well as some impurities. A normal sample may have about 40–64 % resin, 25 % endogenous gum, 10–17 % volatile oil and 1.5–10 % ash. Total sulphur content may vary from 15.3–29.0 % (Anon., 2009). By means of steam distillation, adsorption chromatography and gas chromatography–mass spectroscopy (GC–MS) analysis, Rajnikath *et al.* (1984) characterized seven sulphur components (di-, tri- and tetra-sulphides). The identity of some of the components has been confirmed by GC synthesis and comparison. According to this research, the flavour of asafoetida is largely due to three sulphur compounds:

- (R)-2-butyl-1-propenyl disulphide (mixture of E and Z isomers 7:3);
- 1(1-methylthiopropyl) 1-propenyl;
- di-and 2-butyl-3-methylthioallyl disulphide (both as mixtures of diastereomers).

The last compound, however, is reported as not present in the Afghan variety of asafoetida. Chromatography of the essential oil of Afghan asafoetida gave, in addition to the two compounds mentioned above, a fraction showing the presence of a few components including:

- dimethyl trisulphide;
- 2-butyl methyl disulphide;
- 2-butyl methyl trisulphide;
- di-2-butyl disulphide;
- di-2-butyl trisulphide;
- di-Z-butyl tetrasulphide.

The resinous part of asafoetida has mainly ferulic acid esters (60 %), free ferulic acid (1.3 %), asaresinotannols and farnesiferols (A, B and C), coumarin derivatives (e.g. umbelliferone) and coumarin–sesquiterpene complexes (e.g. asacoumarin A and asacoumarin B). Free ferulic acid is converted to coumarin during dry distillation. The gum fraction contains mainly glucose, monosaccharide and a few polysaccharides such as D-galactose, L-arabinose, L-rhamnose, 4-*O*-methyl-D-glucuronic acid and D-glucuronic acid. The volatile oil obtained by steam distillation has an orange–brown colour and is very pungent. It has largely sulphur-containing

compounds with disulphides as major components and various monoterpenes (Anon., 2007). Further trisulphides and tetrasuphides have also been characterized using GC–MS analysis. The pungent odour of the oil is due to the disulphides (Tiwari and Ankur, 2004).

Galbanum contains about 6 % volatile oil, 67 % resin, 19 % gum and 8 % foreign matter. The volatile oil is mainly a hydrocarbon of the terpene series. The boiling point is between 160 °C and 165 °C. The oil is dextro-rotatory and colourless. It has a specific gravity of 0.884, and forms crystals with gaseous hydrochloric acid. The yellow–brown resin of galbanum may be obtained by extraction with alcohol and distilling of the solvent. Umbelliferon ($C_9H_6O_3$) may be obtained from the oil using boiling water or slightly alkaline water. It is closely related to coumarin. The aroma of galbanum is similar to that of asafoetida and ammoniac, being weaker than the former, but stronger than the latter (Anon., 2012c).

Sagapenum comprises 50–60 % resin, 23–30 % gum and 3–11 % volatile oil. The oil has sulphur compounds, 1–4 % bassorin together with calcium malate, phosphate and some impurities. The resin contains sagaresinotannol and umbelliferone. Sagapenum occurs in yellow or yellowish red, semi-transparent, agglomerated granules, resembling galbanum, but with a darker colour. The odour is alliaceous, somewhat similar to but less disagreeable than that of asafoetida, and more powerful than that of galbanum, becoming more pronounced on heating. The taste is bitter and acrid. Although it resembles galbanum, it can be distinguished by its solubility in petroleum. Sagapenum yields a much larger amount of resin than galbanum. It contains *umbelliferon* but no sulphur (Anon., 2012d).

Sumbul roots are dark outside and yellowish white inside. Sumbul comprises 18–28 % gum, 70 % resin, 1–4 % volatile oil and 1–4 % ash (Culbreth, 1917). It has a highly pungent taste and a fibrous appearance. The odour resembles musk or *Kasturi*. In its main market in India it is often adulterated with roots of jatamansi (*Nardostactys jatamansi*) or tagar (*Valleriana celtica*). Its volatile oil has two balsamic resins, one soluble in alcohol and the other in ether. It has a bitter taste like peppermint and, on dry distillation, yields a bluish oil containing umbelliferone. Unlike asafoetida, it does not show the presence of sulphur (Grieve, 2010). Betaine and umbelliferon are present in the root. Vanillic acid and phytosterol are present in the resin. The volatile acids identified are acetic, butyric, angelic and tiglic acid. The non-volatile acids identified are oleic, linoleic, cerotic, palmitic and stearic acids.

8.3 Cultivation and processing

Before flowering in spring, the plant produces sprouts and foliage from the taproot. After about a month, the green foliage turns yellow. It is at this stage that the taproot is tapped for asafoetida. The process is described as follows (Pruthi, 2001):

1. Soil and stones surrounding the foliage are removed and base of the plant including top of the taproot is exposed.
2. The foliage is then pulled out and upper part of the taproot (which is covered by a brush-like mass) is exposed.
3. Afterwards the taproot is covered with loose earth and gravel and left undisturbed for about five days.

4. When this period is over, the earth and gravel around the taproot are cleared and the brush-like mass is pulled out, completely exposing the top of the taproot. Top of the tap root is then scraped, making an area up to 6.5 cm^2, depending upon its size. When scraping is complete, the taproot is shaded and protected using a construction made with twigs and stones.
5. Two to three days after scraping, the first incision is made and the first flow of sap is collected from top of the taproot. After another 2–3 days, a slightly deeper cut is made about 0.5 cm deep, and sap is collected again. A third cut may be made to induce further flow of sap. The process of cutting the top of taproot and collecting sap is continued for 10–15 cycles until the flow of sap stops.
6. After each cutting, the taproot is covered again with twigs and stones to prevent soil or gravel falling onto the cut surface, and to maintain a cool condition under which the taproot will mature.
7. The sap is stored in a pit dug in the soil. The pit size may vary depending on the amount of resin available in a season, and it can be as large as 1.8 m long, 1.8 m wide and 2.4 m deep. The sides of the pit are plastered with mud and the top covered with stalks of asafoetida plants, leaving an opening of about 0.3 m diameter through which the daily collection of sap can be poured into the pit.

Some Pathans in Afghanistan collect resin from wild plants by cutting the stems which show above ground. They also chop and boil roots and stems of asafoetida plant in water and collect the resin by evaporating the water, but the quality of such resin is definitely inferior.

The average yield of resin has been estimated at roughly 40 g per plant, but certain plants may yield as much as 900 g (Krishnamurthy, 1994). Omidbeygi and Pirmoradei (2006) studied the yield of asafoetida at Khomrout located in Zarand in the Kerman province of Iran, looking at varying root diameters and numbers of incisions. Mean resin yield per plant was 43.34 g, 48.62 g, 55.26 g, 62.54 g, 72.38 g and 89.90 g from roots with 4–5 cm, 5–6 cm, 6–7 cm, 7–8 cm, 8–9 cm and 9–10 cm diameter, respectively. The sequence of incisions also has an influence on the resin yield. Resin yield increased for the first nine incisions and thereafter declined until the 14^{th} incision. The study recommended extraction of resin from roots with diameter of 6 cm and above and limiting the incisions to nine for a better yield.

Asafoetida stored in pits is generally very thick and sticky. At this stage it has pungent odour and tastes bitter and acrid due to the sulphur compounds present. It varies in colour from white to grey or dark red. Resin continues to mature during storage in the pit (Pruthi, 2001). It can be moulded by hand and made into 'tears', 'mass' or 'paste'. 'Tears' constitute the purest form of gum resin and are round and flattened, 5–30 mm in diameter and greyish or dull yellow in colour. There are two types of 'tears', those that retain their original pale colour for years and other that gradually become yellowish brown or dark. 'Mass' asafoetida is the common commercial form of this spice. It comprises 'tears' agglutinated into a more or less uniform mass, often found with fragments of root, soil, etc. 'Paste' is the glue type of resin, and it typically contains some extraneous matter (NIIR Board, 2010).

Traditionally, white asafoetida was packed first in cloth and then in jute bags. Dark red asafoetida was conventionally packed in goat or sheep skin, where it could

Table 8.4 Physical and other characteristics of asafoetida oil

Parameter	Remarks
Odour:	Strong characteristic garlic-like odour.
Solubility:	Soluble in alcohol and oils, insoluble in water.
Specific gravity:	0.906–0.973 @ 20 °C
Optical rotation:	–9°0′ to +9°18′ @ 20 °C
Refractive index:	1.493–1.518 @ 20 °C
Fire/explosion hazard:	Flammable liquid, flash point: 65.5 °C
Reactivity:	Stable
Decomposition:	When heated to decomposition produces acid fumes and carbon monoxide smoke
Toxicity:	Liquid may irritate eyes and skin. Avoid handling during pregnancy

Source: Anon., 2007.

mature further. Today, many people use plastic bags or sheets for initial packing and then place the bags in wooden boxes.

The main products of asafoetida are:

- volatile oil;
- tincture of gum resin;
- compounded asafoetida.

The volatile oil portion is 10–17 %. It is obtained by steam distillation, and yields may vary from 3–10 % depending on the purity of the product. Commercial use of the oil is mainly in the preparation of medicines. Physical properties and other characteristics of the volatile oil of asafoetida are given in Table 8.4.

Asafoetida oil has to be stored in a tightly closed container and the storage room should be cool (15–25 °C), dry and well ventilated, as well as protected from sources of sunlight, heat and ignition. The volatile oil must be handled carefully. Contact with the eyes or skin should be avoided as well as inhalation of fumes from the oil (Anon., 2012a).

Tincture of asafoetida is prepared from asafoetida gum resin with ethyl alcohol. The strength of the tincture depends on the application. However, a regular tincture of 1000 ml is prepared by macerating 200 g asafoetida in 750 ml alcohol (70 %) in a closed vessel for 7 days and shaking occasionally. The liquid is filtered and made into the required volume. The tincture becomes milky on addition of water due to separation of the resin (Anon., 2012e). Flavouring and pharmaceutical industries mainly use alcoholic tinctures (NIIR Board, 2010).

Compounded asafoetida, called Bandhani Hing in India, is a ready-to-use powder preparation designed in particular for making Indian curries because natural asafoetida is so strong and not appropriate for direct use in cooking, being difficult to grate. Compounded asafoetida is composed of asafoetida from one or more sources (Irani or Pathani or both) mixed with gum arabic and edible starch or edible cereal flour. The blending formula varies from manufacturer to manufacturer and is a trade secret. However, the product will generally have 30 % asafoetida (Anon., 2003). The product is popular particularly in South India. Indian

companies such as Laljee Godhoo & Co and Vandevi of Mumbai, or MVM Brand Sun Industries of Chennai, are large producers of compounded asafoetida. It is available in the market in the form of powder or brick (powder compacted).

8.4 Quality issues

Asafoetida is one of the naturally admixed with foreign matter or adulterated agricultural products in the world. It is not strange to find clay, sand or stone in it. Extraction from the plant itself is very crude, and there are abundant opportunities to get the exudate mixed with soil, dirt and plant parts. Sometimes, gypsum is added to increase weight. Other adulterants include rosin, gum arabic and other cheaper kinds of gum resins, barley or wheat flour, slices of potato, etc. Exudates of other species, not necessarily of the same genus, are mixed with and supplied to buyers who are not well versed with the product.

Since India is the major buyer and consumer of asafoetida, regulations to thwart adulteration have been prescribed for Hing, Hingra and compounded asafoetida. According to the Government of India Prevention of Food Adulteration Act of 1954, Hing should not have more than 15 % total ash by weight, ash insoluble in dilute hydrochloric acid not more than 2.5 % by weight, alcohol extract (with 90 % ethyl alcohol) not less than 12 % as estimated by the U.S.P. 1936 method and starch not more than 1 % by weight. Lower quality Hingra should not have more than 20 % total ash by weight, ash insoluble in dilute hydrochloric acid not more than 8 % by weight, alcohol extract (with 90 % alcohol) not less than 50 % as estimated by the U.S.P. 1936 method and starch not more than 1 % by weight (Anon., 2003).

Compounded asafoetida is sometimes adulterated during processing with materials such as chalk and other oleogums like galbanum, ammoniaccum and colophony (Raghavan *et al*., 1974). Officially, compounded asafoetida should not contain colophony, galbanum, ammoniaccum or any other foreign resin/s, coal tar dyes or mineral pigment. The total ash content of compounded asafoetida should not be more than 10 % by weight, acid insoluble ash in dilute hydrochloric acid not more than 1.5 % by weight and alcohol extract (with 90 % of ethyl alcohol) as estimated by the U.S.P. 1936 method should be less than 5 % by weight (Anon., 2003).

8.5 Main uses of asafoetida

The main uses of asafoetida are for flavouring in cooking and in traditional medicine. Both uses are prevalent in India, Iran and Afghanistan. In China and other Asian countries, asafoetida is used to a more limited extent in traditional medicine.

8.5.1 Culinary uses

For flavouring, asafoetida is used most often in its compounded form, either after frying in oil or steeping in water. When asafoetida is added to hot oil, it changes

from its strong and powerful stinging smell to an enticing oniony–garlicky aroma. In India, asafoetida is used extensively to flavour curries, soups, sauces and pickles, most often in conjunction with onion and/or garlic. Some Brahmin communities and Jains in India, who do not eat garlic and onion, use asafoetida as a substitute (Andi *et al*., 1997). Vegetarian preparations of South India that use asafoetida include sambar, rasam and certain lentil curries. It is also added to season some fish dishes and in making certain types of crackers like, pappadam.

In Iran, the whole plant is considered as a fresh vegetable and is used for cooking. Leaves particularly find application in kebabs and egg preparations (Rosetti, 2009). Some people eat the large cabbage-like tops of the plant raw. Asafoetida is also rubbed onto warmed plates prior to serving meat dishes on special occasions (NIIR Board, 1991). Traditionally, Afghan people use asafoetida to make landhi, dried meat prepared for winter use. Fresh meat is rubbed with salt and raw asafoetida and then strung on landhi poles (tall poles with crosspieces) to dry. The dried meat is then consumed during winter months (NIIR Board, 2010). It has been found that the ethanolic fraction of asafoetida inhibits fungal growth (Thyagaraja and Hosono, 1996).

Nutritional value of asafoetida, as reported by the University of Medicine and Dentistry in New Jersey (UMDNJ, 2012), is given in Table 8.5. Asafoetida has minerals like sulphur and phosphorus, and vitamins such as riboflavin and niacin.

8.5.2 Medicinal uses

The therapeutic use of asafoetida is mentioned in a number of classical Ayurvedic texts of the Charaka and Susrutha schools, notably the *Charaka samhita*, *Susrutha samhita* and *Ashtanga Samgraha.* In a review article on asafoetida, Iranshahy and Iranshahi (2011) state that it is traditionally used for treating various diseases, including asthma, gastrointestinal disorders and intestinal parasites. The Ayurvedic preparation, *Hingvashtaka churna*, which contains asafoetida along with other herbs like ginger and long pepper, as well as rock salt, is regarded as a good remedy for indigestion (Anon., 2012b). Asafoetida is used in the Middle East to treat stomach ache (Lev and Amar 2008). It has also been linked to pain relief (Dutt, 1877; Chatterjee and Pakrashi, 1995) and the alleviation of various neurological conditions (Duke, 2003; Bakru, 2007). Antiviral activity has been demonstrated *in vitro* against the influenza A virus (H1N1), but there is, as yet, no clinical evidence to support this observation (Wolters Kluwer Health, 2009). A few asafoetida based preparations have also been used in veterinary medicines (Krishnamurthy 1994).

Table 8.5 Nutritional value of asafoetida

Foods	Wt (g)	Kcal	Protein (g)	Fat (g)	Carbohydrate (g)	Ca (mg)	Fe (mg)	Fiber (g)
Asafoetida	10	297	4	1.1	67.8	690	39.4	4.1

Source: UMDNJ (2012).

There have been a few studies of the potential use of asafoetida in modern medicine. An investigation on the effect of extracts of asafoetida and certain other food additives noted that asafoetida extract inhibited aflatoxin production significantly (more than 90 %) at a concentration of 5–10 mg/ml (Soni *et al*., 1992). The effect of asafoetida on growth of the parasite *Trichomonas vaginalis* was studied *in vitro* and showed that it had a potent antiparasitic effect on *T. vaginalis* compared to the reference drug metronidazole (Ramadan and Al Khadraw, 2003).

While studying the anticarcinogenetic effect of several spices, Unnikrishnan and Kuttan (1990) showed that asafoetida could increase the lifespan of mice by 52.9 %. Saleem *et al.* (2001) also reported that asafoetida demonstrated antioxidant and anticarcinogenic properties in mice. Mallikarjuna *et al*. (2003) studied the effect of asafoetida on *N*-methyl-*N*-nitrosourea (MNU)-induced mammary carcinogenesis in rats. It significantly increased antioxidant activity and inhibited tumour growth, indicating the chemopreventative potential of asafoetida against certain cancers.

There have been a number of studies suggesting the beneficial effects of asafoetida on digestion (Kamanna and Chandrashekara, 1982; Desai and Kalro, 1984; Suresh and Kesavan, 1984; Platel and Srinivasan 2000). Antispasmodic and hypotensive effects of asafoetida were also observed in rats by Mohammad *et al*. (2004) whilst anticonvulsive effects were observed by Ansari *et al*. (2006) and Fatehi *et al*. (2004). Asafoetida has also been linked to improved male fertility and potency (Kassis *et al*., 2009).

There are also some side-effects for consumption of asafoetida. When taken raw and in too large a quantity, it may induce headache, dizziness, nausea, vomiting, throat irritation, swelling of lips and stomach pain. Women during pregnancy should avoid medicines containing asafoetida as it is known to induce miscarriage. Such medicines might also affect the periodicity of menstrual cycle. In a laboratory investigation of infants treated with asafoetida, an oxidizing effect of asafoetida on foetal haemoglobin has been noticed. According to Kelly *et al.* (1984), asafoetida must be considered potentially life-threatening if given to infants.

Asafoetida is very much a wild crop and scientific production methods are yet to be developed. There is scope for increasing productivity by selecting and growing high-yielding varieties and improving agronomic practices. Yield depends greatly on the size the taproot attains by about 4 years of growth. Harvest and post-harvest technology are traditional and need to be improved to increase the amount of oleogum resin tapped and to ensure its quality. Importers often receive contaminated asafoetida as extraction has not been refined. They do not purify the product as modern methods are yet to be adopted.

Producing countries have not looked into the economics of production or the income the crop can generate for producers. Importers generally are not aware of the price at which traders in the producing countries collect asafoetida from villages. Since trade is not organized and controlled by government agencies, both in the producing and consuming countries, some traders follow devious practices to enjoy a major share of the profit.

Even a small amount of asafoetida in a processed product like compounded asafoetida can impart its characteristic smell. There is therefore plenty of scope for adding adulterants in compounded asafoetida or other products to increase income.

Hence there is a need for strict quality specifications and labelling laws together with effective implementation in consuming countries.

There are many applications for asafoetida in traditional medicine in India, China and Arabian countries. The few scientific investigations carried out indicate that it has some potential for exploitation in modern medicine. Studies undertaken on its anticarcinogenic effects, although preliminary, are promising. Hence, there is a need for continued and systematic research into the potential role of asafoetida in the development of effective and easy to use pharmaceuticals for use in disease control and to address physiological problems in the human body.

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9

Allspice

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Abstract: The evergreen tree *Pimenta dioica* provides the culinary spice pimento or allspice of commerce. The dried, mature but not ripe, berries are the spice of commerce. Pimento is also sold as ground spice. The extraction and chemical composition of other commercially important products obtained from the spice, like berry oil, leaf oil and oleoresin, are detailed. Cultivation, propagation and post-harvest processing are reviewed in the chapter. The use of allspice in food, medicine and perfumery and the functional properties are also described. Cleanliness, safety issues and trade specifications are also enumerated in the chapter.

Key words: adulteration, allspice, berry oil, chemical composition, cultivation, oleoresin, quality, trade specifications.

9.1 Introduction

Allspice, *Pimenta dioica* (L.) Merr. (syn: *P. officinalis* Lindl., *Myrtus pimenta* L., *M. dioica* L. and *Eugenia pimenta* DC (Merrill, 1947) is a polygamodioecious evergreen tree, the dried unripe fruits of which provide the culinary spice pimento. It belongs to the family Myrtaceae and is known in English as allspice or pimento, in French as *piment jamaique* or *toute-epice,* in Portuguese as *pimenta da Jamaica* and in Spanish as *pimiento gorda*. The vernacular names of allspice are given in Table 9.1. The name allspice was coined by John Ray, an English botanist, who identified the flavour as a combination of clove, cinnamon and nutmeg.

The word pimento is derived from the Spanish word *pimienta* for black pepper, as allspice resembles peppercorns. It is known as pepper in many languages. In Russian it is known as *Yamaiskiy pjerets* – Jamaica pepper; in French *poivre aromatique* – aromatic pepper; in German *Nelkenpfeffer* – clove pepper; and in Swedish as *kryddpeppar* – condiment pepper. Newspice (German *Neugewurz*) also refers to its origin from the New World and the French *toute-epice* reflects the complex aroma of this spice. However, the berries were widely known as *pimienta,* later anglicized as pimento. The genus name *Pimenta* was derived from the Spanish *pimiento* for black pepper. Since the Spaniards initially called allspice pimiento, the name was also introduced to many European countries along with the spice when the spice was introduced to Europe in the sixteenth century. The species name *dioica* (Greek *di-* from *dyo* 'two', *oikos* 'house') indicates that the functional male and female flowers grow on different plants.

Table 9.1 Vernacular names of allspice (*Pimenta dioica*)

Language	Vernacular name
Arabic	*Bahar, Bhar hub wa na'im*
Danish	*Allehande*
Dutch	Jamaica pepper, piment
English	Jamaica pepper, myrtle pepper, pimento, newspice
Estonian	*Harilik pimendipuu, Vurts*
Finnish	*Maustepippuri*
French	*Piment, piment Jamaique, poivre aromatique, toute-epice, poivre de la Jamaique*
German	*Piment Neugewurz, Allgewurz, Nelkenpfeffer, Jamaicapfeffer, Englisches Gewurz*
Hungarian	*Jamaikai szegfubors, Szegfubors, Pimento, Amomummag*
Icelandic	*Allrahanda*
Italian	*Pimento, pepe di Giamaica*
Norwegian	*Allehande* Polish
	Ziele angielskie
Portuguese	*Pimenta da Jamaica*
Russian	*Yamaiskiy pjerets*
Spanish	*Pimienta de Jamaica, pimienta gorda*
Swedish	*Kryddpeppar*
Turkish	*Yeni bahar*

Source: http://www.uni-graz.at/~katzer/enal/Pime_dio.htm.

9.1.1 Origin and distribution

The family Myrtaceae consists of about 3000 woody species, most of which grow in the tropics. The genus *Pimenta* Lindl. consists of about 18 species of aromatic shrubs and trees native to tropical America (Willis, 1966). The genus is closely related to *Myrtus* L. and *Eugenia* L. The commercially important *Pimenta* spp. is *Pimenta dioica* (L.) Merr. providing the spice pimento (allspice) and *P. racemosa* (Mill) Moore, bay or bay rum tree providing oil of bay. The basic chromosome number for the genus is $x = 11$ and allspice is a diploid with $2n = 22$ (Purseglove *et al*., 1981).

The tree is indigenous to West Indies (Jamaica). The trees are also found in Central America (Mexico, Honduras, Guatemala, Costa Rica and Cuba) and in the neighbouring Caribbean islands, although its original home is in dispute. Christopher Columbus discovered allspice in the Caribbean islands in about 1494. Spanish explorers and later settlers in Jamaica harvested and used the leaves and berries. Reports indicate that, there has been continuous production of berries in Jamaica from about 1509 to the present day. The berries reached London in 1601 as described by Clusius in his *Liber Exoticorum* and the plants were first cultivated in England in a hot house in 1732 (Weiss, 2002). Before World War II, allspice was more widely used than today; however, during the war many trees were cut down and there was a shortage of the spice. Although cultivation was taken up after the war, production never fully recovered.

Allspice was introduced into West Indian Islands (Grenada, Barbados, Trinidad and Puerto Rico) from its place of origin. Attempts to introduce it into countries in tropical regions, namely, India, Sri Lanka, Fiji, Malaysia, Singapore and Indonesia (Java, Sumatra), have, for various reasons, not succeeded fully. In India, there are a few trees in Maharashtra, Tamil Nadu, Karnataka and Kerala.

9.1.2 Production and trade

Jamaica is the largest producer and exporter of pimento, accounting for 70 % of the world trade. The remaining 30 % is produced by Honduras, Guatemala, Mexico, Brazil and Belize. The dried mature but unripe berries, berry oleoresin, berry oil and leaf oil are the products of commercial importance obtained from *P. dioica* and they find varied uses in the food, medicine and perfume industries. Among the pimentos from various geographical locations, Jamaican pimentos are considered to be of high quality because of their flavour, appearance and size and receive a premium price in the market (Table 9.2).

The major importing countries are the USA (Table 9.3), Germany, the UK, Finland, Sweden and Canada. Leaf oil is mainly exported to the USA and the UK. Pimento is generally classified with capsicum in the import statistics of most countries and hence analysis of the market situation is difficult.

9.2 Chemical composition

9.2.1 Berry

The dried, mature but not ripe, berries are the pimento spice used in commerce. Pimento is also sold as ground spice. Berries conforming to international standards should be between 6.5 and 9.5 mm in diameter, medium to dark brown in colour, with an uneven surface and with a pleasant odour, characteristic of the spice and with approximately 13 fruits/g. The dried berry contains aromatic steam volatile oil, fixed (fatty) oil, resin, protein, starch, pigments, minerals, vitamins (Table 9.4), etc. The constituents present in the oil influence the quality and aroma of the spice. The phenolic compounds eugenol and isoeugenol and the sesquiterpene hydrocarbon, β-caryophyllene are the major compounds present in allspice (Table 9.5). Several other compounds have been identified in allspice, which are present in lesser quantities (Table 9.6). The geographical variation, cultivar differences, stage

Table 9.2 Average price of allspice in New York

Country	Year	Price (dollars/pound)
Guatemala/Honduras	1997	0.961
	1998	1.041
	1999	1.920
	2000	2.452
Jamaica	1997	1.180
	1998	1.353
	1999	2.268
	2000	3.579
Mexico	1997	0.583
	1998	1.071
	1999	1.912
	2000	2.360

Source: http://www.fas.usda.gov/htp/tropical/2001/03-01/spcavg.pdf.

Table 9.3 Imports of allspice by the USA

Country	1999		2000	
	Quantity (kg)	Value (dollars)	Quantity (kg)	Value (dollars)
China	99 850	126 658	94 320	69 458
Guatemala	185 349	540 039	269 339	1 077 571
Honduras	424 028	1 044 247	297 831	1 137 345
India	167 379	118 132	62 809	62 136
Jamaica	367 384	1 218 489	359 881	1 789 828
Lebanon	750	3 000	0	0
Mexico	80 849	201 311	387 081	1 205 363
Pakistan	13 230	31 832	28 592	39 483
Portugal	5 000	2 955	0	0
Spain	12 668	52 486	19 800	13 766
Taiwan	1 081	3 400	0	0
Thailand	0	0	4 830	16 325
Turkey	1 326	2 323	2 852	4 808
Other	39 529	91 470	40 164	145 499
Total	1 398 423	3 436 342	1 567 499	5 561 582

Source: http://www.fas.usda.gov/htp/tropical/2001/03-01/tropic.htm.

Table 9.4 Nutrient composition of allspice (per 100 g)

Composition	Quantity
Proximates	
Water	8.5 g
Food energy	262.6 kcal
Carbohydrates	72.1 g
Protein	6.1 g
Fat	8.7 g
Dietary fibre	21.6 g
Ash	4.6 g
Minerals	
Calcium	660.6 mg
Iron	7.1 mg
Magnesium	134.1 mg
Phosphorus	113.3 mg
Potassium	77.0 mg
Sodium	77.0 mg
Zinc	1.0 mg
Copper	0.6 mg
Manganese	2.9 mg
Vitamins	
Vitamin C	39.2 mg
Thiamin B1	0.1 mg
Riboflavin B2	0.1 mg
Niacin	2.9 mg
Vitamin B6	0.3 mg
Folate	36.0 µg
Vitamin E	1.0 mg

Source: http://ndb.nal.usda.gov/ndb/foods/show/220?qlookup=allspice&fg=&format=&man=&lfacet=&max=25&new=1

Table 9.5 Constituents identified in allspice extracts (ppm) using different extraction procedures

Compound	CO_2 extracts				
	150 bar/50 °C	350 bar/50 °C	350 bar/70 °C	SDE	DDE
α-Pinene	40	60	39	50	46
β-Pinene	39	55	37	56	54
Myrcene	38	48	37	79	72
(*e*)-β-Ocimene	23	29	23	48	46
α-Thujene	23	31	21	31	27
Sabinene	24	36	24	26	45
δ-3-Carene	55	74	52	102	95
α-Phellandrene	107	138	101	188	138
Limonene + β-phellandrene	138	188	119	298	233
p-Cymene	85	111	79	193	171
α-Terpinene	16	19	13	38	11
γ-Terpinene	87	111	83	183	150
Terpinolene	146	199	136	261	170
1,8-Cineole	272	355	249	472	403
Linalool	32	43	30	48	37
Terpinen-4-ol	110	160	107	198	123
p-Cymen-8-ol	21	31	22	41	20
α-Terpineol	47	70	47	90	44
Trans-p-Menth-2-en-1-ol- + *cis-p*-menth-2-en-1-ol	16	21	15	31	15
β-Caryophyllene	1749	2534	1595	1915	1838
α-Humulene	423	610	380	452	414
α-Selinene	267	383	236	262	237
β-Selinene	173	243	153	173	161
δ-Cadinene	210	307	188	216	186
β-Elemene	105	150	95	113	113
Allo-aromadendrene	81	118	73	83	82
Germacrene *d*	82	121	77	43	77
Spathulenol	42	54	39	47	34
Caryophyllene oxide + viridiflorol	187	225	147	168	142
Humulene oxide ii	39	55	35	47	34
t-Cadinol + *t*-muurolol	76	103	66	79	55
α-Muurolol	29	38	25	29	21
α-Cadinol	60	90	56	78	51
Selin-11-en-4-ol	131	170	113	120	78
Caryophylla-2(12),6(7)-dien-5-ol	30	37	25	28	29
Eugenol	18176	29976	18178	22240	11135
Methyl eugenol	1822	2670	1661	2025	1424
Chavicol	57	73	58	60	25
Myristicin	33	44	26	31	23
Elemicin	13	19	12	15	10

SDE: simultaneous distillation and extraction using diethyl ether. DDE: direct diethyl ether extract.
Source: Lawrence (1999).

of maturity, etc. also influence the quality of the berry. The quality of the berries from Jamaica is superior to that of berries from other islands and is preferred for trade. Prolonged storage of allspice is detrimental to both oil content and flavour of the spice.

Table 9.6 Minor compounds in allspice berries

Camphene (3 ppm)	*cis*-sabinene hydrate (2 ppm)
(*Z*)-β-ocimene (1 ppm)	Linalool oxide-furanoid (1 ppm)
α-*p*-Dimethylstyrene (1 ppm)	β-phellandren-6-ol (11 ppm)
δ-Elemene (1 ppm)	*trans*-piperitol (1 ppm)
α-Cubebene (10 ppm)	*cis*-piperitol (3 ppm)
α-Ylangene (6 ppm)	Hexanal (<1 ppm)
α-Copaene (35 ppm)	Benzaldehyde (<1 ppm)
β-Cubebene (1 ppm)	Cinnamaldehyde (1–10 ppm)
α-gurjunene (43 ppm)	Vanillin (1–10 ppm)
α-Bulnesene (27 ppm)	Methyl salicylate (3 ppm)
Aromadendrene (31 ppm)	Guaiacol (<1 ppm)
Selina-4,11-diene (35 ppm)	4-vinylguaiacol† (1 ppm)
γ-Muurolene (57 ppm)	Methyl chavicol (6 ppm)
Ar-curcumene (10 ppm)	Safrole (5 ppm)
Zingiberene (25 ppm)	(*E*)-isoeugenol (1 ppm)
α-Muurolene (42 ppm)	Methyl (*E*)-isoeugenol (1 ppm)
Germacrene a (11 ppm)	6-methyloxyeugenol (1–10 ppm)
β-Bisabolene (4 ppm)	Palustrol (1 ppm)
cis-Calamene (10 ppm)	Caryophyll-5-en-12-ol† (1 ppm)
β-sesquiphellandrene (5 ppm)	Isocaryophyllene oxide (1–10 ppm)
Cadina-4,11-diene (11 ppm)	Salvial-4(14)-en-1-one (1 ppm)
α-Cadinene (11 ppm)	Globulol† (12 ppm)
cis-Calacorene (3 ppm)	Humulene oxide (4 ppm)
trans-Calacorene (1 ppm)	Ledol† (1–10 ppm)
Camphor (1 ppm)	Eudesmol* (1–10 ppm)
Ascaridole* (3 ppm)	Selineol*† (1–10 ppm)
Carvone (11 ppm)	Eudesmol* (1–10 ppm)
Geranial (<1 ppm)	Epi-cubenol (21 ppm)
Linalyl acetate (3 ppm)	Caryophylla-2(12),6(13)-dien-5-ol (12 ppm)
α-Terpinyl acetate (10 ppm)	Isospathulenol (7 ppm)
Neryl acetate (2 ppm)	Cubenol† (1–10 ppm)
Geranyl acetate (6 ppm)	*trans*-sabinene hydrate (2 ppm)

* Correct isomer not identified; †Tentative identification.
Source: Lawrence (1999).

9.2.2 Berry oil

Extraction of berry oil can be carried out by different methods. Berry oil is generally obtained by hydrodistillation or steam distillation of dried immature berries. When supercritical CO_2 extraction techniques are employed for extraction of berry oil, the oil obtained is of superior quality and flavour, compared with steam-distilled or hydrodistilled oil. The compositions of berry oils extracted by steam distillation, hydrodistillation and supercritical CO_2 extraction techniques are compared in Table 9.7 (Garcia-Fajardo *et al.*, 1997). The berry oils extracted by supercritical CO_2 method and steam distillation have been characterized based on their physico-chemical properties (Table 9.8).

The yield of berry oil ranges from 3.0–4.5 %. The oil is yellow to brownish yellow with a warm spicy sweet odour and fresh and sweet top-note, and is placed in the warm, sweet spicy group (Arctander, 1960). About 60 constituents have

Table 9.7 Percentage composition of a steam-distilled oil, a hydrodistilled oil and a supercritical CO_2-extract of Mexican allspice

Compound	Steam distilled oil	Hydrodistilled oil	Supercritical CO extract
α-Pinene	trace	0.1	trace
β-Pinene	trace	0.2	trace
Sabinene	0.3	0.3	0.2
Myrcene	17.7	16.5	6.0
δ-3-Carene	trace	trace	–
α-Terpinene	trace	0.1	trace
p-Cymene	0.2	trace	trace
Limonene	0.7	trace	trace
1,8-Cineole	1.9	4.1	1.3
(*Z*)-β-ocimene	trace	1.2	0.9
γ-Terpinene	1.1	0.2	trace
Terpineolene	trace	0.6	0.4
Linalool	0.4	trace	trace
Terpinen-4-ol	0.3	0.5	0.3
Methyl salicylate	trace	trace	–
α-Terpineol	0.7	0.7	0.4
Eugenol	17.3	8.3	14.9
Methyl eugenol	48.3	62.7	67.9
β-Caryophyllene	6.2	2.7	5.2
α-Humulene	1.1	0.2	0.2
γ-Cadinene	0.6	0.1	0.2
β-Selinene	trace	trace	trace
α-Selinene	0.4	trace	trace
δ-Cadinene	trace	trace	trace

Source: Lawrence (1999).

Table 9.8 Physicochemical comparison of liquid CO_2-extracted and steam-distilled allspice berry oils

	Extraction procedure	
	Supercritical CO_2	Steam distillation
Specific gravity at 20°C	0.98 to 1.03	1.027 to 1.048
Refractive index at 20°C	1.505 to 1.525	1.525 to 1.54
Optical rotation at 20°C	–5 to 0	–5 to 0
Solubility in 70 % v/v ethanol at 20°C	1 to 2	1 to 2
Total phenols v/v, minimum	75 %	65 %

Source: Charalambous (1994).

been detected, including phenols, monoterpene hydrocarbons, oxygenated hydrocarbons, sesquiterpene hydrocarbons and oxygenated sesquiterpenes, and about 34 constituents were reported in steam-distilled berry oil using gas chromatography (Nabney and Robinson, 1972). The oil from green berries is similar in composition to that from dried berries, but has a higher monoterpene content (Ashurst *et al.*,

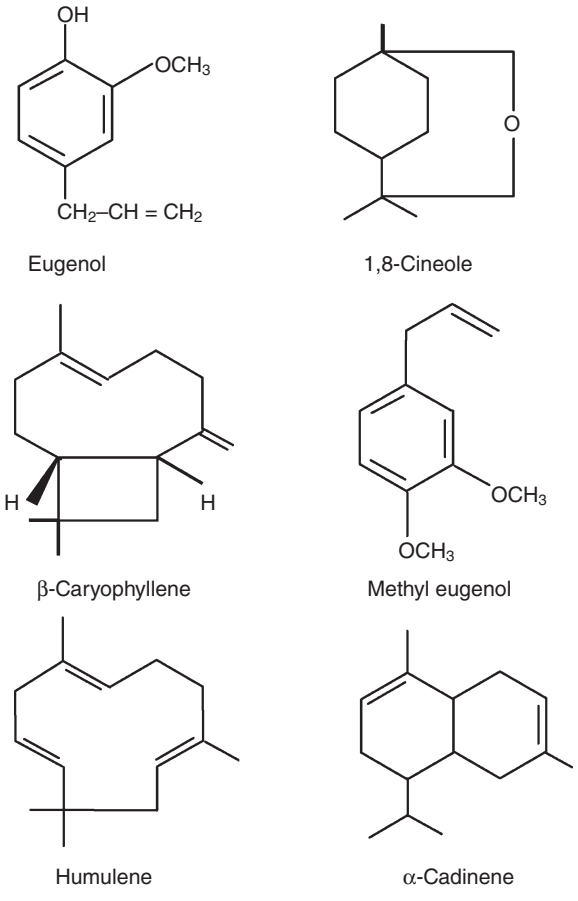

Fig. 9.1 Structures of some of the compounds in allspice.

1973). The principal components are usually eugenol, methyl eugenol, β-caryophyllene, humulene, terpinen-4-ol and 4,5-cineole (Fig. 9.1) (Tables 9.9, 9.10 and 9.11) (Nabney and Robinson, 1972; Purseglove *et al.*, 1981, Lawrence; 1999).

The main constituents affecting taste and flavour are the abundance and ratio of 1,8-cineole (Fig. 9.1) and α-phellandrene. Allspice contains various essential oils (Pino *et al.*, 1989), phenolic acids (Schulz and Herrmann, 1980), flavanoids (Vosgen *et al.*, 1980), catechins and phenyl propanoids (Kikuzaki *et al.*, 1999). The flavonol content of allspice is low and consists mainly of quercetin glycosides (Vosgen *et al.*, 1980). Three new galloylglucosides, (4*S*)-α-terpineol 8-*o*-β-D-(6-*o*-galloyl) glucopyranoside; (4*R*)-α-terpineol 8-*o*-β-D-(6-*o*-galloyl) glucopyranoside and 3-(4-hydroxy-3-methoxyphenyl) propane-1, 2-diol 2-*o*-β-D-(6-*o*-galloyl) glucopyranoside were isolated from berries of *P. dioica* (from Jamaica) together with three known compounds, gallic acid, pimentol and eugenol 4-*o*-β-D-(6-*o*galloyl) glucopyranoside (Kikuzaki *et al.*, 2000).

Allspice berry oil extracted by supercritical CO_2 extraction is light red brown with the full sweetness and fresh natural odour and flavour of the freshly ground

Table 9.9 Constituents identified in Jamaican allspice berry and leaf oils

	Berry oil	Leaf oil
Phenolics	Eugenol	Eugenol
	Methyl eugenol	Methyl eugenol
	Chavicol	Isoeugenol
Monoterpene hydrocarbons	Δ-3-Carene	Limonene
	p-Cymene	*cis*-β-Ocimene
	Limonene	*trans*-β-Ocimene
	Myrcene	α-Pinene
	α-Pinene	α-Phellandrene
	β-Pinene	Sabinene
	α-Phellandrene	γ-Terpinene
	α-Terpinene	Terpinolene
	γ-Terpinene	
	Terpinolene	
	Thujene	
Oxygenated monoterpenes	1,8-Cineole	1,8-Cineole
	Linalool	Linalool
	α-Terpineol	Terpinen-4-ol
	Terpinen-4-ol	
	Terpinen-4,8-oxide	
Sesquiterpene hydrocarbons	Alloaromadendrene	Alloaromadendrene
	γ-Cadinene	δ-Cadinene
	Calamene	β-Caryophyllene
	β-Caryophyllene	α-Copaene
	Ar-curcumene	α-Gurgunene
	β-Elemene	α-Humulene
	α-Humulene	α-Muurolene
	β-Humulene	α-Selinene
	Isocaryophyllene	
	γ-Muurolene	
	α-Selinene	
	β-Selinene	
Oxygenated sesquiterpenes	β-Caryophyllene alcohol	
	Caryophyllene oxide	
	Caryophyllene aldehyde	
	Humulene epoxide ii	

Source: Purseglove *et al.* (1981).

spice. The sensory character of the pimento berry oil obtained by steam distillation and liquid CO_2 extraction is represented in Fig 9.2 (Charalambous, 1994).

The initial impact of the liquid CO_2 extracted oil is sweet, spicy with a distinctly heavy fruity and floral dianthus character. After 6 hours the profile becomes warmer, more fruity and peppery, less phenolic and spicy. These notes are still prominent after 24 hours and continue for several days. The initial profile of steam distilled oil, although strong, is more phenolic, medicinal and less fruity. After 6 hours the profile becomes warmer with increased fruitiness but not attaining the richness of fruit notes of the CO_2 extract. The floral character is hardly noticeable at any stage of evaporation. All these notes are still prominent after 24 hours (Charalambous, 1994).

Table 9.10 Chemical composition of allspice berry oil

Eugenol (80.1 %)	α-Gurjunene (0.1 %)
Methyl eugenol (5.0 %)	Linalool (0.1 %)
β-Caryophyllene (4.5 %)	Terpinolene (0.1 %)
α-Muurolene (1.1 %)	(*E*)-β-Ocimene (0.1 %)
α-Selinene (1.1 %)	Globulol (0.1 %)
Ledene (0.8 %)	γ-Terpinene (0.1 %)
Allo-aromadendrene (0.7 %)	δ-3-Carene (0.1 %)
Calamenene (0.3 %)	*p*-Cymen-8-ol (0.1 %)
p-Cymene (0.3 %)	Copaene (unknown isomer) (0.1 %)
10-α-Cadinol (0.2 %)	α, *p*-Dimethylstyrene (0.1 %)
Methyl chavicol (0.2 %)	Limonene (0.1 %)
Spathulenol (0.2 %)	α-Pinene (0.1 %)
δ-Cadinene (0.2 %)	α-Thujene (0.1 %)
γ-Cadinene (0.2 %)	α-Phellandrene trace
1,8-cineole (0.2 %)	2-methylbutyl acetate trace
Myrcene (0.2 %)	α-Terpinene trace

Source: Guzman and Siemonsma (1999).

Table 9.11 Chemical composition of allspice berry oil from Cuba

Eugenol (87.0 %)	β-Selinene (0.2 %)
1,8-Cineole (3.3 %)	γ-Terpinene (0.2 %)
β-Caryophyllene (2.5 %)	α-Terpineol (0.2 %)
α-Humulene (1.6 %)	Calamenene (0.1 %)
p-Cymene (0.7 %)	Caryophyllene oxide (0.1 %)
Terpinen-4-ol (0.5 %)	α-Copaene (0.1 %)
Terpinolene (0.5 %)	γ-Muurolene (0.1 %)
δ-Cadinene (0.4 %)	β-Phellandrene (0.1 %)
Guaiene (unknown isomer) (0.4 %)	β-Pinene (0.1 %)
Limonene (0.4 %)	α-Terpinene (0.1 %)
α-Phellandrene (0.4 %)	γ-Cardinene (0.1 %)
Camphene (0.2 %)	α, *p*-Dimethylstyrene (0.1 %)
β-Elemene (0.2 %)	Humulene oxide (0.1 %)
Myrcene (0.2 %)	
α-Pinene (0.2 %)	Total 100 %

Source: Guzman and Siemonsma (1999).

9.2.3 Oleoresin

Oleoresin is prepared by extraction of the crushed spice with organic solvents followed by evaporation of the solvent. The composition of the oleoresin depends upon the raw materials and the solvents used for extraction of oleoresin. The oleoresin is a brownish to dark green oily liquid and two grades are normally available, based on the volatile oil content namely, 40–50 and 60–66 ml per 100 g. A US specification requires a minimum of 60 ml per 100 g.

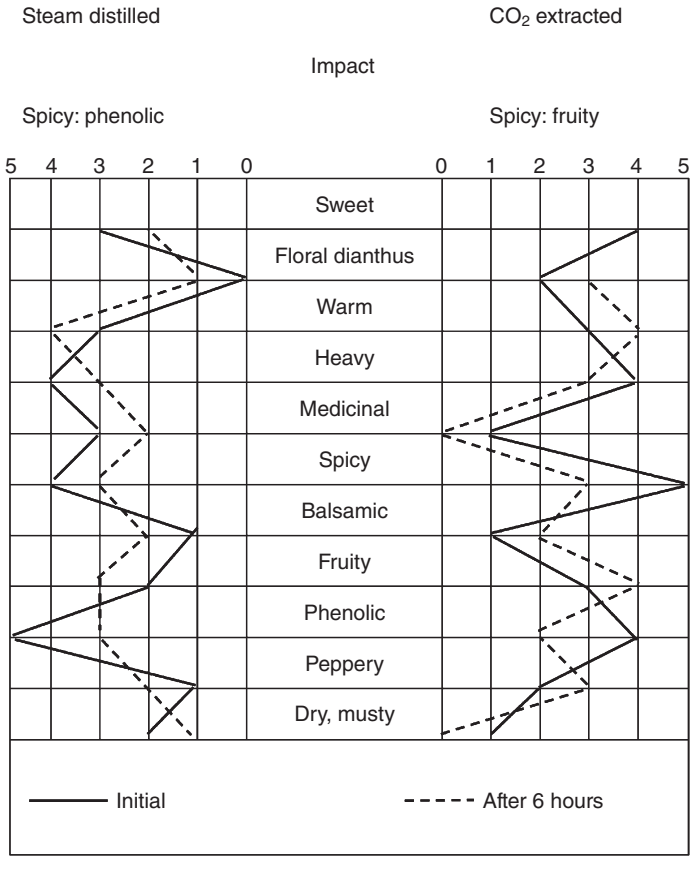

Fig. 9.2 Comparative odour profiles of steam-distilled and CO_2-extracted pimento berry oil. *Source*: Charalambous (1994).

Table 9.12 Leaf oil composition of allspice extracted by supercritical CO_2

Methyl chavicol (0.31 %)	α-Muurolene (0.05 %)
Thymol (1.82 %)	Calamenene* + γ-Cadinene (0.05 %)
Carvacrol (1.08 %)	Caryophyllene oxide (0.07 %)
Eugenol (93.87 %)	*t*-Cadinol (0.17 %)
β-Caryophyllene (1.79 %)	α-Cadinol (0.17 %)
α-Humulene (0.35 %)	α-Amorphene (0.37 %)

* Correct isomer not identified.
Source: Lawrence (1999).

A small quantity is sufficient to get the required flavour and aroma in food. Kollmannsberger and Nitz (1993) compared extracts using supercritical CO_2 extraction at various pressures and temperatures (150 bar and 350 bar at 50 °C, 350 bar at 70 °C) with there using direct diethyl ether extract and simultaneous distillation

Table 9.13 Chemical composition of leaf oil of allspice of Cuban origin

α-Pinene (0.56 %)	γ-Terpinene (0.56 %)
Myrcene (0.19 %)	Terpinolene (1.38 %)
α-Phellandrene (1.12 %)	Menthol (0.56 %)
p-Cymene (1.87 %)	Methyl chavicol (0.09 %)
1,8-Cineole (14.50 %)	Carvone (0.10 %)
Limonene (0.10 %)	Thymol (1.00 %)
Carvacrol (1.00 %)	δ-Cadinene (5.49 %)
Eugenol (28.04 %)	Cadina-1,4-diene (0.49 %)
β-Caryophyllene (1.00 %)	α-Calacorene (1.23 %)
α-Humulene (10.12 %)	Caryophyllene oxide (2.69 %)
Allo-aromadendrene (2.13 %)	α-Eudesmol (0.52 %)
α-Amorphene (2.77 %)	β-Eudesmol (0.82 %)
α-Muurolene (1.76 %)	*t*-Cadinol (6.64 %)
Calamenene* + γ-Cadinene (11.12 %)	α-Cadinol (4.94 %)

* Correct isomer not identified.
Source: Lawrence (1999).

and extraction of pimento berries. Supercritical CO_2 extracted at 350 bar pressure and 50 °C temperature was found to be the best (Table 9.5).

9.2.4 Leaf oil

Pimento leaf oil is produced by distilling fresh or dry leaves. Oil can also be extracted using supercritical extraction, and the extraction procedure with CO_2 has been described (Falconieri *et al*. 2010). Leaves used for distillation may be fresh, withered or dried and stored for 2 or 3 months prior to distilling. Yields from dried and fresh leaves are 0.5–3.0 % and 0.3–1.25 %, respectively. Oliveira *et al*. (2009) also recently reported that the yield of essential oils in leaves and fruits varied from 0.97 to 1.41 % and the major component was eugenol. The leaf oil is a brownish yellow liquid with dry–woody, warm–spicy aromatic odour.

The main composition of the leaf oil of allspice is eugenol. Eugenol content of leaf oil (65–96 %) is somewhat higher than that in berry oil (Pino and Rosado, 1996; Pino *et al*. 1997). The major compounds of the leaf essential oil from Jamaica sample was identified as eugenol (76.02 %), methyl eugenol (7.14 %) and β-caryophyllene (6.47 %) (Jirovetz *et al*., 2007). Leaf oil composition of allspice extracted using the supercritical CO_2 method is given in Table 9.12. The chemical composition of the oil is also influenced by the geographical origin of the spice (Tables 9.9 and 9.13) (Pino *et al.,* 1997).

Variation was observed in the yield and composition of leaf oil from fruiting female and non-fruiting male trees. Oil yields in male pimento trees (2.13 %) were lower than those in female trees (2.67 %). The contents of eugenol ($p < 0.01$), myrcene, α -phellandrene, γ-terpinene, terpinolene and α-thujene ($p < 0.005$) were significantly different in the female and male trees. Non-fruiting and fruiting trees

contained the same volatile components with different relative abundances. Monoterpenoid profles could be used for the early prediction of the berry-bearing ability of pimento trees. (Minott and Brown, 2007).

9.3 Cultivation

9.3.1 Propagation

Seeds

Allspice is traditionally propagated through seeds, but vegetative propagation is also adopted to get true to type plants. High-yielding trees that fruit regularly and have well formed fruit bunches are selected as mother trees. Fresh ripe fruits from such high-yielding trees are collected and seeds are extracted from the ripe fruits after soaking them overnight in water and rubbing them in a sieve to remove the pericarp. Allspice seeds lose their viability soon after harvest and hence seeds are planted immediately after extraction. If seeds are to be transported or kept for a few days, it is advisable not to extract the seeds from the fruits. It is reported that the viability of the seeds can be maintained at 50 % for nine weeks by storing them at 21–30 °C (Devadas and Manomohandas, 1988).

Seeds are sown in beds of 15–20 cm height, 1 m width and convenient length made of loose soil–sand mixture over which a layer of sand (about 5–8 cm thick) is spread. Seeds are sown at 2–3 cm spacing and depth of about 2 cm. The seed bed has to be protected from direct sunlight. If only a small quantity of seeds is available for sowing, they can be sown directly in polybags filled with a soil–sand–cowdung mixture and kept in the shade.

The beds may be mulched with dried leaves or straw to hasten germination. Watering should be done regularly. Germination commences in about 9–10 days and continues over a month. All the mulching on the seed bed must be removed as the seeds start germinating. The seedlings are transplanted into polythene bags (25 cm × 15 cm) containing a mixture of soil, sand and well-decomposed cowdung (3:3:1) at the three- to four-leaf stage. The seedlings are ready for transplanting to the field at 9–10 months old, when they are 25–40 cm high.

Vegetative propagation

Allspice is polygamodioecious, and it is difficult to identify the functional male and female trees until they flower. Hence clonal propagation is necessary to obtain uniformly high-yielding trees. Cuttings of allspice could be rooted in 7–8 months with hormonal application. Air layering of softwood and semi-hardwood shoots with hormone application (indolebutyric acid 4000 ppm + naphthalene acetic acid 4000 ppm) aided in rooting of allspice (Rema *et al.* 1997, 2008). Studies on air layering in Maharashtra indicated that rooting is a slow process, taking 18–28 months and that January is the best season for rooting (Haldanker *et al.*, 1995). Propagation of allspice through chip budding is also possible although the percentage of success is low (30 %). Approach grafting of allspice was reported with 90 % success in Jamaica (Chapman, 1965). Approach grafting on its own rootstock was also successful in India.

9.3.2 Climate and soil

The natural habitat of allspice in Jamaica is limestone forest. Although allspice is planted on a wide range of soils, a well-drained, fertile, loam limestone soil with a pH of 6–8 suits the crop best. Pimento grows well in semi-tropical lowland forests with a mean temperature of 18–24 °C, a low of 15 °C and a maximum of 32 °C. Allspice flourishes well up to 1000 m above sea level. An annual rainfall of 150–170 cm evenly spread throughout the year is desirable, but allspice grows well with a rainfall of 120–250 cm.

9.3.3 Planting and after-care

The spacing recommended for allspice is 6 m × 6 m. Pits of about 60 cm deep and 30 cm wide are dug and are filled with topsoil to which well-rotted manure or compost are incorporated. Although permanent shade trees are not considered necessary for allspice, they may be required in very exposed conditions. Transplanting should be done at the beginning of the rainy season. For vegetatively propagated trees, one male tree should be planted for every ten females for adequate pollination. When trees are grown for leaves to produce oil, the sex of the tree is not important.

The base of the young seedlings should be kept free of weeds. After 3–4 years of growth, slashing once or twice annually around the tree would be sufficient. The larger weeds in the plantation may be controlled from time to time by slashing. The branches cut from the trees during harvesting can be used as mulch. Allspice has to be irrigated until it is 2 or 3 years old. Generally, fully grown trees of allspice are not irrigated. However, in a severe summer, irrigating trees on alternate days at 10 l/tree is recommended. Very little is known about the manurial requirements of pimento. As the tree is found mainly on soils derived from limestone, it is generally assumed that it is a lime-demanding plant and there are indications that the crop requires a soil relatively high in potash (Ward, 1961). A fertilizer dose of 20 g N, 18 g P_2O_5 and 50 g K_2O/tree in the first year after planting is progressively increased to 300:250:750/year for a grown tree of 15 years or more. The fertilizers are to be applied in two equal doses (May and September), in shallow trenches dug around the plant about 1.0–1.5 m away from the tree. The Department of Agriculture, Jamaica, recommends 1 kg of 10:10:10 or 15:15:15 NPK mixture applied during February and September at 0.4 kg/tree/application. Young plantations can be intercropped for 1–3 years with crops such as banana or any other low-growing plants such as pulses.

9.3.4 Harvesting, processing and storage

The clonally propagated plants start flowering in 3 years and seedlings in 5–6 years under well-managed conditions. Seedling trees take 18–20 years to come into full bearing. The berries are harvested when fully grown, but still green, about 3–4 months after flowering. The time and extent of flowering are affected by the local conditions and climate, particularly the time of onset of the spring rains, so that the time of harvesting varies between seasons and places. It normally occurs from August–September in Jamaica, July–August in Guatemala and Honduras and

September–October in Mexico. Allspice does not produce fruits in the plains. Spraying paclobutrazol was reported to induce flowering in allspice and further spraying of indoleacetic acid + benzylaminopurine induces fruit set in allspice (Krishnamoorthy *et al.*, 1995).

A healthy, well-managed tree would produce on average 10 kg green berries/tree annually after 10 years. Allspice gives a good crop once every 3 years. Care must be given while harvesting berries to be used as spice as the quality of the berry is assessed mainly on appearance, colour, flavour and essential oil content. Berries for distillation require less care.

The harvested berries are taken to the drying shed and left in heaps up to 5 days to ferment. Berries are then spread in drying yards and turned frequently to ensure uniform drying. It takes about 5–10 days for drying (12–14 % moisture content) depending upon the weather. Well-dried fruits should be brownish black in colour and rattle when a handful is shaken. About 55–65 kg berries is obtained from 100 kg green. The dried berries are cleaned and stored in a clean dry place. In Guatemala, berries are blanched in boiling water for 10 minutes. This process reduces contamination and produces an attractive colour in the dried spice. Because of frequent shifting of the berries in and out of the sheds during rainy days, many berries break and, hence, mechanical drying is preferred. Artificial drying is adopted in places where the berries mature during the rainy season. Solar energy dryers and many other simple dryers using firewood and forced air dryers are available for drying allspice. A small-scale unit of hot air drying can dry 250 kg (550 lb) of green pimento in 8 hours (Breag *et al.,* 1973). A maximum temperature of 75 °C is recommended for obtaining good-quality allspice without any loss in essential oil content. Microbial contamination is also reported to be minimum in artificial drying.

The dried fruits should be stored in poly-lined corrugated cardboard containers or in air-tight containers and kept in a cool, dry area with a maximum temperature of 21 °C and maximum humidity of 70 %. Excessive heat volatilizes and dissipates aromatic essential oils and high humidity tends to cake them. Dried fruits should be stored off the floor and away from outside walls to minimize the chances of dampness. The product has to be kept away from heavy aromatic materials. The essential oil is stored in sealed opaque containers. The industry standard has recommended a shelf-life of 24 months.

9.3.5 Diseases

Leaf rust

The most serious disease of pimento in Jamaica is the leaf rust, caused by *Puccinia psidii* Wint. The young leaves, shoots, inflorescence and young fruits are covered by a bright yellow powdery mass of urediospores in the infested trees. Severe infection results in defoliation of the young leaves, with successive attacks culminating in the death of the tree. Leaf rust has also been reported in Florida. The variety of *P. psidii* reported on allspice in Jamaica is different from that found in south Florida (Marlatt and Kimbrough, 1979). The disease is severe during late winter and early spring on flushes of new growth in Florida. The symptoms are observed on both upper and lower surfaces of the leaves. Mature leaves bear circular, brown, necrotic lesions covered with urediospores.

Die back
The tree is also affected by die back or canker, caused by *Ceratocystis fimbriata* Ell. and Halst. The disease is usually localized and spreads to other parts of the tree. Bark canker and dark streaking of the wood with drying of the leaves is observed in infected trees. When primary infection occurs below a fork in the tree, death occurs within few months. The disease can be controlled by pruning and removal of all dead and infected branches and application of 1 % Bordeaux mixture.

Leaf rot
A leaf rot disease caused by *Cylindrocladium quinqueseptatum* was reported in India. The disease is severe during June–September. The disease can be controlled by a prophylactic spraying of 1 % Bordeaux mixture in June (Anandaraj and Sarma, 1992).

9.3.6 Pests

Borer
The larvae of red borer *Zeuzera coffeae,* Nietner (*Cossidae lepidoptera*) damage allspice by tunnelling into the collar region (Abraham and Skaria, 1995). The branches wither and wilt. Swabbing the main stem with a suspension of 0.25 % carbaryl was found to be effective against the pest.

Tea mosquito
The tea mosquito *Helopeltis antonii* has been reported to attack allspice in Kerala (Devasahayam *et al*., 1986). The bug causes necrotic lesions on young shoots of allspice. The pest can be controlled by spraying quinalphos 0.05 % on tender flushes.

Leaf-damaging pests
Caterpillars of the bagworm *Oeceticus abboti* and related species feed on young leaves and shoots of allspice. Young leaves are also damaged by whiteflies, *Aleyrodidae,* and the redbanded thrips, *Selenothrips rubrocintus.* Adults of the weevils *Prepodes* spp. and *Pachnaeus* spp. also feed on leaves and their larvae damage roots. Scale insects, soft and hard, are frequently present on trees but normally do little damage (Purseglove *et al*., 1981).

Fruit fly
The fruit fly *Anastrepha suspensa* is reported to occur on allspice in Jamaica (Van Whervin, 1974) and cause damage to the berries.

9.4 Main uses of allspice

Whole spice, ground spice, berry oil, leaf oil and oleoresin are the major products obtained from pimento. The Mayans used allspice to embalm and preserve the bodies of their leaders. Allspice was more popular in the early twentieth century than it is today. It is reported that during World War II a shortage of the spice occurred in Europe and its popularity never recovered (Tainter and Grenis, 1993).

The major use of allspice is in the food industry (65–70 %). A small quantity is used for domestic purpose (5–10 %), for production of pimento berry oil (20–25 %), for extraction of oleoresin (1–2 %) and in the pharmaceutical and perfume industries.

9.4.1 Uses in the food industry

Allspice is mostly used in western cooking and is less suitable for eastern cooking. It is most used in British, American and German cooking. The dried mature fruits are mainly used as a flavouring and curing agent in processed meats and bakery products and as a flavouring ingredient for domestic and culinary purposes. Whole fruits are preferred in prepared soups, gravies and sauces. Whole ripe berries are an essential component of the local Jamaican drink *Pimento dram* and as an ingredient of the liqueurs Chartreuse and Benedictine.

Ground spice

The major use of allspice in the ground form is for flavouring processed meats, baking products, fruit cakes, pies, desserts, pickles, sauces, salads, vegetables, soups, fish, poultry, sausages, meats, marinades, mulled wine and preserves. For domestic culinary use, pimento is often mixed with other ground spices.

Oleoresin

Oleoresin is also used in the meat processing and canning industries in the same way as ground spice is used. Allspice oleoresin is prepared in very small quantities and has not become a substitute for ground spice in the food industry. However, it has an advantage over ground spice in that it avoids the risk of bacterial contamination and its strength and quality are more consistent.

Essential oil

The berry oil contains all the odour principles of the ground spice and oleoresin but lacks some of the flavour principles. Essential oils from leaf oil and berry oil are used as a flavouring agent in meat products and confectioneries. The maximum permitted level of berry oil in food products is about 0.025 %.

9.4.2 Deodorizing effect

The major function of allspice is to flavour food, but it has a sub-function of deodorizing or masking unpleasant odours. The concentration of methyl mercaptan is a major cause of bad breath, and it was observed that allspice has a deodorizing rate of 61 % (deodorizing rate is the percentage of methyl mercaptan (500 ng) captured by methanol extract).

9.4.3 Uses in traditional medicine

Allspice is not only valued as a spice to add flavour to food but has medicinal, antimicrobial, insecticidal, nematicidal, antioxidant and deodorizing properties. The powdered fruit of allspice is used in traditional medicine to treat flatulence,

dyspepsia, diarrhoea and as a remedy for depression, nervous exhaustion, tension, neuralgia and stress. In small doses it can also help to cure rheumatism, arthritis, stiffness, chills, congested coughs, bronchitis, neuralgia and rheumatism. It has anaesthetic, analgesic, antioxidant, antiseptic, carminative, muscle relaxant, rubefacient, stimulant and purgative properties (Rema and Krishnamoorthy, 1989). It is also useful for oral hygiene and in cases of halitosis. An aqueous suspension of allspice is reported to have anti-ulcer and cytoprotective activity by protecting gastric mucosa against indomethacin and various other necrotizing agents in rats (Rehaily *et al.*, 2002).

9.5 Functional properties

9.5.1 Antioxidant properties

Antioxidants help to preserve foods from oxidation and deterioration and to increase their shelf-life. They can also be used as a natural preservative. Spices and herbs are recognized as sources of natural antioxidants (Mariutti *et al.*, 2008) and thus play an important role in the chemoprevention of diseases resulting from lipid peroxidation (Chung *et al.*, 1997). Allspice has a strong hydroxyl radical-scavenging activity (Nakatani, 2000). The berries of *P. dioica*, showed strong antioxidant activity and radical-scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (Miyajima *et al.,* 2004).

The antioxidant properties of *P. dioica* essential leaf oil were assayed by study of the capacity to counteract DPPH (2,2-diphenyl-1-picrylhydrazyl), hydroxyl (OH) and superoxide radicals (Jirovetz *et al.*, 2007). The ethyl acetate-soluble part of the berries showed strong antioxidant activity and radical-scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. From the ethyl acetate-soluble part, two new compounds, 5-galloyloxy-3,4-dihydroxypentanoic acid and 5-(5-carboxymethyl-2-oxocyclopentyl)-3*Z*-pentenyl 6-*O*-galloyl-β-D-glucoside, were isolated together with 11 known polyphenols. Quercetin and its glycosides showed remarkable activity for scavenging DPPH radical and inhibiting peroxidation of liposome. Two new compounds also exhibited strong DPPH radical-scavenging activity and inhibitory effect on the peroxidation of liposome as myricetin (Oliveira *et al.*, 2009).

The antioxidant properties of the essential oil were compared to those of the synthetic antioxidant propyl gallate and it was observed that the free radical-scavenging activity of the essential oil was concentration dependent and was higher than that of the synthetic antioxidant propyl gallate (Feng Xue *et al.*, 2010). Allspice had high concentration of antioxidants (i.e. > 75 mmol/100 g)(Dragland *et al.*, 2003).

Compounds that markedly inhibit the formation of malondialdehyde from 2-deoxyribose and the hydroxylation of benzoate with the hydroxyl radical were isolated from methanol extracts of allspice. These compounds were identified as pimentol and had a strong antioxidant activity as hydroxyl radical scavengers at 2.0 μm (Oya *et al.*, 1997). A phenylpropanoid, threo-3-chloro-1-(4-hydroxyl-3-methoxyphenyl) propane-1,2-diol, isolated from berries of *P. dioica* inhibited auto-oxidation of linoleic acid in a water–alcohol system (Kikuzaki *et al.*, 1999). Allspice was screened for superoxide anion radical (O_2^-) scavenging activity and it

was observed that allspice decreased the yield of DMPO-O_2^-. The mechanism of O_2^- scavenging activity was by the inhibition of the formation of O_2 (Yun *et al*., 2003).

Four new phenolic lycosides, (2-hydroxy-3-methoxy-5-allyl) phenyl β-D-(6-*O*-*E*-sinapoyl) glucopyranoside (1), (1'*R*,5'*R*)-5-(5-carboxymethyl-2-oxocyclopentyl)-3*Z*-pentenyl β-D-(6-galloyl) glucopyranoside (2), (*S*)-α-terpinyl [α-L-(2-*O*-galloyl) arabinofuranosyl]-(1->6)-β-D-glucopyranoside (3) and (*R*)-α-terpinyl [α-L-(2-*O*-galloyl) arabinofuranosyl]-(1->6)-β-D-glucopyranoside (4), were isolated from the berries of *P. dioica* together with eight known flavonoids. All the four glycosides showed radical-scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. (Kikuzaki *et al.*, 2008). The effect of different allspice extracts (ethanol, chloroform, diethylether, benzene and hexane) on the stability of rapeseed oil was examined. The ethanol extract exhibited a remarkable antioxidant effect and the antioxidant effectiveness of various extracts was in the order ethanol extract > chloroform extract > diethylether extract > benzene extract > hexane extract (Vinh *et al.*, 2000).

9.5.2 Toxicity

Allspice oil should only be used in low dilutions since it is found to irritate the mucous membrane, owing to the presence of eugenol in allspice oil. It is also reported to cause dermal irritation. At low doses, it is non-toxic, non-irritant, non-sensitizing and nonphototoxic.

9.5.3 Fungicide

Essential oil of allspice is reported to have antifungicidal activity (Farina *et al.*, 2007). The antifungal potential of extracts of allspice was tested *in vitro* against the field fungus (*Fusarium oxysporum*) and six storage fungi (*Aspergillus candidus, A. versicolor, Penicillium aurantiogriseum, P. brevicompactum, P. citrinum* and *P. griseofulvum*) and *in situ* against the initial mycoflora of wheat grains after harvest (mainly *Fusarium* spp., *Alternaria* spp. and *Cladosporium* spp.). Allspice suppressed the growth of all the above fungus *in vitro* (Scholz *et al*., 1999). Essential oil inhibited the activity of *A. niger*, *Candida albicans*, *C. blanki*, *C. cylindracea*, *C. glabrata*, *C. krusei*, *C. tropicalis* and *Saccharomyces cerevisiae.* The minimum inhibitory concentration range for essential oil of allspice was 0.31–1.25 μL mL^{-1} (Kamble and Patil, 2008).

9.5.4 Bactericide

Allspice had a strong bactericidal effect against *Yersinia enterocolitica* (Bara and Vanetti, 1995). The minimum inhibitory concentrations (%) of hexane extracts of allspice for several pathogenic bacteria are given in Table 9.14. (Hirasa and Takemasa, 1998). A study testing thymol (thyme and oregano), eugenol (clove, pimento and cinnamon), menthol and anethole (anise and fennel) on three pathogenic bacteria, *Salmonella typhimurium, Staphylococcus aureus* and *Vibrio parahaemolyticus*, showed that all these spice components inhibited the bacteria to

Table 9.14 Minimum inhibitory concentration of hexane extracts of allspice for pathogenic bacteria

Bacteria	Minimum inhibitory concentration (%)
Escherichia coli	10
Salmonella sp.	>10
Staphylococcus aureus	10
Bacillus cereus	10
Camphytobacter	10

Source: Hirasa and Takemasa (1998).

different extents. Eugenol was more active than thymol, which was more active than anethole. Eugenol is also sporostatic to *Bacillus subtilis* at 0.05–0.06 % level (Tainter and Grenis, 1993).

Allspice was also reported to suppress *Escherichia coli, S. enterica* and *Listeria monocytogenes* (Friedman *et al.,* 2002). Essential oil was found to reduce the intracellular and extracellular verocytotoxin produced by *E. coli* (Takemasa *et al.*, 2009). Edible films made from fruits or vegetables containing essential oils can be used commercially to protect food against contamination by pathogenic bacteria. Apple-based films with allspice were active against three foodborne pathogens namely, *E. coli, S. enterica*, and *L. monocytogenes* both by direct contact with the bacteria and indirectly by vapours emanating from the films (Du *et al*., 2009).

9.5.5 Insecticide

Allspice is reported to have insecticidal properties. The effect of 103 plant powders on the mortality and emergence of adults of *Sitophilus zeamais* and *Zabrotes subfasciatus* was evaluated in the laboratory. Powdered allspice caused > 20 % mortality of *S. zeamais*. Allspice oils at all concentrations inhibited egg hatch of *Corcyra cephalonica* compared with the control (Bhargava and Meena, 2001). Essential oil of allspice had very strong insecticidal activities against the Japanese termite, *Reticulitermes speratus* Kolbe (Seo *et al.*, 2009).

9.5.6 Nematicide

The nematicidal activity of the essential oil of allspice (*P. dioica* L. Merr.) leaves and its major constituent eugenol was tested against *Meloidogyne incognita.* The essential oil and eugenol exhibited promising nematicidal activity at 660 µg/ml (Leela and Ramana, 2000). Essential oil of allspice was effective against the pine wood nematode *Bursaphelenchus xylophilus* (Park *et al*., 2007).

9.5.7 Perfumery

The oil is used in perfumery, notably for oriental fragrances. It is used as a fragrance component in perfumes, cosmetics, soaps and aftershaves.

9.6 Quality issues and adulteration

9.6.1 Specifications

Cleanliness, safety issues (microbes and moisture levels) and economic parameters aroma, flavour and granulation) are the main quality aspects dealing with spice. The cleanliness specifications have been set out in laws such as Food and Drug Administration Defect Action Levels (FDADALs) (USA) or in trade practices such as the American Spice Trade Association (ASTA), European Spices Association (ESA), etc.

Description

As per the ISO specifications, allspice is described as the dried, fully mature but unripe, whole berry of *P. dioica* (L.) Merrill, 6.5–9.5 mm in diameter, a dark brown colour, the surface somewhat rough and bearing a small annulus formed by the remains of the our sepals of the calyx. Allspice may also be in the pure ground form.

Odour and taste

The odour and taste of pimento, either whole or ground, shall be fresh, aromatic and pungent. It shall be free from any foreign taste or odour, including rancidity or mustiness.

Freedom from moulds, insects, etc.

Allspice, whole or ground, shall be free from living insects and moulds and shall be practically free from dead insects, insect fragments and rodent contamination visible to the naked eye with such magnification as may be necessary in any particular case. In case of dispute, the contamination of ground pimento shall be determined by the method specified in ISO 1208 (ISO, 1982).

Extraneous matter

All that does not belong to the fruits of allspice and all other extraneous matter of animal, vegetable and mineral origin shall be considered as extraneous matter. Broken berries are not considered as extraneous matter. The total percentage of extraneous matter in whole dried allspice shall not be more than 1 % (m/m) when determined by the method described in ISO 927 (ISO, 2009).

Product and cleanliness specification

The standard specifications of various countries for berries, leaf oils and berry oil of allspice are given in (Tables 9.15–9.20) and the cleanliness specifications are given in Tables 9.21 and 9.22.

9.6.2 Sampling

Sampling shall be carried out in accordance with the method specified in ISO 948 (ISO, 1980).

Table 9.15 Chemical requirements of allspice

Characteristics	Requirement		Methods of test
	Whole	Ground	
Moisture content, % (m/m), max.	12	12	ISO 939
Total ash, % (m/m) on dry basis, max.	4.5	4.5	ISO 928
Acid insoluble ash, % (m/m) on dry basis, max.	0.4	0.4	ISO 930
Volatile oil, % (ml/100 g) on dry basis, min.			ISO 6571
Group A, more than	3	2	
Group B, min.	2	1	
max.	3	2	
Non-volatile ether extract, % (m/m) on dry basis, max.	–	8.5	ISO 1108
Crude fibre, % (m/m) on dry basis, max.	–	27.5	ISO 5498

Source: Purseglove *et al.* (1981).

Table 9.16 US government standard specifications for allspice

Moisture, not more than	10 %
Total ash, not more than	5 %
Acid-insoluble ash, not more than	0.3 %
Volatile oil, ml per 100 g, not less than	3
Sieve test	
US standard sieve size	No. 25
Percentage required to pass through, not less than	95

Source: Purseglove *et al.* (1981).

Table 9.17 Canadian government standard specifications for allspice

Total ash, %, not more than	6.0
Ash insoluble in HCl, %, not more than	0.4
Crude fibre, %, not more than	25
Quercitannic acid, calculated from the total oxygenabsorbed by the aqueous extract, % not less than	8

Source: Purseglove *et al.* (1981).

Table 9.18 European Spice Association (ESA) product specification for allspice

Product	Ash % w/w (max.)	Acid insoluble ash % w (max.)	Moisture % w/w (max.)	Volatile oil % v/w (min.)
Jamaica	5 (ESA)	0.4 (ISO)	12 (ISO)	3.5 (ISO)
Other origins	5 (ESA)	1 (ESA)	12 (ISO)	2 (ESA)

ISO = International Organization for Standardization.
Source: Sivadasan and Kurup (1998).

Table 9.19 British Standards Institute specifications for allspice

	Berry oil	Leaf oil
Apparent density, g/ml at 20 °C	1.025 to 1.045	1.037 to 1.050
Optical rotation at 20 °C	0 °C to –5 °C	–
Refractive index at 20 °C	1.526 to 1.536	1.531 to 1.536
Phenolic* % volume, minimum	65	80
Solubility in ethanol at 20 °C (70 % v/v)	2 volumes	2 volumes

* Determined by absorption with 5 % KOH.
Source: Purseglove *et al.* (1981).

Table 9.20 Essential oil association of the USA specification

	Berry oil EOA No. 255	Leaf oil EOA No. 73
Specific gravity at 25 °C	1.018 to 1.048	1.018 to 1.048
Optical rotation at 20 °C	0° to –4 °C	–0°30' to –2°
Refractive index at 20 °C	1.527 to 1.540	1.5319 to 1.5360
Phenols*, % by volume, minimum.	65	50 to 91
Solubility in 70 % alcohol at 25 °C	2 volumes	2 volumes

* Determined by absorption with 1N KOH.
Source: Purseglove *et al.* (1981).

Table 9.21 American Spice Trade Association (ASTA) cleanliness specifications for allspice

Total extraneous matter, determined by sifting and by hand picking, % by weight	0.5
Mammalian excreta, mg/lb	2.0
Other excreta, mg/lb	5.0
Whole insects, dead (by count) per lb	2.0
Insect-bored or otherwise defiled berries, % by weight	1.0
Mouldy berries, % by weight	2.0

Source: Sivadasan and Kurup (1998).

Table 9.22 Dutch regulations regarding cleanliness for allspice

Ash content (max %)	6.0
Sand content (max %)	1.5

Source: Sivadasan and Kurup (1998).

9.6.3 Packing

Allspice, whole or ground, shall be packed in clean and sound containers made of a material that does not affect the product but that protects it from the increase or loss of moisture and volatile matter. The packaging shall also comply with any national legislation relating to environmental protection.

Table 9.23 Maximum permissible limits of trace metals in allspice

Metal	Concentration (in ppm)
Aluminium	73
Arsenic	0
Barium	4.8
Beryllium	0.037
Bismuth	0
Boron	8.6
Cadmium	0
Copper	5.1
Lead	0
Lithium	0
Magnesium	1300
Manganese	11
Molybdenum	0.4
Nickel	0.57
Selenium	0.16
Silicon	18
Strontium	2.4
Tin	7.8
Titanium	1.6
Zinc	9.4

Source: Sivadasan and Kurup (1998).

9.6.4 Marking

The following particulars shall be marked directly on each package or on label attached to the package: name of the product (type: whole or ground) and trade name; name and address of the producer or packer and trademark, if any; code or batch number; net mass; grade; producing country; any other information requested by the purchaser, such as the year of harvest and date of packing (if known).

9.6.5 Pesticide residues

The limits for pesticide residue prescribed for other agricultural products are generally followed for spices. Maximum permitted limits of trace metals in allspice are given in Table 9.23.

9.6.6 Adulteration

Ground pimento is sometimes adulterated with powdered clove stem or with the aromatic berries of the Mexican tree *Myrtus tobasco*, known as 'pimienta de tobasco'. The powdered berries of the aromatic shrub *Lindera benzoin* (called wild allspice) has a strong spicy flavour in bark and berries and is used as a substitute for allspice by the Americans. A mixture of pimento leaf oil and clove stem and leaf oils can serve as a relatively inexpensive substitute for berry oil. Pimento berry oil is sometimes adulterated with eugenol from cheaper sources. Samples are also considered adulterated or of poor quality for trade if they contain an average of 30 or more

insect fragments per 10 g, or an average of one or more rodent hairs per 10 g or an average of 5 % or more mouldy berries by weight.

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10

Capers and caperberries

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Abstract: This chapter on capers and caperberries gives a detailed account of the plant description, distribution, important cultivars, chemical composition, flavour volatile profiles, cultivation practices, reproductive biology, propagation, production technology, caper grading system and post-harvest technology. It also deals with its uses in food, processing, functional and health benefits, nutritional properties, health-promoting and therapeutic characteristics, quality issues and future trends.

Key words: *C. orientalis*, *C. sicula*, *C. spinosa*, caper bush, caperberries, *Capparis*, cultivation, flavour, glucosinolate, orchard establishment, pests and diseases, plant nutrition, postharvest technology; reproductive biology, volatile profiles.

10.1 Introduction

The caper bush (*Capparis spinosa* L., Capparidaceae) is a perennial winter-deciduous species that bears rounded, fleshy leaves and large white to pinkish white flowers. It is widespread in Mediterranean Europe, Africa, Asia and Australia. Its young flower buds, known as capers, are used for food seasoning, and different parts of the plant are used in the manufacture of medicines and cosmetics (Sozzi, 2001; Rivera *et al*., 2003). The plant is also known for its fruit (caperberry) which are usually consumed pickled. Other species of *Capparis* are also picked along with *C. spinosa* for their buds or fruits (Bouriche *et al*., 2011).

The economic importance of the caper plant led to a significant increase in both the area under cultivation and production levels during the late 1980s. The main production areas are in harsh environments found in Morocco, the south eastern Iberian peninsula, Turkey and the Italian islands of Pantelleria and Salina. The species has developed special mechanisms in order to survive in Mediterranean conditions, and introduction in semi-arid lands may help to prevent the disruption of the equilibrium of those fragile ecosystems. This drought-tolerant perennial plant has a favourable influence on the environment and it is utilized for landscaping and reducing erosion along highways, steep rocky slopes, sand dunes or fragile semi-arid ecosystems (Lozano Puche, 1977). The caper plant has low flammability and may

play a role in cutting down forest fires (Neyişçi, 1987). It favours rural economies in marginal lands in many Mediterranean countries and neighbouring regions: Turkey, Morocco, south eastern Spain, Italy (especially the Mediterranean island of Pantelleria, the Aeolian island of Salina, and Sicily), Tunisia, France (Provence), Greece, Algeria, Egypt, Asia Minor, Cyprus and the Levant. Whether the species is indigenous to the Mediterranean or not is still unknown (Zohary, 1960). Considerable genetic variation for the caper bush and its relatives exists, mainly in dry regions in west or central Asia. The genus *Capparis* could be of a subtropical or tropical origin later naturalized in the Mediterranean basin (Pugnaire, 1989).

The caper bush is a perennial shrub 30–50 cm tall. Its roots can be 6–10 m long (Reche Mármol, 1967) accounting for 65 % of the total biomass (Singh *et al*., 1992). Caper canopy is made up of four to six radial decumbent branches from which many secondary stems grow. In wild bushes, Singh *et al*. (1992) observed up to 47 branches per plant. Branches are usually 2–3 m long. Stipular pale yellowish spines are often hooked and divaricate but sometimes weakly developed or absent. Leaves are alternate, 2–5 cm long, simple, ovate to elliptic, thick and glistening, with a rounded base and a mucronate, obtuse or emarginate apex. Flower bud appearance is continuous so that all transitional stages of development, from buds to fruit, can be observed simultaneously. The first ten nodes from the base are usually sterile and the following ten only partially fertile; the subsequent nodes have a caper each, almost to the tip of the stem. Flowers are hermaphroditic, 5–7 cm across, axillary and solitary, with purplish sepals and white petals. Stamens are numerous, with purplish filaments. The gynophores are approximately as long as the stamens. The ovary is superior, one-locular, with five to ten placentas. The fruit (caperberry) is ellipsoid, ovoid or obovoid, with a thin pericarp. The fruit bursts when ripe, exposing many seeds embedded in a pale crimson flesh. Seeds are 3–4 mm across, grey-brown and reniform. The embryo is spirally in-curved. Germination is epigaeal. A thousand seeds weigh 6–8 g (Gorini, 1981; Akgül and Özcan, 1999; Li Vigni and Melati, 1999).

The caper bush is the most important member of the Capparidaceae in economic terms. It has been suggested that *Capparis* and its relatives form a basal paraphyletic complex within the Brassicaceae group (Zomlefer, 1994; Judd *et al*., 2007) on the basis of molecular (Rodman *et al.*, 1993) and morphological (Judd *et al.*, 1994) cladistic analyses. Taxonomists have long agreed that the caper family is very closely related to Brassicaceae based on some major shared characters, particularly the original bicarpellate ovary with parietal placentae, the vacuolar and utricular cysternae of the endoplasmic reticulum, the presence of myrosin cells and glucosinolate production. Species identification in the highly variable *Capparis* genus is difficult; the continuous flux of genes (Jiménez, 1987) throughout its evolution has made it hard to reach conclusions in the field of systematics. Besides, there have been divergent opinions concerning the rank assigned to the different taxa and to their subordination (Zohary, 1960; Jacobs, 1965; St. John, 1965; Bokhari and Hedge, 1975; Rao and Das, 1978; Higton and Akeroyd, 1991; Fici and Gianguzzi, 1997; Rivera *et al*., 1999; Fici, 2001). *C. spinosa* is morphologically closely related to *C. orientalis* Duhamel and *C. sicula* Duhamel (Inocencio *et al*., 2005), and some authors have included those taxa as belonging to *C. spinosa* (Higton and Akeroyd, 1991; Fici, 2001).

Identification and characterization of cultivars and species have traditionally been based on morphological and physiological traits. However, such traits are not

always available for analysis and are affected by varying environmental conditions. Molecular marker technology offers several advantages over just the use of phenotypic traits. Molecular markers developed for *Capparis* are also a powerful tool for phylogenetic studies. Genetic variation in capers from Italy and Tunisia was estimated by means of random amplified polymorphic DNA techniques (Khouildi *et al*., 2000). On the basis of amplified restriction fragment length polymorphism fingerprinting, Inocencio *et al*. (2005) suggested that *C. spinosa* could be a cultigen-derived form of *C. orientalis* with some introgression from *C. sicula*.

10.2 Chemical composition

A considerable body of literature exists on the phytochemical constituents of caper bush, capers and caperberries (reviewed by Sozzi, 2001). The chemical composition of capers and caperberries is affected by the genotype, harvest date, size, environmental conditions and preservation procedures (Nosti Vega and Castro Ramos, 1987; Rodrigo *et al.*, 1992; Özcan and Akgül, 1998; Özcan, 1999a, b; Inocencio *et al*., 2000). Capers and caperberries are a good source of K, Ca, S, Mg, and P (Özcan, 2005) (Table 10.1). High salt brine treatments greatly affect their chemical composition. Protein and fibre, as well as mineral (Mg, K, Mn) and vitamin (thiamine, riboflavin, ascorbic acid), contents drop during preservation procedures, while ash increases due to the addition of NaCl.

Both capers and caperberries are rich in unsaturated fatty acids. Oleic, linoleic and linolenic acid represent 58–63.5 % of total fatty acids in flower buds (Nosti Vega

Table 10.1 Proximate composition of raw *Capparis spinosa* fruit and flower bud

Constituent	Fruits (caperberries) (%)	Flower buds (capers) (%)
Water (%)	79.6[A]; 82.7[B]	78.4[C]; 76.8–80.3[D]
Protein (%)	4.6[A]; 3.34[B]	6.31[C]; 4.59–6.79[D]
Lipid (fat) (%)	3.6[A]	0.47[C]; 1.51–1.77[D]
Carbohydrate (%)	3.2[A]	–
Fibre (%)	7.2[A]	2.0[C]; 4.5–5.9[D]
Ash (%)	1.8[A]	1.7[C]; 1.33–1.84[D]
Rutin (%)	–	0.28[C]
Minerals		
Calcium (mg/100 g)	28[A]	183[C]; 49–134[D]
Iron (mg/100 g)	0.9[A]; 0.54[B]	1.37[C]; 0.9–2.1[D]
Magnesium (mg/100 g)	39[A]	57[C]; 46.9–81.1[D]
Manganese (mg/100 g)	0.72[B]	0.29[C]
Phosphorus (mg/100 g)	116.8[B]	103.6[C]; 16.6–26.4[D]
Potassium (mg/100 g)	383[A]; 326.9[B]	504.9[C]; 502.4–598.3[D]
Sodium (mg/100 g)	18[A]; 12.1[B]	5.9[C]; 19–28.5[D]
Vitamins		
Ascorbic acid (mg/100 g)	23[A]	26[E]
Thiamine (mg/100 g)	0.69[A]	0.7[C]
Riboflavin (mg/100 g)	–	0.22[C]

Sources: [A]Brand and Cherikoff (1985); [B]Özcan (1999b); [C]Nosti Vega and Castro Ramos (1987); [D]Rodrigo *et al*. (1992); [E]Lemmi Cena and Rovesti (1979).

and Castro Ramos, 1987; Rodrigo *et al*., 1992) and 73 % in fruit (Özcan, 1999b). The oil content of the seeds ranges from 27.3–37.6 % in *C. spinosa* and from 14.6–38.0 % in *C. ovata*, linoleic being the main fatty acid in both species (25–50 %; Matthäus and Özcan, 2005). These authors found that seed oils show high contents of Δ^5-avenasterol (138.8–599.4 mg/kg); this compound has been suggested as an antioxidant and antipolymerization agent in cooking oils.

Capers are a good source of natural antioxidants. Antioxidant effectiveness of caper methanolic extracts is conserved even after removal of glucosinolates, thus suggesting that the radical scavenging properties of capers are mainly due to other metabolites such as phenolic compounds and flavonoids (Germanò *et al*., 2002) (Table 10.1): rutin (quercetin 3-rutinoside), quercetin 7-rutinoside, quercetin 3-glucoside-7-rhamnoside, kaempferol-3-rutinoside, kaempferol-3-glucoside, and kaempferol-3-rhamnorutinoside (Rochleder and Hlasiwetz, 1852; Zwenger and Dronke, 1862; Ahmed *et al*., 1972a; Tomás and Ferreres, 1976a, b; Ferreres and Tomás, 1978; Artemeva *et al*., 1981; Rodrigo *et al*., 1992; Sharaf *et al*., 1997; Inocencio *et al*., 2000). Rutin and kaempferol-3-rutinoside are probably the most abundant flavonoids, followed by kaempferol-3-rhamnorutinoside in significantly lower concentrations (Rodrigo *et al.*, 1992; Sharaf *et al*., 1997). Sharaf *et al*. (2000) identified a quercetin triglycoside (quercetin 3-*O*-[6‴-α-L-rhamnosyl-6″-β-D-glucosyl]-β-D-glucoside) in methanolic extract of the aerial part of the caper bush. Two different 1*H*-indole-3-acetonitrile glycosides, as well as (6*S*)-hydroxy3-oxo-α-ionol glucosides, have been isolated in methanolic extracts of caperberries (Çaliş *et al.*, 1999, 2002). Total flavonoids are greatly variable (1.82–7.85 mg/g) (Inocencio *et al*., 2000). A serving of capers (10 g) will provide 65 mg flavonoid glycosides or its equivalent, 40 mg quercetin as aglycone (Inocencio *et al*., 2000).

Capers are rich in glucosinolates whose hydrolysis to glucose, sulphuric acid and isothiocyanates is catalyzed by the enzyme myrosinase. Guignard (1893b) first reported the presence of this enzyme in *C. spinosa*. Isothiocyanates are well known for the important role they play in plant defence mechanisms, and also in human health as cancer-preventing agents (Verhoeven *et al*., 1997). The high levels of glucosinolates found in caper buds are only comparable with those of Brussels sprouts; other widely-consumed glucosinolate-containing vegetables such as cabbage or broccoli show lower amounts (Matthäus and Özcan, 2002). Brassicaceae are usually considered a major source of glucosinolates (Kjoer, 1963; Kjoer and Thomsen, 1963; Rosa *et al*., 1997). The presence of glucosinolates is synapomorphic for members of this family and lends additional support to the new phylogenetic classification (Judd *et al*., 2007). In fact, the conclusion that Capparidaceae and Brassicaceae should remain together, based on the presence of glucosinolates, was drawn over 50 years ago (Hegnauer, 1961; Kjoer, 1963).

Methyl glucosinolate (glucocapparin) is the most common glucosinolate in the *Capparis* genus (Ahmed *et al*., 1972b). Moreover, it accounts for 90 % of the total glucosinolates in *C. spinosa* buds (Matthäus and Özcan, 2002). Nevertheless, other glucosinolates have also been detected in and isolated from caper plants. Those include 2-propenyl glucosinolate (sinigrin), 3-methylsulfinylpropyl glucosinolate (glucoiberin), indol-3-ylmethyl glucosinolate (glucobrassicin), and 1-methoxyindol-3-ylmethyl glucosinolate (neoglucobrassicin) (Ahmed *et al*., 1972a; Matthäus and Özcan, 2002). There are qualitative and quantitative differences in glucosinolate

composition in different caper tissues (Schraudolf, 1989; Matthäus and Özcan, 2002), as happens with most glucosinolate-containing species (Rosa *et al*., 1997). Methyl glucosinolate was reported to be present at levels in the range of 38–268 mg/kg in capers treated with dry salt, brine or oil (Sannino *et al*., 1991). Interference in the determination of dithiocarbamate residues in capers has been reported and seems to be due to the presence of methyl glucosinolate (Sannino *et al*., 1991). However, thiocyanates and isothiocyanates (odoriferous breakdown products of glucosinolates), as well as other volatile compounds, do not interfere in those pesticide tests (Brevard *et al*., 1992).

The flavour volatile profile of capers is complex. Analysis of the volatiles present in the pickled flower buds indicated at least 160 different components (Brevard *et al.*, 1992). The nature of the volatiles involved is also very diverse and includes esters, aldehydes, alcohols and other chemical groups. Elemental sulphur (S_8) was identified in the volatile fraction of capers, in addition to sulphur-containing compounds (e.g., thiocyanates and isothiocyanates) and raspberry-like components (α-ionone, β-ionone, frambinone, zingerone). Also, the main constituents of the caperberry volatile oil are isopropyl isothiocyanate (~52 %) and methyl isothiocyanate (~42 %) (Afsharypuor *et al*., 1998).

10.3 Cultivation of capers and caperberries

10.3.1 Environmental requirements

The caper bush requires a semi-arid climate. Mean annual temperatures in areas under cultivation are over 14 °C and rainfall varies from 200 mm/year in Spain to 460 in Pantelleria Island and 680 in Salina Island (Barbera, 1991). The caper bush can withstand strong winds and temperatures over 40 °C in summer, but it is sensitive to frost during its vegetative period. It survives low temperatures and has been found at 1000 m above sea level in the foothills of the Alps, although it is usually grown at lower altitudes (Barbera *et al*., 1991).

The caper bush is a rupiculous species adapted to xeric areas. It is widespread on rocky areas and is grown on different soil associations, including alfisols, regosols and lithosols (Barbera, 1991; Fici and Gianguzzi, 1997). In different Himalayan and trans-Himalayan locations, *C. spinosa* tolerates both silty clay and sandy, rocky or gravelly surface soils, with less than 1 % organic matter (Ahmed, 1986; Kala and Mathur, 2002). It grows on bare rocks, crevices, cracks and sand dunes in Pakistan (Ahmed and Qadir, 1976), the Adriatic region (Lovric, 1993) and Egypt, Libya and Tunisia (Ayyad and Ghabbour, 1993), in transitional zones between the littoral salt marsh and the coastal deserts of the Asian Red Sea coast (Zahran, 1993), in the rocky arid bottoms of the Jordan valley (Turrill, 1953), in calcareous sandstone cliffs at Ramat Aviv, Israel (Randall, 1993) and in coastal dunes of Australia (Specht, 1993) and Israel (Levin and Ben-Dor, 2004). It also grows spontaneously in wall joints of buildings, antique constructions and monuments (Sozzi, 2001, and references cited therein).

Deep and well-drained soils with sandy to sandy-loam textures are favoured (Barbera and Di Lorenzo, 1982, 1984; Ahmed, 1986; Özdemir and Öztürk, 1996), although caper bush adapts to calcareous accumulations or moderate percentages

of clay (González Soler, 1973). It shows a good response to volcanic (Barbera and Di Lorenzo, 1982) or gypseous soils (Font Quer, 1962) but is sensitive to poorly drained soils. Soil pH between 7.5 and 8 is optimum (Gorini, 1981), although pH values from 6.1 to 8.5 are tolerated (Duke and Terrel, 1974; Duke and Hurst, 1975; Ahmed, 1986). The caper bush is usually not considered to be a halophyte, but it has been detected in the loamy solonchacks of the coastal lowlands of Bahrain, where the conductivity may reach 54 dS/m (Abbas and El-Oqlah, 1992).

Aerosols from sea-water-fed cooling towers produced leaf chlorosis or necrosis, probably due to chloride toxicity (Polizzi *et al*., 1995). In contrast, caper bush withstands chronic levels of some other toxic gaseous pollutants. Krishnamurthy *et al*. (1994) reported an unusual 93 % retention of leaves when caper bush was exposed to a mixture of sulphur dioxide, oxides of nitrogen, ammonia and suspended particulate matter, although the photosynthetic area per leaf was reduced by 61 % and the fresh weight by 67 %.

The caper bush has developed a series of features and mechanisms that reduce the impact of high radiation levels, high daily temperature and insufficient soil water during its growing period (Rhizopoulou, 1990; Levizou *et al*., 2004). *C. spinosa* has devolved a very effective system to offset limited water resources (deep roots and highly conductive wood). It is a stenohydric plant (Rhizopoulou *et al*., 1997) with a highly specialized conducting tissue (Psaras and Sofroniou, 1999) and also thick amphistomatous and homobaric leaves bearing a multi-layered mesophyll, thick outermost epidermal cell walls and small leaf intercellular cell space percentage (Rhizopoulou and Psaras, 2003). Levizou *et al*. (2004) found that *C. spinosa* assimilates up to 3.4 times more CO_2 per m^2 during its growth period than other species in Mediterranean ecosystems. Caper bush also displays characteristics of a plant adapted to poor soils (Pugnaire and Esteban, 1991). Its high root/shoot ratio and the presence of mycorrhizae serve to maximize the uptake of minerals in poor soils. Different N_2-fixing bacterial strains have been isolated from the caper bush rhizosphere playing a role in maintaining high reserves of that growth-limiting element (Andrade *et al*., 1997).

10.3.2 Reproductive biology

Caper bush is a perennial plant with a relatively short juvenile period. The biotype Mallorquina can yield 1 kg/plant in the second year of cultivated growth. Temperature is the main environmental factor affecting caper bush flowering. A positive correlation between temperature and productivity has been observed (Luna Lorente and Pérez Vicente, 1985). Fertility of the nodes is maximum (close to 100 %) during the hottest periods and lower at the beginning and end of the season (Barbera *et al*., 1991). *C. spinosa* is night flowering (Petanidou *et al*., 1996). The white petals open concomitantly with increasing relative humidity and declining temperature and exposure to sunlight (Rhizopoulou *et al*., 2006). It blossoms for approximately 16 hours, from *c*. 18:00 h to *c*. 10:00 h the next morning (Ivri, 1985; Petanidou *et al*., 1996) and most nectar secretion is nocturnal.

Caper flowers attract different insects, among them hawk-moths and bees (Kislev *et al*., 1972; Eisikowitch *et al*., 1986; Dafni *et al*., 1987; Dafni and Shmida, 1996). In Greece, flowers are mainly pollinated by bees (Petanidou, 1991). *C. spinosa* has not

evolved specific mechanisms to prevent self-pollination. Nevertheless, the flower architecture, anthesis, colour and odour indicate that self-pollination is not regularly found in caper bush. *C. spinosa* is an important nectar source for pollinators in semi-arid ecosystems (Eisikowitch *et al*., 1986), since it grows and flowers entirely during the most stressful period of the year, when the surrounding flora exhibits minimum growth rates. This performance provides *C. spinosa* with a competitive advantage against other species (Rhizopoulou *et al*., 2006). Flower reward in genus *Capparis* is affected by the location and year (Petanidou *et al*., 1996) and differs significantly among taxa. *C. aegyptia* has a higher pollen grain weight and its nectar is richer in total amino acids (Eisikowitch *et al*., 1986). On the other hand, higher nectar concentration and volume are found in *C. ovata* (Eisikowitch *et al*., 1986; Dafni *et al*., 1987). Amino acid content and concentration, as well as hexose concentration, increase with flower age while sucrose concentration decreases (Petanidou *et al*., 1996).

The juicy fruit is consumed by birds (Seidemann, 1970; Danin, 1983) like *Sylvia conspicillata*, *Oenanthe leucura* (Hódar, 1994) and *Chlamydotis* (*undulata*) *macqueenii* (van Heezik and Seddon, 1999) that disperse the seeds. Harvester ants (Luna Lorente and Pérez Vicente, 1985; Li Vigni and Melati, 1999) and lizards like *Lacerta lepida* (Hódar *et al.*, 1996) feed on the fruit and carry off fragments together with the hardcoated seeds. Wasps are attracted by mature caperberry scent and also act as dispersal agents (Li Vigni and Melati, 1999).

10.3.3 Propagation

Caper bush yields a large amount of seeds per generative shoot. Poor caper seed germination performance has been observed in Argentina (Sozzi and Chiesa, 1995), Armenia (Ziroyan, 1980), Cyprus (Orphanos, 1983), India (Singh *et al*., 1992), Italy (Cappelletti, 1946; Barbera and Di Lorenzo, 1984; Macchia and Casano, 1993), Spain (Reche Mármol, 1967; Luna Lorente and Pérez Vicente, 1985; Pascual *et al*., 2003, 2004), Turkey (Yildirim, 1998; Söyler and Arslan, 1999; Tansi, 1999) and the USA (Stromme, 1988; Bond, 1990). However, caper bush propagation is usually carried out by seed owing to the serious rooting problems associated with cuttings. Low germination percentages (5–15 %) are obtained within 2–3 months of seeding. Different treatments have been used to improve the germination percentage, including mechanical scarification (sand paper, ultrasound, etc.), stratification, soaking in concentrated H_2SO_4 or H_2O_2, or in 0.2 % $KMnO_4$, 0.2 % KNO_3, gibberellin (GA_4+7) or gibberellic acid (GA_3) aqueous solutions, and manipulation of the environmental conditions (light/dark, temperature) (Reche Mármol, l967; Ministerio de Agricultura, 1980; Orphanos, 1983; Singh *et al*., 1992; Macchia and Casano, 1993; Sozzi and Chiesa, 1995; Yildirim, 1998; Söyler and Arslan, 1999; Tansi, 1999). A soaking period of 30 days or longer enhances seed germination: final germination values range from 95–99 %, reducing the time to reach 50 % of final germination and consequently the duration of germination tests (Pascual *et al*., 2009).

Caper seed germination depends on the covering structures (Sozzi and Chiesa, 1995). The seed of the genus *Capparis* is bitegmic (Corner, 1976). The testa is 0.2–0.3 mm thick, with all its cell walls somewhat lignified; its tegmen consists of an outer fibrous, lignified layer four to ten cells thick, with a lignified endotegmen

composed of contiguous cuboid cells, with strongly thickened radial walls. Only the mesophyll between exo- and endotegmen is unlignified (Guignard, 1893a; Corner, 1976). As the integrity of the covering structures is very important for dormancy persistence in caper seeds, the seed coats are very likely to be the main cause for the seed low germination rate (Sozzi and Chiesa, 1995). A physiological dormancy could also explain the response to GA_3 (Pascual *et al*., 2004). Nevertheless, the viable embryos germinate within 3–4 days after partial removal of the lignified seed coats (Sozzi and Chiesa, 1995), while GA_3-treated seeds germinate within 20–70 days (Pascual *et al*., 2004).

The seed coats and the mucilage surrounding the seeds may be ecological adaptations to avoid water loss and conserve seed viability during the dry season (Scialabba *et al*., 1995). Seeds lie without order in the pericarp, each of them surrounded by an adherent layer of pulp. They can be obtained by rubbing and washing followed by drying in the shade. Large or medium-size fruits set in the central or apical region of the stems are adequate sources of dull brown mature seeds (Pascual *et al*., 2003). Those seeds are over 90 % viable (Orphanos, 1983; Sozzi and Chiesa, 1995; Tansi, 1999) for 2 years if held at 4 °C and low relative humidity. Seeds obtained from small not-yet-opened fruits are generally light brown and immature. The final germination percentage is also affected by fruit position on the plant and fruit weight (Pascual *et al*., 2003). Commercial lots of seed are usually pre-germinated in February or March in boxes or bins (Luna Lorente and Pérez Vicente, 1985). Seeds are packed in moist river sand, or compost made of two parts turfy loam and one part leaf mould and sand, or in mixtures with vermiculite or perlite (Foster and Louden, 1980; Kontaxis, 1989). Small lots can be pre-germinated in boxes; moderate to large lots are usually pre-germinated in bins located in a protected place. Two to four layers of seed are packed in each bin and covered with a sand layer. Seeds are sprinkled with water and treated with captan or captafol. Sprouted seeds are obtained and planted after 25–50 days. After proper cultivating, seeds (1.5–2 g/m) are planted about 1.5 cm deep, in rows 30 or 40 cm apart. Yields of 45–50 seedlings per metre may be obtained after 30 days.

Caper bush is a difficult-to-root woody species and successful propagation requires careful consideration of biotypes and seasonal and environmental parameters. Rooting percentages up to 55 are possible when using 1-year-old wood, depending on cutting harvest time and substrate utilized (Pilone, 1990a). Propagation from stem cuttings is the standard method for growing 'Mallorquina' and 'Italiana' in Spain and 'Nocella' in Salina. Hardwood cuttings vary in length from 15–50 cm and the diameter of the cuttings may range from 1–2.5 cm. Another possibility is to collect stems during February through the beginning of March, treat them with captan or captafol and stratify them outdoors or in a chamber at 3–4 °C, covered with sand or plastic. Moisture content and drainage should be carefully monitored and maintained until planting (Luna Lorente and Pérez Vicente, 1985). Softwood cuttings are prepared from 25- to 30-day shoots, each cutting containing at least two nodes. Cuttings are planted in a greenhouse under a mist system with bottom heat; 150–200 cuttings m^{-2} may be planted. Dipping the cutting basal end into 1500–3000 mg/l auxin solution may enhance rooting (Pilone, 1990b), but results depend on the type of cutting. Hardwood cuttings do not seem to respond to indole-3-butyric acid or α-naphthaleneacetic acid (NAA) pre-treatments. On the other

hand, dipping the herbaceous cutting base in a 2000 ppm NAA yielded rooting percentages of 83 % (Luna Lorente and Pérez Vicente, 1985).

Successful *in vitro* culture was achieved from nodal shoot segments. 6-benzylaminopurine stimulated proliferation and shoot development; when combined with indoleacetic acid (IAA) and GA_3, formation of proliferating clusters was enhanced (Rodríguez *et al*., 1990). High rooting response was obtained by using 30 μM IAA (Rodríguez *et al*., 1990). The presence of abnormal vitrified shoots was observed in some cases and could be prevented by means of alternate culture in cytokinin-enriched and hormone-free media, or normalized by using sucrose-enriched medium (Safrazbekyan *et al*., 1990). Because of the difficulties of caper bush conventional propagation, micropropagation may be a promising alternative technique. Micropropagation of caper has been standardized by Chalak *et al*. (2003) and Carra *et al*. (2007) with a high rooting percentage. Grafting is a less common method of propagation for caper bush. In Spain, acceptable results (60 % scion take) were obtained using bark grafting in plantings. Nurseries generally whip-graft with survival rates of 70–75 % (Luna Lorente and Pérez Vicente, 1985).

10.3.4 Orchard establishment

Caper plantings over 25–30 years old are still productive. Thus, physical properties of the soil (texture and depth) are particularly important. Mouldboard ploughing and harrowing are usual practices prior to caper plant establishment (Luna Lorente and Pérez Vicente, 1985). Soil-profile modification practices, such as slip ploughing operating 0.6–1 m deep, can ameliorate some restrictions (Massa Moreno, 1987).

In Pantelleria, digging backhoe pits for each shrub was found to be the most effective means of cultivating caper in rocky soils (Barbera, 1991). Two planting designs are used: square/rectangle and hedgerow system. Spacing is determined by the vigour of the biotype, fertility of the soil, equipment to be used and the irrigation method, if any. Bush spacing of 2.5 × 2.5 m (Barbera and Di Lorenzo, 1982) or 2.5 × 2 m (Bounous and Barone, 1989) is common in Pantelleria. In Salina, 3 × 3 m is satisfactory for 'Nocella'. In Spain, 4 × 4 or 5 × 5 m is satisfactory for 'Mallorquina'. Spacing of 2–2.5 m is appropriate if *C. spinosa* is used to control soil erosion on slopes.

Nursery plants, propagated as seedlings or rooted cuttings, are dug in the nursery row during the dormant season. Caper bush may be transplanted either bare-root or containerized. Most plants are handled bare-root and replanted immediately in their permanent location or heeled-in in a convenient place with the roots well covered. Containerized plants are used only where lack of irrigation is the chief factor limiting transplanting success.

10.3.5 Pruning and trellising

Caper bush is usually dormant pruned. After removal of dead tissue, it must be pruned of weak, non-productive wood and water sprouts. The caper bush benefits from a short and heavy spur pruning which reduces branches to a length of 1–3 cm or 5–10 cm when the plant is young and vigorous (Barbera and Di Lorenzo, 1982, 1984; Luna Lorente and Pérez Vicente, 1985). It is important to leave several buds

on the spur as only the 1-year-old stems will bear flower buds for the current season. Early summer pruning involves thinning out weak stems when the caper bush is in active shoot growth, 30–40 days after budding. Summer pruning also involves heading back a few of the new shoots to induce flower bud formation.

If the caper plants could be trellised rather than allowed to sprawl on the ground, picking and management would be easier (Trewartha and Trewartha, 2005). The choice of a caper-support method is an economic decision. Trellising would keep plants off the ground, increase usable space and lessen harvest difficulties. The primary disadvantages of all trellis systems are the high cost of establishment and the necessary commitment to extensive, detailed canopy manipulations.

10.3.6 Plant nutrition

Fertilization should begin 20–30 days before planting. At that time, 100 kg/ha ammonium sulphate, 400 kg/ha single superphosphate and 150 kg/ha potassium chloride have been suggested in Spain (Massa Moreno, 1987). Fertilizers may be broadcast on the surface and incorporated by tilling or cultivating, or applied in a surface band. In Pantelleria, plots are enriched with organic or inorganic fertilizers applied to the backhoe pits (Barbera, 1991).

The types of fertilizer used and application rates should be related to plant age and soil nutrient content (Sozzi, 2001). Measurement of the total concentration of a nutrient in the plant and extraction of different elements from soil are useful to diagnose mineral deficiencies (Sozzi, 2001). Phosphate and potassium fertilizers are generally applied every 2–3 years. Instead, ammonium fertilizers are incorporated annually into the soil, late in winter before sprouting. In Pantelleria and Salina, N–P–K fertilizers are applied during winter (December and January) at a rate of 200–300 g/plant (Barbera and Di Lorenzo, 1982; Barbera, 1991). Bounous and Barone (1989) suggested that fertilizations with 150–200 kg/ha of ammonium sulphate and additional P–K applications would be appropriate for mature plantings.

10.3.7 Irrigation

Caper bush is cultivated mostly in poorly-irrigated soil. Irrigation is, however, specially important during the first year when the caper bush is highly sensitive to water stress. In Pantelleria and Salina, irrigation is impossible due to the lack of water (Barbera and Di Lorenzo, 1984). Nevertheless, a type of mulching – which may include placing stones around the young plants – is utilized to protect them from wind action and thus reduce evaporation. In Spain and Argentina, additional water is usually provided during the first year. The caper bush shows its productive potential under irrigation (longer vegetative cycle, larger bud production that begins earlier and shorter intervals between harvests), although the plant tends to be more prone to diseases (Jiménez Viudez, 1987). In Spain, irrigation begins in January when caper bush is grown with almond trees, or in February or March when grown alone, and it ends in August in either case (Jiménez Viudez, 1987). Yields were doubled and even tripled when irrigation was used in Almería (it rains 96 mm from February through August), Jaén (284 mm) and Murcia (156 mm). In 1984, the

average yield in Spain was 1365 kg/ha in irrigated plantings and 650 kg/ha in non-irrigated plantings (Ministerio de Agricultura, Pesca y Alimentación, 1989). In 1988, 837 ha were irrigated in Almería, Murcia, and Jaén (Ministerio de Agricultura, Pesca y Alimentación, 1988). In 1995, only 41 ha (mainly in Murcia, Córdoba and Valencia) were still under irrigation due to the increasing competition from caper grown in Turkey and Morocco (Ministerio de Agricultura, Pesca y Alimentación, 1997). A point source sprinkler system may be utilized. Total volumes of 12–140 l/plant week, depending on the climatic conditions, are supplied under irrigation (Jiménez Viudez, 1987).

10.4 Pests and diseases

C. spinosa is not very sensitive to pest damage when growing wild. Nevertheless, some phytophagous species attack caper in its main production areas. Insecticide treatments are restricted by the short interval between harvests (7–10 days): only low-persistence active principles can be used.

In Pantelleria, the caper moth (*Capparimyia savastanoi* Mart.) and the caper bug (*Bagrada hilaris* Bm.) are considered the most important pests. The control of caper moth relies on the removal of infested leaves, combined with the use of poisoned hydrolyzed protein baits in summer when populations are high (Longo and Siscaro, 1989; Longo, 1996). The caper bug was first found on wild plants (Carapezza, 1981) and, later on, attacking cultivated caper plantings (Genduso, 1990). The pale creamy oval eggs, which turn to orange as the insect develops (Mineo and Lo Verde, 1991), are laid singly on the ground, in the cracks of the bordering field walls and, more rarely, on the leaves. At the beginning of spring it attacks different wild plants, among them caper bush which grows weak and rapidly yellows. Pyrethroid formulations are used to control this insect. The chemicals are applied either to the walls or to the plants after harvest is finished (Barbera, 1991). The painted bug (*Bagrada picta* Fabr.; Pentatomidae) is a pest of cruciferous oilseed crops and has been reported to thrive on caper bush at Tandojam during summer (Mahar, 1973).

The larval form of the weevil *Acalles barbarus* Lucas causes damage to the root system (Liotta, 1977). In general, its targets are weak adult plants previously affected by other insects. The only effective control is the removal of the attacked plants. Other insect pests in Italy are *Phyllotreta latevittata* Kutsch (Chrysomelidae) which causes oval to round erosions in leaves, leaf yellowing and stem decay, and *Asphondylia spp.* (Cecidomyiidae) and *Cydia capparidana* Zeller (Tortricidae) which alter the morphology of buds (Harris, 1975; Orphanides, 1975, 1976). The braconid *Chelonus elaeaphilus* Silv., a promising parasite of *Prays oleae* (an olive pest), was also recovered from *C. capparidana* infesting caper bush (Fimiani, 1978). Rapisarda (1984–5) reported the occurrence of *Aleurolobus niloticus* Priesner & Hosny (Aleyrodidae), a polyphagous species that feeds only on caper bush leaves in Sicily.

Caper bush is the only larval host plant available in southern Spain during the dry season for different Pieridae: cabbage small white (*Pieris rapae* L.) and large

white (*Pieris brassicae* L.) butterflies, and desert orange tip (*Colotis evagore* Klug.) (Fernández García, 1988; Jordano *et al*., 1991). *P. rapae* also attacks in California (Kontaxis, 1990) and in the Badkhyzskii Reserve, Turkmen (Murzin, 1986). The larvae of *P. rapae* and *P. brassicae* usually use cruciferous plants in the rainy season and caper bush in summer when Brassicaceae are dry (Fernández García, 1988). Oviposition takes place preferentially on the ground or on dried material around the host plant. *C.evagore* larvae are unable to survive on alternative cruciferous hosts (Jordano and Retamosa, 1988; Jordano *et al*., 1991), but they complete their lifecycle successfully in certain coastal enclaves where caper bush provides sufficient resources throughout the year. The adult lays red eggs singly, on young leaves, stems and inert supports next to the food plant (Fernández *et al*., 1986; Fernández Haeger and Jordano Barbudo, 1986).

Caper bush and other related species are also the commonest food plants of other *Pieridae* in Saudi Arabia, such as *Anaphaeis aurota* F., *Colotis fausta fausta* Olivier and *Colotis liagore* Klug. (Pittaway, 1979, 1980, 1981, 1985). These species deposit the eggs on isolated bushes in rocky scarps and cliffs. Eventually, caper plants may be completely stripped of foliage, the resulting bare branches carrying pupae and larvae. Pyrethroids can be used to control all of these *Pieridae* pests (Massa Moreno and Luna Lorente, 1985). Larvae of *Lampides boeticus* L. (Lycaenidae), which have anthophagous and carpophagous habits, have also been found to feed on caper buds (Jordano Barbudo *et al*., 1988).

The pentatomid bug *Eurydema ornata* L. attacks caper bush leaves and may cause serious damage (Fernández *et al*., 1986). The green stink bug *Nezara viridula* L. has caused some damage in Spain and Argentina. All these Hemiptera can be controlled by using trichlorfon, endosulphan, dimethoate or chlorpyriphos. Other insect pests detected in caper include *Ceuthorhynchus* sp. (*Curculionidae*) and *Heliothis-Helicoverpa* (Noctuidae). Many ant species (*Camponotus* spp., *Plagiolepis pygmaea*, *Crematogaster auberti*, *C. sordidula*, *Formica subrufa*, *Tetramonium hispanica* and *Cataglyphis viaticoides*) have been found feeding on caper plants (Fernández *et al*., 1986). In California, caper bush can be damaged by cabbageworm, black vine weevil and flea beetle, as well as gophers, snails and slugs (Kontaxis, 1998).

Damping-off diseases, caused by several fungi (*Pythium* spp., *Fusarium* spp. *Verticillium* spp., etc.), may be severe. Frequently, caper seedlings are completely destroyed either when they are placed in seedbeds or after being transplanted. Seedlings are usually attacked at the roots or in the stems at or below the soil line, and the invaded areas soon collapse. These diseases can be controlled through the use of sterilized soil and chemically treated seeds.

The most important fungus attacking caper leaves and flowers is probably the white rust disease (*Albugo capparidis* De By.). A list of fungi affecting caper bush was given by Ciferri (1949). *Neoramularia capparidis* spec. nov. produces small greyish white leaf spots with narrow brown margin in India (Bagyanarayana *et al*., 1994). Caper bush is also a host of *Leveillula taurica* (Lev.) G. Arnaud, causal agent of the powdery mildew (Gupta and Bhardwaj, 1998; Kavac, 2004). Caper plants were reported to have been infected with *Botrytis* spp. and *Pythium* spp. in California (Kontaxis, 1990). A caper vein banding virus (CapVbV) was reported in Sicily and was tentatively assigned to the carlavirus group (Majorana, 1970). Gallitelli and Di Franco (1987) showed that this virus infects caper plant symptomlessly and

suggested the name caper latent virus (CapLV, genus *Carlaviruses*, family Flexiviridae). The real causal agent of vein banding may be a rhabdovirus, the caper vein yellowing virus (CapVYV), that may infect caper bush simultaneously to the CapLV (Di Franco and Gallitelli, 1985). New serological tests have shown that CapVYV is indistinguishable from the pittosporum vein yellowing virus (PVYV, genus *Nucleorhabdovirus*, family Rhabdoviridae) (Nuzzaci *et al*., 1993). *C. spinosa* is also a natural host of the cucumber mosaic virus (CMV, genus *Cucumovirus*, family Bromoviridae) (Tomassoli *et al*., 2005).

10.5 Main cultivars and world production and trade

10.5.1 Main cultivars

The commercial product known as 'caper' is actually being obtained from different species (*C. spinosa*, *C. orientalis*, *C. sicula*, etc.) with intermediate biotypes and similar genetic background (Inocencio *et al*., 2005). This fact complicates quality control and challenges researchers to develop new simple methods to discriminate different cultivars or species (Inocencio *et al*., 2002). The main caper germplasm collections are located in Italy and Spain.

Many biotypes have been chosen by growers owing to some advantageous characteristics. Features of interest in caper bush improvement programmes are: (i) high productivity (long stems, short internodes and high node fertility, short and uniform flowering periods); (ii) deep green spherical flower buds, with close non-pubescent bracts and late opening; (iii) absence of stipular spines and easy stalk separation to simplify harvest and post-harvest operations; (iv) processed product with an agreeable appearance; (v) capacity for agamic reproduction; (vi) resistance to water stress, cold and pests; (vii) oval fruit with light green pericarp and few seeds; (viii) thick and tender stem tip (food use).

Caper biotypes are commonly referred to as *C. spinosa*, but many of them belong to other taxa (Inocencio *et al*., 2005). The most attractive Italian commercial biotypes are 'Nocellara' or 'Nuciddara' (a cultivar within *C. orientalis*) and 'Nocella' or 'Nuccida' (Barbera *et al*., 1991; Fici and Gianguzzi, 1997). Both are highly productive and yield high-quality capers (almost spherical shape, mustard green colour, strong aroma and conserved integrity after brining). 'Nocellara' does not bear spines and 'Nocella' has very small harmless ones. On the other hand, 'Nocella' does not resist drought. Other Italian biotypes are 'Senza spine' and 'Inermis' – Italian selection forms, without stipular spines –, 'Ciavulara' (Barbera *et al*., 1991; Fici and Gianguzzi, 1997), 'Testa di lucertola' (Barbera *et al*., 1991), 'Spinoso di Pantelleria' (Barbera *et al*., 1991; Fici and Gianguzzi, 1997) and 'Spinosa comune' or 'Spinoso di Salina' (a cultivar within *C. sicula* subsp. *sicula*; Barbera *et al*., 1991; Fici and Gianguzzi, 1997; Rivera *et al*., 2003).

'Ciavulara' is less productive and its buds tend to open precociously; capers are flatter and flake easily during post-harvest treatments, giving a poor aspect to the final product. 'Testa di lucertola' ('Lizard's head') produces capers with a lengthened pyramid shape. 'Spinoso of Pantelleria' and 'Spinoso of Salina' have conspicuous axillary spines. In 'Spinoso of Pantelleria', the leaf tips also bear a small thorn. 'Spinoso of Salina' is less productive; its capers are flattened pyramidal and tend to

flake during post-harvest curing. Other Italian biotypes are 'Tondino' (Caccetta, 1985), grown in Pantelleria and Salina, 'Aculeata' and 'Dolce di Filicudi e Alicudi' (Alkire, 2001). A complete description of all cultivars can be found elsewhere (Rivera *et al.*, 2003).

The most important Spanish biotypes are 'Común' or 'del País' and 'Mallorquina' (Luna Lorente and Pérez Vicente, 1985; Rivera *et al.*, 1999). 'Común' is a heterogeneous population with spiny stems which dry out completely in winter. 'Mallorquina' has long spiny stems, bright green leaves and small seedy fruit. 'Mallorquina' is highly productive, presents a vigorous growth and has extraordinary yields under irrigation. Other biotypes within *C. spinosa* are cultivated to a lesser extent in the Balearic Islands: 'Redona', 'Roses', 'De las Muradas', 'Figues Seques' and 'Peluda' (Rivera *et al.*, 1999). 'Redona' is a spiny but highly productive biotype, yielding high-quality capers. On the other hand, 'Fulla Redona' is a biotype within *C. orientalis*, with no spines. It is considered a promising biotype by the quality and quantity of its produce.

10.5.2 World production and yield

The economic importance of the caper bush led to a significant increase in both the area being cultivated and production levels during the late 1980s. Global trade in capers involves around 60 countries, and the average annual production is estimated to be around 10 000 t: 3500–4500 t are produced in Turkey, 3000 t in Morocco, 500–1000 t in Spain and 1000–2000 t in other countries. Turkey is the leading caper-exporting country. The USA was one of the most important caper consumers during the 1990s. Harvest represents two-third of the total labour in the crop management process as it is done manually, and it is time-consuming due to: (i) the decumbent character of the branches; (ii) the presence of stipular spines in some biotypes; (iii) high temperatures and solar radiation during summer in caper-producing areas; (iv) the small diameter of flower buds. Since flower buds are arranged along twigs which have an indeterminate growth habit, twigs should not be cut.

Caper bush yields are highly variable depending on the growing environment, cultural practices and biotype, but a maximum yield is expected in the fourth year. A mature caper plant may produce 4–5 kg/year. According to Lozano Puche (1977) a wild growing plant yields 2–3 kg/year in Spain, but the same caper bush has the potential to produce 6–9 kg/year when cultivated in irrigated fertile soils (Jiménez Viudez, 1987). Great differences in yield are attributed to genetic variations. A 3-year-old 'del País' planting yields 1–1.5 t/ha year, but this production may be doubled and even tripled by using 'Mallorquina'. Bounous and Barone (1989) indicated average annual yields of 1–1.5 kg/plant and yields as high as 4 kg/plant in the third and fourth years of cultivated growth. Barbera and Di Lorenzo (1982) reported average annual yields of 1–1.5 kg/plant in Pantelleria (maximum yields of 4–5 kg/plant) and 2–3 kg/plant in Salina in 3-year plantings (average annual yields of 3–4 t/ha). On the other hand, Caccetta (1985) estimated annual yields of 1.2–2.5 t/ha in Pantelleria and 1.8–2.6 in Salina. Global growth in caper trading is estimated to be around 6 % per annum. In some countries such as Australia, opportunities exist in import replacement of high-quality capers for a niche market as well as in export (Trewartha and Trewartha, 2005). The caper crop can create new jobs in harvesting

as well as in the processing and distribution industries. Every hectare of capers planted is estimated to produce six to eight permanent jobs (Trewartha and Trewartha, 2005).

10.6 Post-harvest technology and uses in food processing

10.6.1 Post-harvest technology

Different physicomechanic characteristics of capers and caperberries have been assessed, and this information will help to develop more efficient handling and processing systems (Özcan and Aydin, 2004; Özcan *et al.*, 2004). After harvest, capers are placed in shallow vats. In Spain, post-harvest conditioning is generally performed by local traders, cooperatives or producer associations. After removing the leaves and pedicels, a first selection of capers takes place and blemished and open buds are discarded. Then, capers are subjected to a first sieving, which generally grades them into two size groups, with diameters lower or higher than 8–9 mm. Capers are valued in proportion to the smallness of their size. This first classification provides an incentive for re-collection of smaller capers and makes the subsequent industrial steps easier. Fresh capers have an intensely bitter taste, and one of the purposes of the pickling process, besides preservation, is to remove this unpleasant flavour. This is due to the presence of the glucoside glucocapparin, which is readily hydrolyzed to by-products completely lacking the bitter taste. After aeration in a well-ventilated place, capers are packed in wooden or polyvinyl chloride (PVC) barrels, fibreglass tanks or large casks and treated with high salt brine (*c.* 16 % NaCl w/v at the equilibrium, increasing to 20 % after changing the first brine). After filling, the casks are hermetically closed and placed in the sun. In order to reach the equilibrium in salt concentration, barrels are rolled during the early stage of brining. Periodical salt checks should be performed, also ensuring that the brine completely covers the material. This 'wet' curing process lasts 20–30 days (Luna Lorente and Pérez Vicente, 1985), but capers may be stored under such conditions for several months, until final industrial conditioning takes place.

Capers may be classified as fully brined vegetables (Ranken, 1988). Brines with a high salt content are increasingly being objected to (Alvarruiz *et al.*, 1990; Rodrigo *et al.*, 1992). Organoleptic characteristics and preservation of the final product proved to be the same over at least 27 months when capers had been pretreated with 10, 15 or 20 % NaCl at equilibrium (Alvarruiz *et al.*, 1990). High salt concentrations inhibit both the growth of undesirable microorganisms and the activity of lactic acid bacteria. Lower NaCl brines (i.e. 5 %) are more likely to permit growth of coliform bacteria, yeasts and moulds (Özcan and Akgül, 1999a).

Fermentation takes place at a higher rate when pickling small (≤ 8 mm) buds (Özcan and Akgül, 1999a). In Italy, growers arrange capers in cement tanks, PVC or wooden barrels, or open drums, between layers of solid salt (10–15 % w/w). This promotes the extraction of water from the raw product by osmosis and generates saturated brine. This treatment lasts 7–8 days. Then, the brine is removed and the capers are submitted to the same process once or twice more (Barbera, 1991). Capers are also pickled in vinegar (at least 4 % acidity as acetic acid) in a 1:1 (w/v) ratio (Reche Mármol, 1967). Regular topping up with vinegar ensures that all the

Table 10.2 Caper grading system

Diameter (mm)	Commercial denomination	Number of flower buds/kg	
		According to Barbera (1991)	According to Luna Lorente and Pérez Vicente (1985)
< 7	Non Pareil	5500	7000
7–8	Surfine	4000	4000
8–9	Capucine	3250	4000
9–10	Capote	2600	2000
10–11	Capote	2200	2000
11–12	Fine	1900	1300
12–13	Fine	1600	1300
13–14	Grosse	–	800

capers remain covered. This pickling process lasts 30 days. Only 10 % of vinegar is absorbed by the product, the remainder being discarded at the end of the period.

Following the completion of the curing period, the industrial processing is completed in three steps. First, capers are drained and rinsed with several changes of water to dislodge and remove all sediment. Second, damaged buds are disposed of and capers are carefully size-graded according to a grading system (Table 10.2). Finally, capers are prepared in a variety of ways and packed as a finished product. Pasteurization (80 °C, 15 minutes) of the final product is used to prevent the development of pathogens. These heat treatments can further prevent the development of certain spoilage-causing microorganisms (Ranken, 1988; Alvarruiz *et al*., 1990).

Without pasteurization, 6–10 % NaCl and 1 % acidity as acetic acid (w/v) are required in the final product to avoid the risk of spoilage (Alvarruiz *et al*., 1990; Özcan and Akgül, 1999b). In some cases, NaCl is avoided and covering capers with diluted acetic acid or distilled malt vinegar (4.3–5.9 % acetic acid) serves as an alternative. In Italy, the final product is treated with dry salt. Such preparation decreases the cost of transportation and gives a more intense flavour. In Spain, a similar treatment is carried out with capers of large diameter. Capers are drained and mixed with dry salt (20 % maximum). The caper industry discontinued the use of olive oil in caper preparations due to its high cost. Other special preparations, including wine vinegar, with or without the addition of tarragon, *Artemisia dracunculus* L. (Vivancos Guerao, 1948), are also expensive and exclusively utilized with capers of small diameter. Sweetening ingredients like sugar are added to those capers exported to Denmark or some northern European countries (González Soler, 1973).

Capers are generally packed in PVC or wooden barrels of 180–200 kg for the pickle industry but 40 kg barrels are used for packing ‘non pareil’ and ‘surfine’ capers, depending on the country importing them. For retail sale, capers are packed in various kinds of glass or plastic flasks containing 20 g to 5 kg, or translucent sachets of 0.1–1 kg. Five-kilogram flasks and sachets are usually sold to restaurants and coffee shops.

Traditionally, caperberries are fermented by dipping in water for 4–7 days. This immersion produces a strong fermentation accompanied by a colour change (from green to yellowish) and loss of texture due to flesh breakdown and gas accumulation. This step affects the value of the product and has proven to be unnecessary (Sánchez *et al*., 1992). Lactic acid bacteria show faster growth rates at low NaCl concentrations (Sánchez *et al*., 1992) but, as for capers, undesirable microorganisms can grow in 5 % NaCl brines (Özcan, 1999a). In order to protect caperberries from spoilage during fermentation, 4–5 % NaCl brines may be adequate (Sánchez *et al*., 1992), but fermentation must be continuously controlled (Özcan, 1999a). Fermentation should last 20–25 days. Brines with 10 % (Sánchez *et al*., 1992) to 15 % (Özcan, 1999a) NaCl at equilibrium create a favourable environment for pickled caperberry storage. Sorbic and benzoic acids, as well as their corresponding sodium and potassium salts, are used as preservatives during final packing. A method combining steam distillation (extraction) and high-performance liquid chromatography (HPLC) determination could be used to control the levels of those preservatives in caperberries (Montaño *et al*., 1995).

10.6.2 Uses in food processing

Consumption of capers and caperberries has a long history. Direct evidence of the consumption of *Capparis* spp. from 18 000–17 000 years ago was obtained by archaeological excavations from Palaeolithic sites (Wadi Kubbaniya, west of the Nile Valley, Upper Egypt) (Hillman, 1989). Prehistoric remains of wild caperberries were also recovered from sites in south west Iran and in Iraq (Tigris) and dated to 6000 BC (Renfrew, 1973). Also, remains of caper seeds were recovered in quantity from different archaeological sites and dated to 9000–8000 BC (van Zeist and Bakker-Heeres, 1982, 1986; Willcox, 1996). A Bronze Age jar bearing carbonized flower buds and unripe fruit was found at Tell es Sweyhat (Syria) and suggests the consumption of pickled capers during the Bronze Age (van Zeist and Bakker-Heeres, 1988). The caper bush was utilized by ancient Greeks, Hebrews and Romans (reviewed by Sozzi, 2001; Rivera *et al*., 2002), and both capers and caperberries are recognized as safe products when used as spices for natural seasoning.

There are almost 550 recipes that include capers (CondéNet, 2005), most of them compiled from specialized journals (Gourmet, Bon Appetit). Capers have a sharp piquant flavour and are mainly used as a seasoning to add pungency to: (i) sauces (e.g., tartare, remoulade, ravigote, vinaigrette, sauce gribiche, tarragon sauce and caper sauce); (ii) dressings and salads (e.g., caponata, a cold eggplant salad with olives and capers); (iii) cold dishes (vithel tohnné), or sauces served with salmon, herring, whiting or turbot; (iv) pasta, pizzas and canapés; (v) cheeses (e.g., liptauer cheese); and (vi) lamb, mutton, pork or chicken preparations (Hayes, 1961; Knëz, 1970; Machanik, 1973; Nilson, 1974; Baccaro, 1978; Stobart, 1980). A complex organoleptic profile is responsible for caper flavour (Brevard *et al*., 1992). Caperberries and tender young shoots of the caper bush are also pickled for use as condiments, as previously described. The unripe seeds or pickled buds of other species (*Tropaeolum majus* L., *Caltha palustris* L., *Cytisus scoparius* (L.) Link., *Zygophyllum fabago* L., *Euphorbia lathyrus* L.) are sometimes suggested as

substitutes for capers (Redgrove, 1933; Vivancos Guerao, 1948; Seidemann, 1970; Mitchell and Rook, 1979; Stobart, 1980; Bond, 1990).

10.7 Functional properties and health benefits

Different organs of the caper plant have been used as folk remedies for various diseases (Pernet 1972; Kirtikar and Basu, 1975; Boulos, 1983; Duke, 1983; Jain and Puri, 1984; Abbas *et al*., 1992; Husain *et al*., 1992; Al-Said, 1993; Ghazanfar and Al-Sabahi, 1993; Ghazanfar, 1994; Bhattacharjee, 1998). It is traditionally utilized in diabetes control and treatment in Morocco (Jouad *et al*., 2001; Eddouks *et al*., 2002). Liv.52, an Indian traditional polyherbal formulation that contains different plant extracts, among them 24 % of *C. spinosa*, is a 'liver stimulant' with some protective action against hepatotoxic substances (ethanol, acetaldehyde and carbon tetrachloride), radiation sickness and dermatitis. The health benefits of Liv.52 related to *C. spinosa* have been extensively reviewed (Sozzi, 2001), and recent studies confirm its efficacy on liver cirrhotic patients (Fallah Huseini *et al*., 2005).

Caper has been used in folk medicine as carminative, anti-escorbutic, antispasmodic, diuretic and vermifuge. The decoction of caper bush has hypoglycaemic properties and may be useful in antidiabetic therapy (Ageel *et al*., 1985; Yaniv *et al*., 1987). Aqueous extracts of *C. spinosa* have a potent anti-hyperglycaemic activity in streptozotocin diabetic rats (Eddouks *et al*., 2004). No changes were observed in basal plasma insulin concentrations following treatment of normal or diabetic rats with *C. spinosa* aqueous extracts, thus indicating that the underlying mechanism of its pharmacological activity seems to be independent of insulin secretion (Eddouks *et al*., 2004). Another beneficial effect observed in diabetic rats being administered *C. spinosa* extract was the reduction in plasma cholesterol which is usually high in patients with diabetes mellitus (Eddouks *et al*., 2005). High levels of plasma lipids represent a risk factor for coronary heart disease.

The oral administration of a caper root decoction or tincture to guinea pigs revealed strong desensitizing effects against various plant and animal allergens (Khakberdyev *et al*., 1968). Cappaprenol-12, -13 and -14 in ethanol extracts of caper leaves are anti-inflammatory compounds (Al-Said *et al*., 1988; Jain *et al*., 1993). It has recently been shown that methanolic extracts of *C. spinosa* flowering buds possess a marked anti-allergic and antihistaminic effect (Trombetta *et al*., 2005). *C. spinosa* is also used in phytomedicine as antifungal (Ali-Shtayeh and Abu Ghdeib, 1999), antihepatotoxic (Gadgoli and Mishra, 1995, 1999), anti-inflammatory (Ageel *et al*., 1986) chondroprotective/antidegenerative (Panico *et al*., 2005) and antileishmania (Jacobson and Schlein, 1999). A role for the plant in the epidemiology of leishmaniasis has been suggested (Schlein and Jacobson, 1994a, b). In fact, extracts of *C. spinosa* caused extensive parasite agglutination, apparently due to caper plant lectins (Jacobson and Schlein, 1999). Methanolic extracts of *C. spinosa* showed some antimalarial activity when assayed *in vitro* against a multi-drug resistant strain of *Plasmodium falciparum* (K1) (Marshall *et al*., 1995). Extracts of the whole plant or its aerial part also exhibited variable degrees of antimicrobial activity, as well as antifungal activity (Ali-Shtayeh *et al*., 1998).

A number of caper extracts have anticarcinogenic activity. The hydrolysis products of some glucosinolates have anticarcinogenic effects (Mithen *et al*., 2000) and different antioxidant compounds (e.g. quercetin, rutin) may also contribute to cancer prevention. A methanolic caper extract showed strong antioxidant/free radical scavenging effectiveness in different *in vitro* tests and, when topically applied, afforded significant *in vivo* protection against UV-B light-induced skin erythema in healthy human volunteers (Bonina *et al*., 2002).

Antidermatophytic activity in caper extracts is comparable with that of griseofulvin preparations (often used as a standard in evaluating antibiotic potential), suggesting a possible use against dermatophytic infections in humans (Ali-Shtayeh and Abu Ghdeib, 1999). In contrast, the green parts of caper plant have been considered to be potentially irritating to the skin because of its glucosinolates (Mitchell, 1974; Mitchell and Rook, 1979; Cronin, 1980; Foussereau *et al*., 1982). Caper leaf and fruit extracts, applied as wet compresses to inflamed skin, may produce acute contact dermatitis (Angelini *et al*., 1991). Nevertheless, Lemmi Cena and Rovesti (1979) pointed out that caper extracts may be used for treating enlarged capillaries and dry skin. Barbera (1991) suggested that they could be utilized for cosmetic preparations (creams, shampoos, lotions and gels), due to the presence of some active principles: rutin and quercetin (flavonoids that produce effects similar to those of vitamin P), glucocapparin (rubefacient action), pectins (moisturizing and protecting effects), phytohormones and vitamins.

10.7.1 Health-promoting and therapeutic characteristics

C. spinosa bud extract may be considered as an interesting source of antioxidants and antibiotics and as a strong scavenger against free radicals for therapeutic or nutraceutical industries (Tlili *et al*., 2011). Phytochemical studies of caper have shown the presence of many beneficial compounds such as spermidine, rutin, quercetin, kaempferol, stigmasterol, campesterol, tocopherols and carotenoids. Biological studies reveal significant antimicrobial, antioxidative, anti-inflammatory, immunomodulatory and antiviral properties.

Considering the effect of different preservation treatments on *C. spinosa* buds, the antiradical activity decreases in the following order: fresh capers > pickled capers > buds dried at 55 °C > salt-dried buds. The highest retention of antiradical activity is observed when capers are treated with vinegar (62 % of the activity in fresh material). Results indicate that both flower buds and leaves can be considered a promising source of flavonoids in general and rutin in particular, even after the preservation treatments (Gonzalez *et al.*, 2010).

Sher and Aleymeini (2010) pointed out the ethnobotanical and pharmaceutical importance of *C. spinosa* and explored its agro-industrial potential for the Kingdom of Saudi Arabia. *C. spinosa* proved to be a multipurpose plant used for curing various human ailments including gastrointestinal problems, inflammation, anaemia, liver dysfunction and rheumatism. It has been used as an antispasmodic analgesic; anthelmintic; antihaemorrhoidal; aperient; deobstruent; depurative; diuretic; expectorant; and general body tonic in indigenous, Ayurvedeic, Chinese and Unani systems of medicine. This study concluded that *C. spinosa* had economic significance for Saudi Arabia.

Table 10.3 Nutritional value of caperberries

Serving size	100 g of caperberries	
% Daily requirements		
Total calories	23	1 %
Calories from fat	7.2	
Total fat	0.9 g	1 %
Saturated fat	0.4 g	1 %
Mono-unsaturated fat	0.1 g	
Polyunsaturated fat	0.4 g	
Trans fat	0 g	
Cholesterol	0 g	0 %
Total carbohydrate	4.9 g	2 %
Dietary fibre	3.2 g	13 %
Sugars	0.4 g	
Protein	2.4 g	5 %
Minerals		
Calcium	40 mg	4 %
Iron	1.7 mg	9 %
Magnesium	33 mg	8 %
Phosphorus	10 mg	1 %
Potassium	40 mg	1 %
Sodium	2964 mg	123 %
Zinc	0.3 mg	2 %
Copper	0.4 mg	19 %
Manganese	0.1 mg	4 %
Selenium	1.2 mcg	2 %
Vitamins		
Riboflavin	0.1 mg	8 %
Niacin	0.7 mg	3 %
Folic acid	23 mcg	6 %
Vitamin A	138 IU	3 %
Vitamin C	4.3 mg	7 %
Vitamin E	0.9 mg	4 %
Vitamin K	24.6 mcg	31 %
Phytosterols	48 mg	

Caperberries are high in vitamin content (Table 10.3) and are recommended for good health for the following reasons:

- They are very low in calories, have minimal amounts of fats and no cholesterol.
- They are a good source of B-group vitamins like thiamine, riboflavin, niacin, B6 and folic acid that are essential to enhance the energy production from food.
- They are a good source of vitamin C, a natural water-soluble antioxidant that enhances the immune system, and vitamin K which prevents internal and external bleeding.
- They are a moderate source of vitamin A, which enhances the eyesight, and vitamin E and selenium, natural antioxidants that scavenge the free radicals that oxidize fats, preserve the integrity of cell membranes and protect the body.

- They are a good source of minerals like calcium, iron, potassium, phosphorus, magnesium, zinc and manganese, which play a very important role in maintaining proper metabolic activities.
- They are a good source of soluble dietary fibre, that adds roughage to the contents of the intestines, promotes satiety, promotes the health of the colon and also helps in relieving constipation, haemorrhoids, diverticular disorders, etc.
- They are a very good source of rutin and quercetin (180 mg/100 g), second only to tea leaf. Both compounds are powerful antioxidants. Rutin strengthens capillaries and inhibits platelet clump formation in the blood vessels. Both actions help in smooth circulation of blood in very small vessels. Rutin has been used for haemorrhoids, varicose veins and in bleeding conditions such as haemophilia. It has been found to reduce low-density lipoprotein (LDL) cholesterol levels in obese individuals. Research studies suggest that quercetin has antibacterial, anticarcinogenic, analgesic and anti-inflammatory properties.

10.8 Quality issues and future trends

Consumer satisfaction and repeat purchases of food are dependent upon flavour and nutritional quality. Many studies exalt the nutritional value of caper flowering buds, which are widely used as a source of flavour. Capers are rich in antioxidant compounds. Moreover, caper isothiocyanates are well known as cancer preventive agents and different caper extracts have hypoglycaemic properties and protective effects against hepatotoxic substances. In addition, capers and caperberries could be part of new therapeutic strategies based on natural products. Increasing amounts of capers are being consumed in different countries, and this trend appears likely to be sustained for coming years, the interest in new tastes presumably accounting for most of the increase.

Success in caper bush cultivation depends mainly on five fundamental points: (i) biotypes of high quality and production; (ii) adequate propagation; (iii) good control of cultivation practices, particularly harvest; (iv) adequate post-harvest processing and storage; and (v) efficient marketing systems and strategies. Caper yields are much higher in irrigated plantings, with N–P–K fertilization, although much more research is required to determine the optimal cultivation conditions for this species. Diseases and pests do not seem to be a great problem in general but need to be researched. Two major expenses are expected, implantation and harvesting. The latter may be the stumbling block in high-input systems, and the possibility of a semi-mechanical operation should be considered in order to remove this limiting factor. Moreover, further improvement in caper quality may be obtained by regulating harvesting dates. There is an assortment of opportunities for plant breeders to contribute to domestication of caper bush for agricultural purposes. Determination of the genetic bases for productivity, ease of propagation, absence of stipular spines and flower bud quality and conservation are high-priority research needs. In Australia, Trewartha and Trewartha (2005) consider that research and development could support the expansion of a viable caper industry, undertaking investigation in order to reduce picking costs (through harvest management, mechanization and

trellising), select optimum varieties and diversify and add value through product innovation. Finally, marketing research remains an area of great importance. Marketing of capers without prearranged contract with processing or exporting companies could be very risky. Market promotion and the ability of handlers to provide a high-quality product at times that will yield a competitive price have become essential factors. Producers and handlers will be challenged to develop new and expanded markets for capers.

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11

Caraway

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Abstract: Caraway (*Carum carvi* L.) is grown for its seeds (botanically fruits) which are used in food, cosmetics, beverage and pharmaceutical industries. The chapter details the classification, chemical structure and production system of caraway including post-havest handling. The main products from caraway and their uses in food are depicted. Several processed products from caraway whole seed, such as seed powder, essential oil, fatty oil, oleoresins and caraway carvone, find application in the food and medicinal industries. The chapter discusses the bioactive compounds present in essential oil from the seeds and describes the major functional propeties of caraway – antiflatulent, antispasmodic and antidyspepsic, antimicrobial, emmenogogue and lactogogue, antioxidant, anticarcinogenic and insecticidal. Toxicity information and quality specifications for different caraway products and adulteration are also given.

Key words: caraway, cultivation, chemical composition, main products, functional propeties, medicinal uses, toxicity, quality specification, adulteration.

11.1 Introduction

Caraway has been used for centuries as a culinary spice and medicinal agent. Its use as digestive aid was first mentioned in the Egyptian *Eberus Papyrus* about 1500 BC and by Dioscorides (AD 40–90), a Greek physician. Caraway seeds have been found in prehistoric food remains from 3500 BC. In Shakespeare's *Henry IV*, the character Falstaff is invited to have a serving of baked apples and caraway to aid the digestion and relieve gas. Nineteenth-century American eclectic herbal physicians, such as Harvey Felter, pointed out that caraway seeds not only promote digestion but also ease the symptoms of children suffering from digestive disorder. Caraway (*Carum carvi* L.) of the Apiaceae family is one of the oldest herbs known and is native to Asia, Europe and North Africa (Rosengarten, 1969; Levetin and McMohan, 1999). Evidence of caraway was found in Middle Eastern Asia about 5000 years ago. The plant was well known to the ancient Egyptians and was introduced about 1000 years ago from northern Africa into Europe (Rubatzky *et al*., 1999). Caraway seeds have been mainly used as condiment for flavouring of food preparations in Europe and the Middle East from ancient times.

Caraway is known by different names in different countries. It is called *carvi* in French and Italian, *kummel* in German, *alcaravea* in Spanish, *karvy* in Dutch, *kminek*

in Polish, *komeny* in Hungarian, *karve* in Norwegian, *tmin* in Russia, *kummin* in Swedish and *siah zeera* in India (Anon., 2012a). All European countries have their own, albeit to some extent, similar words for this species, which may derive from the Arabian '*karauya*' from the twelfth century (Rosengarten, 1969). It is also called as '*sushva*', *krishna jiraka* or black cumin in India.

There are about 192 species of *Carum* known to occur (Anon., 2010) of which *Carum carvi* L. is the most important economically and is cultivated in several regions worldwide. The caraway (*C. carvi* L.) is usually confused with black caraway (*C. bulbocastanum* Koch, *Bunicum persicum* Boiss) and Nigella (*Nigella sativa* L.) because of their common vernacular names, but otherwise they all are botanically different from each other. Caraway is classified as a mild spice and categorized as a seed spice because the seeds (botanically fruits) are used raw, powdered or in the form of essential oil or oleoresins. As per the taxonomic classification, the caraway belongs to order Apiales, family Apiaceae, genus *Carum* and species *carvi* and is a cross-pollinated crop with somatic chromosome numbers 2n = 20.

11.1.1 Chemical composition

The ground seed of caraway yields up to 5–7.5 % volatile oil, consisting primarily of 60 % δ-carvone and 15 % fixed oil, of which oleic, linoleic, petroselinic and palmitic are the major fatty acids. The chemical composition varies with variety-region, stage of harvest and method of distillate/analysis and has been reported ranging from 1 % to 9 % (Sedlakova *et al*., 2001; Lozykowska et al., 2010). Caraway grown in the northern latitudes yields higher quantities of volatile oil than that cultivated in the warmer climates.

Ten compounds have been identified in the seed's oil and are given in Table 11.1 (Begum *et al*., 2010). The oil is characterized by the presence of a high content of thymol (48.20 %), *o*-cymene (19.29 %), γ-terpinen (17.61 %), trimethylene dichloride (8.81 %), β-pinene (3.08 %), 2-(1-cyclohexenyl) cyclohexanone (0.68 %), β-phellandrene (0.67 %), 3-carene (0.57 %), α-thujene (0.55 %) and linalool (0.54 %). The chief constituents of essential oil of caraway to which the odour and flavour are chiefly attributed are carvone (47–81.17 %) and limonene (9.4–48.7 %). The essential

Table 11.1 Constituents of seeds essential oil of *Carum carvi*

Name of constituent	Amount (%)
α-Thujene	0.55
β-Pinene	3.08
o-Cymene	19.29
β-Phellandrene	0.67
γ-Terpinen	17.61
3-Carene	0.57
Linalool	0.54
2-(1-Cyclohexenyl)cyclohexanone	0.68
Thymol	48.20
Trimethylene dichloride	8.81

Source: Begum *et al*. (2010).

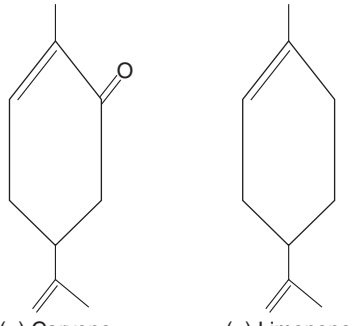

Fig. 11.1 The major compounds of caraway essential oil.

oil concentration of seeds was in the range 2.9–5.1 % (v/w). The carvone and limonene contents of the essential oils were in the range 59–77 % and 26–41 %, respectively, from *C. carvi* (cv. Sylvia) in Norway (Dragland and Aslaksen, 1996). The concentrations of limonene and carvone were 31.41 and 36.24 mg/g, respectively, in variety Plewicki, and 17.60 and 22.46 mg/g, respectively, in variety Konczewicki (Zawirska *et al*., 2000). Chemical structures of carvone and limonene, the major compounds of caraway essential oil, are shown in Fig. 11.1.

It has been reported that twice as much oil occurs in biennial caraway as compared to annual types with similar size of seeds (Bouwmeester *et al*., 1995b). In field tests carried out over several years in Vienna, Austria, essential oil content was 2.8–3.3 % in annual and 3.9–5 % in biennial caraway cultivars. In caraway, *cis*- and *trans*-dihydrocarvone and some isomers of carveol and dihydrocarveol were present in the range 0.5–1 % each (Bailer *et al*., 2001). The total content of essential oils studied in the fruits of four varieties (Gintaras, Rekord, Chmelnickij and Prochana) varied from 1.9 to 4.3 ml 100 g-1. Percentage concentrations of the main caraway compounds, limonene and carvone, were in the range 38.2–52.3 % and 45.7–59.7 %, respectively (Venskutonis *et al*., 1999). A low-resolution gas-phase Fourier transform–infrared (FT-IR) method for the fast analysis of supercritical CO_2-extracted caraway fruit oils has been developed (Ahro *et al*., 2001) and advocated for quality recovery of essential oil. Tunisian caraway ecotypes contained relatively low levels of essential oil ranging from 0.86–1.20 % (w/w). Forty-one volatile compounds were identified, mainly carvone (76.78–80.53 %) and limonene (13.05–20.29 %), but they were found rich in total fatty acids (2.95–5.68 %) (Laribi *et al*., 2010).

The secondary metabolites, viz. terpenes, flavonoids, coumarins and phenolic constituents, add value to *C. carvi* due to their antioxidative properties. The stabilizing effect of some spices on food, especially meat products, is considered to be due to the presence of a high content of phenolic substances. The phenolic functional group is known to have antimicrobial properties and to be capable of retarding the oxidation of active substances. The phenolic compounds identified in *Carum* seed are flavonoids, glycosides, derivatives of quinic acid, proteids and tannins. Rahman and Hossain (2003) isolated and characterized a flavone from the methanolic extract of the seeds of *C. carvi* as 4′,5,7-trihdyroxy-2′-methoxyflavone. The constituents responsible for the antioxidant properties of *Carum* are attributed to carvacrol

Table 11.2 The constituents of caraway essential oil

Constituents	Contents (% of dry weight)	
	El Wakeil *et al*. (1986)	Puschmann *et al*. (1992)
Essential oil	0.99	5.36
Carvone	80.17	50.46
Limonene	9.75	47.66
α-Pinene	0.10	–
β-Pinene	0.40	–
Terpinolene	0.20	–
Myrecene	0.06	0.35
Paracymene	0.06	–
Caryophyllene	0.11	–
trans-Dihydrocarvone	0.59	0.18
cis-Dihydrocarvone	0.11	
Cuminaldehyde	0.08	–
cis-Perrilyl alcohol	0.14	–
trans-Carvone	0.01	–
cis-Carvone	0.14	–
Dihydrocarveol	0.04	0.56
Cuminyl alcohol	0.02	–
Carrylaceate	–	0.16
Unidentified compounds	8.17	Less than 1.00

(Lagouri and Boskau, 1995), dihydroderivatives of main terpenes-dihydrocarbon and dihydrocarvecol – being the important stereoisomer mixtures. The content of other minor and trace substances in the oil may vary within broad limits as shown in Table 11.2 which is a presenting analysis of seed samples of Egyptian origin and of mid-European countries. Upon hydro–distillation by Chowdhury (2002), the seeds gave 3.5 % oil on dry weight basis and, upon GC-MS examination, the oil was found to contain carvone as a major constituent (81.5 %). The other constituents identified were citronellyl acetate, dihydrocarvone, eugenol, isolimonene and limonene oxide, δ-3-carene, camphene, caryophyllene, carveol, *p*-cymene, dihydrocarveol, linalool, *p*-mentha-2,8-dien-1-ol, myrecene, α-pinene, β-pinene, phellandrene, sabinene, α-terpinene and terpinelene which were isolated in trace amounts.

Flavonoids (flavonoid glycosides) are the other important secondary metabolites of *Carum*, and seed flavonoids occur in the form of 3-*O*-glycosides in *C. carvi*. Some compounds with terpenoid constituents were obtained from methanolic extract of caraway seed via crystallization techniques (Glidewell, 1991; Kunzemann and Herrmann, 1977; Ruszkowska, 1998). An aromatic compound glucoside and a glucide were isolated from the water-soluble portion of the methanolic extract of caraway fruits together with 16 known compounds. Their structures were identified as 2-methoxy-2-(4′-hydroxyphenyl) ethanol, junipediol A 2-*O*-β-δ-glucopyranoside and *L*-fucitol, respectively (Matsumura *et al*., 2002). The important flavonoids isolated are quercetin 3-glucuronide, isoquercitrine = quercetin-3-*O*-B-glucopyranoside, quercetin-3-*O*-Caffeoyl-glucoside and kaempferol-3-glucoside. Isoquercitrine is the predominant constituent (80 mg/kg dry weight) present in caraway seed while others are found in quantities 2–10 times lower. The above-mentioned flavonoids

are recognized as possessing antioxidative properties and are able to activate enzymes detoxifying carcinogenic substances and metabolites in the cells.

The coumarins in caraway seed have been identified as umbelliferone, coumarin and scopoletin (Nielsen, 1970). The furocoumarins identified are 8-methoxypsoralen (8-MOP) and 5 methoxypsoralen (5-MOP), detected in caraway seeds in quantities as low as 0.005 μg/g of dry weight (Ceska *et al*., 1987). The coumarins and furocoumarins are known to have antibacterial properties and they are potent photosensitizers when activated by near UV light. Thus they are phototoxic, mutagenic and photocarcinogenic and they also exhibit strong seed germination inhibiting action. Due to such properties as described by Ruszkowska (1998) coumarins have been identified for utilization in psoriasis treatment and in sunscreen lotions and preparations.

11.2 Production and international trade

The Netherlands is the principal commercial supplier of caraway seed, followed by Poland and Germany. Other source countries are Denmark, Bulgaria, Canada, Germany, the UK, India, Morocco, Romania, Russia and Syria (Weiss, 2002). Countries importing caraway include the USA, Switzerland, Austria and Hungary. About 3500 tonnes of caraway seeds and value added products are imported annually into the USA, around 80 % of this tonnage arriving from the Netherlands with the remaining amount from Poland and Denmark. Switzerland and Austria get about 500 t caraway (70 % of the total imported) from the Netherlands and the remainder from Poland.

The major producers of winter-type caraway are the Netherlands, Poland, Hungary and Russia; the spring type is produced mainly by Syria, Morocco, Egypt and western India. Average annual world production of caraway oil ranges from 30–40 t, with a total value of more than $1 million. The Netherlands is the major producer and exporter of caraway essential oil. International prices of careway oil vary from 55–60 USD per kg. Approximately 350 kg of caraway oil and 400 t of caraway seeds, worth 93 450 USD are exported annually from India. According to an estimate by Lozykowska (2009), caraway cultivation in Poland is about 1500 ha and annual demand for caraway seeds is 12 000 kg. Around 30 t of essential oil of caraway is traded yearly in the world market. The world production of seeds is around 15 000 t. Production, however, fluctuates from year to year both in quantity and in price.

11.2.1 Cultivation

Caraway grows as an annual at lower attitudes and as a biennial at higher altitudes up to 4000 m above sea level. It prefers a lot of sunshine and moderate temperatures (16/20 °C) for flowering and seed setting in biennial types (winter types), whereas annual types require more heat for seed production (Svab, 1992; Bouwmeester *et al*., 1995b). Annual caraway thrives in the cool short days of the Eastern Mediterranean winter and in the Indian plains (Arganosa *et al*., 1998). A cold temperature (8/5 °C, day/night) for 7 weeks was best for achieving 100 % flowering in biennial type caraway plants in Hungary (Nemeth *et al*., 1998).

The cultivars recommended for different regions are: Kepron, Prochan, Rekord (Czech Republic), Noord-Hollandsche, Mansholts, Volhouden, Karzo, Springcar, Bleija (The Netherlands), Arterner, Niederdeutscher, Rekord, Konczewicki, Plewicki (Poland), Gintaras (Lithuania), SZK-1 (Hungary), Bi-An and Newe Yaar (Israel) and Sylvia (Norway) (Bouwmeester *et al*., 1995a, 1998,1999; Dragland and Aslaksen, 1996; Putievsky *et al*., 1994; Sedlakova *et al*., 2001).

Caraway is propagated through seeds and is usually sown with a row spacing of 30–40 cm, during March–April in temperate areas and October–November in subtropical areas. About 6–8 kg good quality seed is required for sowing in 1 ha. A seed rate of 8–10 kg /ha is recommended for sowing in Poland in the month of April (Lozykowska, 2009). Depending on region and cultivar, biennial types are harvested from July–September. The annual crop is ready for harvest in March–April after 4–5 months. However, in temperate areas the plant flowers only after over-wintering and thus the crop is harvested in July about 15 months after planting. Caraway yield fluctuates widely from 1–3 t/ha for biennial types and 0.7–1 t/ha from annual types, depending on fertilizer content, cultivation area and cultivars (Venskutonis *et al*., 1999; Lozykowska, 2009).

11.2.2 Post harvest handling

After threshing and mechanical cleaning, the fruits should be re-dried down to 10–12 % moisture content. Raw material so prepared is packed into sacks and, if inadequately stored, it can go musty and mouldy, thus becoming useless (Weglarz, 1998). Spices should thus be stored in a dry, cool and dark place in order to retain the aroma as long as possible. Shade-dried seed contains more oil content than sun-dried seed. The seed can be cleaned easily with a screening mill followed by a gravity separator. The fresh seed should be taken to an oil extraction unit for recovery of essential oil content (Malhotra, 2006). Ground and powdered caraway should be stored in refrigerator in airtight containers and should be used as early as possible since it loses its flavour quickly once opened.

11.3 Main uses in food

The main processed products of caraway are whole seed, essential oil, oleoresin and powder. It is used in the food, cosmetics, beverage and pharmaceutical industries.

11.3.1 Uses of the whole seed

Caraway seeds are botanically fruit, and they have a distinct warm, slightly sweet, sharp but pleasant aroma. Caraway seed is processed for drying, cleaning and grading and is mostly sold as whole in the international market. Due to its inherited preserving qualities, it is known to possess good storage life. Caraway seed is used as a spice for seasoning at both household and commercial levels.

Caraway seeds are extensively used as a common spice in European and Mediterranean cooking. Germans use caraway seed in many of their baked goods, breads, piecrusts, sauces and their famous sauerbraten. Austrians like caraway in stews,

while Italians boil hot chestnuts with caraway seed before roasting them. Caraway masks the smell of heavy foods like spareribs, roast goose, pork, mutton, oxtail stew and other meat dishes, and adds an interesting sweetness to apples, pound cake and cheeses. It is commonly used in canapés, onion bread, cheese spreads, omelets, coleslaw, cooked pastas, rye bread, soups, salad dressings, sauces, rice, boiled seafoods, cabbage and potato soups, sauerkraut, cucumber salad, poultry dressings, stews, homemade sausage and vegetables such as beets, carrots, cabbage, cucumbers, onions, turnips, green beans, potatoes, cauliflower and zucchini (Farrell, 1999). In order to keep the fragrance and flavour intact, caraway seed is generally ground just before preparing dishes or whole seeds are lightly roasted before using them in a recipe.

In the bakery industry, caraway seed is not only mixed into white and rye bread but is also sprinkled on the dough before baking to create a better aroma and taste (Daffertshofer, 1980). The flavouring of different kinds of alcoholic beverages has a long tradition, particularly in Denmark and other Scandinavian countries. The popular products described as *akvavit* or *aquavit* are flavoured using neutral alcohol distillates of caraway. Caraway is only added before distillation (Ney, 1987) and this means that the flavour of the drinks is attributable to the distillates of caraway. Some well-known alcoholic beverages worldwide which include caraway are listed in Table 11.3. In American gin, the flavour additives mostly used include juniper berries and cardamom as well as caraway seeds (Cole and Nobel, 1995). Similarly, caraway schnapps – based on the seeds from the caraway plant – is very aromatic, mild and smooth. It is mostly served with crayfish, marinated herring, fish, Danish smorgasbord, pork, stews, cheese and salads. Caraway schnapps is also added to soups, stews, goulash, sauerkraut, coleslaw, barbecue sauces, breads, cheese spreads and salad dressings. The recipe for caraway schnapps is given here (Anon., 2012b):

- Put 2 teaspoons caraway seeds in a clean glass jar with tight-fitting lid.
- Cover the seeds with 1–2 decilitre clear, unflavoured vodka – 40 % (80 proof).

Table 11.3 Popular alcoholic beverages using caraway

Beverage name	Origin	Remarks	Reference
Akvavit or aquavit	Scandinavia	Caraway with aniseed and fennel 40 % alcohol	Clutton (1995)
Allash	Russia	Sweet kummel with bitter almonds and aniseed	
Cloc	Denmark	Kummel 31 % alcohol, colourless	
Kummel	Netherlands	Caraway with some anise and cumin, minimum 5 % alcohol, one of the oldest liqueurs with digestive properties.	
American gin	USA	Flavour additives include juniper berries, cardamom and caraway seeds	Cole and Nobel (1995)
Caraway schnapps		Caraway seeds in unflavoured vodka (40 %)	Anon. (2012b)

- Let steep for a week in a dark place at room temperature, 18–20 °C (64–68 °F).
- Shake lightly and taste it from time to time.
- Strain and filter your infusion into a clean glass bottle or jar with tight-fitting lid.
- Store (*age*) for a month or so in a dark place at room temperature before serving.

11.3.2 Uses of ground caraway

Ground caraway is produced by grinding dried, cleaned and sterilized fruits. The fine powder product is mostly used for seasoning of foods whereas the coarse product is used for the purpose of extraction of essential oils and oleoresin. Pre-chilling and reduced grinding can be used to overcome the loss of volatiles. Cryo-grinding can help to reduce oil loss during grinding and maintain optimum particle size (Russo, 1976). Moreover, cryo-ground caraway disperses more uniformly in spice formulations and is thus used as a spice for seasoning at both household and commercial levels. The ground caraway is mostly used for adding taste and aroma to various food preparations as well as in bakery products and alcoholic beverages.

11.3.3 Uses of the essential oil

Caraway essential oil is obtained by steam distillation or hydrodistillation of seeds or other parts of the plant as per market requirement and intended use. However, the essential oil extracted from seed is superior in quality and commercially more valuable. In general, the essential oil content in caraway seed ranges from 2.9–5.1 % the major components being δ-carvone upto 65 % and δ-limonene up to 40 %, but these proportions are variable. High-quality seed may contain up to 7 % volatile oil and up to 15 % fixed oil. Sedlakova *et al*. (2003b) reported that caraway seed samples collected before maturation had lower essential oil content than samples harvested at full ripeness and that recovery of essential oil content was also greater with supercritical fluid extraction than with steam distillation. Ground caraway yielded more essential oil (81 %) compared to whole seeds (66 %) in both of the extraction methods. In whole caraway extracts, the carvone content was 81.53 %; in ground caraway extracts, the carvone content declined to 66.37 %. Among the three types of seed mills (ETA 0067 with millstones, splintery VIPO mill and cryogenic mill Vibrom) evaluated by Sedlakova *et al*. (2003a), the highest amount of extracted essential oil was obtained with the splintery mill VIPO (2.55 %).

The fresh seed can be crushed and enable immediately processed for distillation to avoid evaporation losses and enable recovery of more essential oil content. The average essential oil yield, as assessed on laboratory scale, was around 70 kg/ha with top yield of 160 kg/ha (Dachler and Pelzmann, 1999). The oil has a strong characteristic odour due to the carvone content, and the rectified oil obtained using a double distillation process is colourless to pale yellow and has a strong odour and more biting taste. Caraway essential oil is used in the food, cosmetic and pharmaceutical industries. It is used in all major categories of foods and beverages, including alcoholic and non-alcoholic beverages, frozen dairy desserts, candy, baked goods, gelatins and puddings, meat and meat products, condiments, relishes and others. The oil has virtually replaced the seed in processed foods. The highest average maximum

use level is reported to be about 0.02 % in baked goods. It is also used as a fragrance component in cosmetic preparations including toothpaste, mouth wash, soaps, creams, lotions and perfumes, with maximum use level of 0.4 % reported in perfumes (Leung and Foster, 1996), shampoos, mouth washes and aftershaves. Whey protein-based microencapsulation of essential oil of caraway is also done to convert liquid food flavouring into a dry and free-flowing powder form which is easy to handle and incorporate in food (Bylaite *et al.*, 2001). Caraway essential oil has been used with animal food (pasture) for milking cows and sheep (Heeger, 1956), but this is less common now.

After crushing, dried seeds are processed for distillation in order to get a better yield and high-quality oil. Crushed seeds are spread evenly on perforated grids provided in the still to allow complete penetration by the steam. It takes about 6–8 hours for optimum distillation of one batch. According to Bentley and Trimen (1999), caraway derived from a northern or elevated area yields the most oil. Dutch oil is also regarded as better than that distilled in the southern parts of Germany.

11.3.4 Uses of the fatty oil

The fatty oils produced from the distillation process for caraway seed have been reported to be particularly rich in petroselinic acid. The fatty acid profiles of the oils were analysed by automated GC, and petroselinic and *cis*-vaccenic acid were identified as the major components (Reiter *et al.*, 1998). Petroselinic acid is an important raw material for oleochemical processes and can be easily processed into lauric and adipinic acid. Caraway seed oil sourced from Tunisia is rich in petroselinic acid of potential industrial significance (Laribi *et al.*, 2010).

Caraway fatty oils are primarily used in the soap industry for flavouring and in the manufacture of perfumed disinfectant soaps. Fatty acid composition in the late stages of fruit development was 1 % stearic acid, 4 % palmitic acid, 35 % linoleic acid and 60 % petroselinic and oleic acids for annual caraway and 0.5 % stearic, 5 % palmitic, 38 % linoleic and 57 % petroselinic/oleic for the biennial form (Bouwmeester *et al.*, 1998).

11.3.5 Uses of caraway chaff oil

Caraway chaff oil is distilled from the husks and stalks that remain after threshing and is considered inferior in quality comparing with oil extracted from seeds. The dried and pulverized caraway chaff contains 20–23.5 % crude protein of which 75–85 % is digestible and 14–16 % is fat and is ideal for use as cattle feed. The chaff oil is obtained by steam distillation of material left after threshing of fruits. It contains less carvone and more terpenes and thus has less of the characteristic odour of the seed oil and is harsher with a somewhat bitter taste.

11.3.6 Uses of herb and root oil

Caraway herb oil is obtained by steam distillation from fresh whole plant, stalks, leaves and seeds. The top stem is usually prepared for distillation. Growers differ on whether the plant should be harvested before or after flowering. Caraway herb

oil is similar in flavour to oil extracted from seed. Thus it could quickly expand to commercial production as an alternative to seed. The root oil can also be obtained by distillation of minced roots and consists mainly of oxygenated compounds with aldehydes up to 70 %, including octanal, nonanal, *cis*-dec-4-enal and *trans*-dec-2-enal. Complete analysis of herb oil and root oil in comparison to essential oil extracted from seed is not available, but it is considered to be inferior in quality to seed oil.

11.3.7 Uses of caraway carvone

The essential oil constituent *d*-carvone is a nearly colourless to pale yellow liquid, which darkens with age. The odour of caraway carvone is warm, herbaceous, bread-like, spicy, slightly floral. The carvone reportedly has certain cancer-preventive and anthelmintic properties. Pure carvone is prepared by decomposing crystalline compounds of carvone with hydrogen sulphide. Carvone is used in the soap industry to add natural aroma to soap. The demand for carvone is specialized, but it could become an alternative to caraway seed in the food processing and pharmaceutical industries.

11.3.8 Uses of decarvonized oil

Decarvonized oil consists of limonene with traces of carvone and is sold in the market as light oil of caraway. It finds use in scenting cheap soaps. At the beginning of distillation, the essential oil has higher carvone, whereas at the end limonene predominates. The reason is that carvone, as an oxygen-containing compound, is several times more soluble in water than limonene.

11.3.9 Uses of caraway oleoresin

Caraway oleoresin is one of the most valuable flavouring agents as it imparts a warm, aromatic and pleasing flavour to food products. It contains essential oil, organically soluble resins and other related materials present in the original spices. It normally contains 20–25 % volatile and 60–75 % fixed oil as reported by Weiss (2002). The oleoresin is prepared by extraction of crushed dried seed with suitable volatile oil solvents like food-grade hexane ethanol, ethyl acetate or ethylene dichloride, filtration and desolventization under vacuum. The organic solvent should be recovered completely from the oleoresin as set out in ISO and national standards for fixed maximum permissible limits for approved solvents. The effects of different polar solvents or modifiers (methanol, ethanol, acetone, acetonitrile, hexane, dichloromethane [methylene chloride], chloroform or toluene) on yield have been studied (Sedlakova *et al.*, 2003b). The variety used was Kepron, and all the modifiers significantly increased essential oil yield. The use of chloroform was most effective, increasing the amount of extracted essential oil by approximately 91 % compared to steam distillation. Commercial samples in the USA require a minimum of 60 % volatile oil with a dispersion rate of 5 %. The high fixed oil content usually requires the addition of an antioxidant to the legal limit.

11.3.10 Uses of caraway honey

Each caraway plant produces thousands of tiny flowers (florets) that are held in compound umbels. Both nectar and pollen are produced by caraway florets and constitute a valuable source of protein for honeybees, particularly during colony buildup in spring. Honeybees (*Apis mellifera* L.) collect both pollen and nectar from flowers; the former serves as their chief protein source, the latter their source of carbohydrate (Herbert, 1992). Nectar is stored and converted into honey, an important agricultural commodity. The Prairie Provinces produce about 75 % of Canada's honey crop, annually. Caraway honey is a potentially valuable commodity with a number of medicinal properties (Langenberger and Davis, 2002).

11.3.11 Uses of natural potato sprout inhibitor

Besides the use of caraway seeds, caraway seed powder and essential oils in the food and pharmaceutical industries, caraway has proved an important natural sprout inhibitor in potato, extending its dormancy period and quality after storage. Caraway as a natural sprout inhibitor had a positive effect on reduction in respiration intensity, dry matter, reducing sugars and starch contents after 7 months during storage (Zabaliuniene *et al*., 2003). A few monoterpenes from caraway, including S-carvone, were found to suppress sprout growth for more than a year, depending upon the amount applied (Hartmans *et al*., 1995). S-carvone as a commercial suppressant for ware potatoes under the tradename 'Talent' is available in the Netherlands. Ground seed treatment with caraway carvone was the most effective treatment for reducing the number of sprouts on potato tubers and preventing weight loss of tubers (Sanli *et al*., 2010).

11.4 Nutritional and functional benefits

Caraway fruits are recognized as containing beneficial nutrients, minerals, antioxidants and vitamins (Table 11.4). Caraway seeds are a rich source of dietary fibre, 100 g of seeds providing 38 g of fibre. Caraway fruit is mentioned by the pharmacopoeias of numerous European countries, the USA and others as a digestive, carminative and galactagogue. According to Chevalier (2001), the seeds are expectorant and tonic and are frequently used in bronchitis and cough remedies, especially those for children. Different caraway-only preparations (Ozarowski and Jaroniewski, 1987; Lutomski and Alkiewiez, 1993) and/or in composition with other herbs and spices (Sadowska and Obidoska, 1998) are given in Table 11.5. Caraway seeds and extracts are known to possess a number of functional properties:

- antiflatulent, antispasmodic and antidyspepsic;
- antimicrobial (antibacterial and antifungal);
- emmenogogue and lactogogue;
- antioxidant;
- anticarcinogenic and other properties;
- insecticidal.

Table 11.4 Caraway seed (*Carum carvi*), nutritional value per 100 g

Principle	Nutrient value	Percentage of RDA
Energy	333 Kcal	17 %
Carbohydrates	49.90 g	38 %
Protein	19.77 g	35 %
Total fat	14.59 g	48 %
Cholesterol	0 mg	0 %
Dietary fibre	38 g	100 %
Vitamins		
Folates	10 mg%	2.5 %
Niacin	3.606 mg	23 %
Pyridoxine	0.360 mg	28 %
Riboflavin	0.379 mg	29 %
Thiamin	0.383 mg	32 %
Vitamin A	363 IU	12 %
Vitamin C	21 mg	35 %
Vitamin E	2.5 mg	17 %
Vitamin K	0 mg	0 %
Electrolytes		
Sodium	17 mg	1 %
Potassium	1351 mg	29 %
Minerals		
Calcium	689 mg	69 %
Copper	0.910 mg	101 %
Iron	16.23 mg	203 %
Magnesium	258 mg	64.5 %
Manganese	1.300 mg	560.5 %
Phosphorus	568 mg	81 %
Zinc	5.5 mg	50 %
Phytonutrients		
Carotene-ß	189 mg	–
Crypto-xanthin-ß	58 mg	–
Lutein-zeaxanthin	205 mg	–

Source: USDA (2010).

12.4.1 Antiflatulent and antispasmodic properties

A liniment formed by adding a few drops of caraway oil to a small quantity of olive oil, which is then rubbed over the pit of stomach or the abdomen, is known to alleviate colic (Ozarowski and Jaroniewski, 1987; George, 1996). Caraway seeds also alleviate colic if consumed as an oil. Being antispasmodic, the seeds soothe the digestive tract, acting directly on the intestinal muscles to relieve colic and griping as well as bloating and flatulence. The presence of *d*-limonene and *d*-carvone probably contributes towards caraway's antispasmodic action.

Duke *et al*. (2002) confirmed an antispasmodic effect with ED 50 caraway oil at a dose of 20 mg/l. In a study, tablets containing a combination of 100 mg each of peppermint leaves, caraway and fennel fruits and 30 mg gentian root were administered to patients with idiopathic dyspepsia immediately after a meal. Three tablets were sufficient to reduce symptoms after an hour (Uehleke *et al*., 2002). An enteric-coated combination preparation consisting of one capsule containing 90 mg

Table 11.5 Key preparations from caraway and their application in medicine

Preparation	Dose formulation	Properties as medicine	Dose
1. Caraway seed preparations (Ozarowski and Jaroniewski, 1987)			
a. Caraway honey	1 g caraway fruit powder and one TSP honey	Carminative	2–4 times a day
b. Caraway tea	Pour 1.5 glass (capacity 0.35 l) of boiling water over 1 TSP of pulverized fruits	Carminative	Drink 0.5 glass 2–3 times a day after meals
c. Caraway syrup	Pour 1 glass (capacity 0.25 l) of boiling water over 1 TSP of pulverized fruits, keep covered for 30 min, strain and add honey	Carminative for children	Serve 1 TSP after each meal
2. Caraway herbal composition (Lutomski and Alkiewick, 1993)			
a. Mix fruits of caraway, anise, peppermint chamomile and thyme in equal proportions	Pour a glass (capacity 250 ml) of boiling water over 1 TSP of herbs, keep covered for 30 min	Carminative	Drink 0.5 glass 2 times a day after meals
b. Mix fruits of caraway, anise and fennel in equal proportions	Pour a glass (250 ml) of boiling water over 1 TSP of herbs, keep covered for 30 min	Carminative and galactagogue	Drink 0.5 glass 2 times a day
c. Mix fruits of caraway, anise, fennel and coriander in equal proportions	Pour a glass (capacity 250 ml) of boiling water over 1 TSP of herbs, keep covered for 30 min	Carminative	Drink 0.5 glass 2 times a day
d. Mix double proportions of caraway fruit, fennel fruit, yarrow herb, thistle herb and root of liquorice in equal proportions	Pour a glass (capacity 500 ml) of boiling water over 1.5 TSP of herbs in thermos, keep covered for 1 hr	Digestive (improves appetite)	Drink 0.5 glass 30 min before meals

Table 11.5 *Continued*

Preparation	Dose formulation	Properties as medicine	Dose
3. Caraway herbal composition (Sadowska and Obidoska, 1998)			
a. Mix fruits of caraway, anise, peppermint, chamomile and thyme in equal proportions	Pour 0.75 l of dry, white wine over 3 TSF of herbs for 2 weeks (shaking from time to time)	Digestive (improves appetite)	Drink about 50 ml two times a day after meals
b. Mix fruits of caraway, yarrow, root of valerian herb of St. John's wort, leaves of buckbean and leaves of bahu in equal proportions	Pour 0.5 l of boiling water over 2 TSP of herbs in a thermos and keep closed for 30 min	Digestive (improves appetite)	Drink about 500 ml three times a day between meals
4. Liniment of external use (George, 1996)	Dissolve 10 g of caraway essential oil and 5 g of thyme essential oil in 15 ml of 95 % ethanol. mix with 150 g castor oil or some other plant oil	Scabies and mycosis	Apply liniment over affected area as skin
5. Liniment of caraway oil	Few drops caraway oil and olive oil	Anticolic	Rub the liniment over intestinal muscle
6. Liniment for external use (Pruthi, 2001)	5 parts each of caraway oil and alcohol in 75 parts of castor oil	Scabies	Apply over affected area on skin
Caraway formulations (Duke *et al*. 2002)			
a. Caraway seed	1.5–6 g fruit	Antiseptic, anti-anaemic, antibacterial, anticancer, antihistamine, antispasmodic, arminative	2–4 times a day between meals
b. Caraway seed powder	1–2 TSP crushed seed/cup water or chew 1 tsp seed	Digestive, stimulant	3–4 times a day
c. Caraway seed	0.5–2 g powdered seed	Stimulant	3 times a day
d. Caraway concentrated seed water	0.05–0.2 ml concentrated seed water or 0.5–1 tsp tincture or 3–4ml liquid extract	Stimulant	3–4 times a day
e. Caraway essential oil	3–6 drops oil or 0.05–0.2 ml	Stimulant	–

peppermint oil + 50 mg caraway oil consumed each day provided an effective treatment for functional dyspepsia (Freise and Kohler, 1999; Madisch *et al*., 1999). Fruits of caraway ingested orally produce an effect on the digestive tract, bile ducts, liver and kidneys. They have spasmolytic properties, affecting the bile ducts and the sphincter and regulating the flow of bile and pancreatic juices to the duodenum. They act as a cholagogue and increase the secretion of gastric juices, resulting in appetite and digestion stimulation. Commercial caraway seed supplements in the form of an encapsulated powder are available with the recommended dose of two capsules, one or two times a day with water at meal times (Anon., 2010).

11.4.2 Antimicrobial (antifungal and antibacterial) properties

Owing to its antifungal and antibacterial properties, caraway essential oil or carvone is recommended for external use for control of dermal mycosis and scabies. Caraway has been reported to inhibit *Staphylococcus aureus*, *Esherichia coli*, *Salmonella typhi*, *Vibrio cholerae* (Syed *et al*., 1987) and *Mycobacterium tuberculosis* (Mishenkova *et al*., 1985). The application of caraway essential oil has shown an inhibitory effect on three strains of gram-negative and four of gram-positive bacteria, making it a safe and effective antimicrobial agent to prevent deterioration of stored foods by bacteria (Farag *et al*., 1989b). For the treatment of scabies, a solution containing five parts each of alcohol and oil of caraway in 75 parts of castor oil is recommended to be taken orally (Pruthi, 2001). In Indonesia, the leaves mixed with garlic and placed on the skin are recommended to treat inflamed eczema (Perry, 1980). The essential oil showed promising inhibitory activity against all the test bacteria, even at 2 µl/disc and showed 100 % inhibition of radial mycelial growth of all the test fungi at 100 ppm (Begum *et al*., 2010).

The vapours of the essential oils (80–160 ppm) of caraway (*C. carvi*) exhibited antifungal properties against *Mycocentrospora acerina*, *Fibularhizoctonia carotae [Rhizoctonia carotae]* and *Sclerotinia sclerotiorum,* three important post-harvest pathogens of carrots (Horberg, 1998; Regina and Tulasi-Raman, 1992). The extent of inhibition of fungal growth and mycotoxin production was dependent on the concentration of essential oils used. Caraway oil was inhibitory at 2000 ppm against *Aspergillus flavus* and *A. parasiticus,* and at 3000 ppm against *A. ochraceus* and *Fusanium moniliforma*, the mycotoxigenic fungi (Soliman and Badeaa, 2002). Use of caraway oil (4 %) also showed high antimicrobial activity against *Agrobacterium tumefaciens, Ralstonia solanacearum* and *Erwinia carotovora* (Hassanein and Eldoksch, 1997). *C. carvi* essential oil also causes inhibition of mycelial growth and aflatoxin production of *A. parasticus* and can be used as an alternative to chemical preservatives such as potassium fluoride, acetic acid and potassium sulphite addition in foods (Farag *et al*., 1989a). The antibacterial, fungicidal and insecticidal properties of caraway, and their non-toxicity for humans, gives them great potential in crop production and food preservation under the organic production systems. Skrinjar *et al*. (2009) also reported inhibitory effect of caraway extractives on aflatoxin.

11.4.3 Emmenogogue and galactogogue properties

Use of caraway fruits by breast-feeding women and bovines favours milk secretion and enhances lactation and has an indirect, beneficial effect on the baby's digestive

system, because of anti-griping properties. The components acting as a galactagogue in caraway seed have not been identified but may well be limonene and carvone, the main components of caraway seed with these properties (Molnar *et al*., 1997). The addition of 50 g caraway seeds to the basic daily diet of lactating buffalo, continued for 12 weeks of lactation, increased the milk yield, daily fat, Solid-not-fat (SNF), lactose and protein yield significantly (El-Alamy, 2001). A daily supplement of ground caraway seeds had a favourable effect on enhancement of milk yield and milk quality in cows (Portnoi, 1996).

11.4.4 Antioxidant properties

Caraway possesses antioxidant properties. Farag and El-Khawas (1998) reported that essential oils extracted from the gamma-irradiated (10 KGy) caraway fruits were effective as antioxidants, more so than those produced from microwaved fruits. Caraway supplementation at a dose of 60 mg kg^{-1} had a modulatory role on tissue lipid peroxidation, antioxidant profile and prevented 1,2 dimethylhydrazine-induced histopathological lesions in colon cancer rats (Kamaleeswari and Nalini, 2006) and inhibited tumorigenesis (Kamaleeswari *et al*., 2006). Caraway seed extracts can potentially reverse the 2,3,7,8-tetra chlorodibenzo-*p*-dioxin (TCDD) dependent induction in cytochrome, the carcinogenic metabolite (Naderi-Kalali *et al*., 2005). A significant relationship between the concentration of flavonoid compounds and the age of caraway roots has been observed. During the vegetative season, the concentration of flavonoids in caraway roots gradually increases and ranges from an average level of 0.153 mg g^{-1} of air-dry matter (a.d.w.) in June to 0.512 mg g^{-1} a.d.w. in October. The largest amounts of these compounds were contained in the roots of the plants (an average of 0.312 mg g^{-1} a.d.w.) (Najda *et al*., 2008). The antioxidant and antimicrobial properties of caraway have also been reviewed by Damasius *et al*. (2007).

11.4.5 Anticarcinogenic and other properties

Caraway has been recommended for masking unpleasant tastes in medicines and is recommended as a remedy for digestive tract disorders such as stomach gas. Caraway has been demonstrated as improving intestinal iron absorption (El Shobaki *et al*., 1990). The essential oil from caraway has been reported to be potentially anticarcinogenic (Zheng *et al*., 1992). This cancer chemopreventive property of caraway oil is probably due to the induction of the detoxifying enzyme glutathione 5-transferase (GST). Zheng *et al*. (1992) reported that carvone and limonene are the compounds responsible. Higashimoto *et al*. (1993) also reported antimutagenic activity of caraway extracts against N-methyl-N-nitro-N-nitrosoguanidine-induced cancers in experimental animals. Thus abundance of cancer chemopreventive substances (carvone) in the diet may even inhibit the early stages of carcinogenesis. Besides, anticarcinogenic effect 10 mg/kg BW of caraway oil is the safe dose that can be used in managing *Diabetes mellitus* (Ene *et al*., 2007).

11.4.6 Insecticidal properties

Caraway essential oil has proved toxic to mites and insects. It has been reported to inhibit allergy causing mites such as *Dermatophagoides pteronyssinus*, *D. farinael*,

Euroglyphus maynei, *Acarus siro*, *Tyrophagus putrescentiae*, *Glycyphagus domesticus*, *Lepidogoly phus destructor* and *Ghiera fusca* (Ottoboni *et al*., 1992). The petroleum ether extract of caraway seed inhibits the *Tyrogphagus putrescentiae* mite (Afifi and Hafez, 1988) and shows toxicity to some insects, causing larval inhibition in *Musca domestica*, *Culex pipiens,* fatigans and mosquito (Deshmukh and Renapurkar, 1987) and the larvae of *Spodoptera littoralis* (Antonious and Hegazy, 1987). Caraway has shown to cause 100 % and 60 % mortality in *Callosobruchus maculatus* at 10 µl and 1 µl, respectively, while 25 µl was needed to kill 68 % of *Sitophilus granarius* adults (Pascual-Villalobos *et al*., 2003).

11.5 Toxicity

Caraway and its products are generally safe for internal use and do not appear to have any significant toxicity in humans. Although it is non-toxic and non-sensitizing, it may cause skin irritation if used in high concentration. Caraway oil should not be used directly on the skin. The oil should first be mixed with diluting or carrier oil. Herbalists have advised a skin patch test before the diluted oil is applied to skin. However, Lewis (1977) while discussing the problem of allergy, mentioned carvone as sensitizing substance and classified caraway among plants causing contact dermatitis. Furocoumarins such as 5-methoxypsoralen and 8-methoxypsoralen, the known potent photosensitizing substances were detected in traces (Ceska *et al*., 1987). It has been suggested that essential oil of caraway is not safe to use in its purified form by children below two years, because it may cause skin and mucous membranes irritation. In very high dose, the oil can cause abortion and it may be neurotoxic. It should, therefore, be avoided by pregnant women.

The residues of nitrate, nitrite and pesticides in herbs can be transformed by bacteria to toxic nitrites which can cause blood circulation disorder and methemoglobinaemia. Analysis of caraway samples has shown no presence of nitrites but small quantities of nitrates (Gajewska *et al*., 1995). Similarly, analysis of a caraway from reputable suppliers for pesticides residues through gas liquid and thin layer chromatography tests showed that HCH was the main compound found but that the residue did not exceed the maximum limit of 0.2 mg/kg (Duke *et al*., 2002). Caraway is contraindicated in inflammation of the kidneys, since apiaceous essential oils may increase the inflammation as a result of epithelial irritation. Overdoses for long periods can lead to kidney and/or liver damage.

11.6 Quality specifications

11.6.1 Specification for whole seeds

Caraway whole seed means the dried seed of the plant (*C. carvi* Linn). Extraneous matter, including foreign edible seeds, chaff, stem straw, dust, dirt, stones and lumps of earth, should not exceed 5 % by weight. The amount of insect-damaged matter should not exceed 5 % by weight. The physical quality of caraway seeds depend mainly on:

- Quantity of mature, undamaged seeds with appropriate colour, uniformity of size, shape and texture.
- The colour of the crescent-shaped, hard seeds is greyish tan to dark brown marked with five light-coloured ridges and length. Whole fruits are 3–7 mm long, 1–2 cm thick and slightly curved.
- The aroma from seeds should be very aromatic, sweet, spicy, fresh, slightly minty with an aroma resembling anise.
- Seed weight of 1000 grains of biennial type caraway is 3–4.5 g and annual type is around 5.2 g (Franz, 1996).

The minimum specific quality indices for caraway seed are given below (Farrell, 1999):

- total ash: 8.0 %
- acid soluble ash: 1.0 %
- seed moisture: 10 %
- volatile oil: 3 %

The general characteristics of quality standards as laid down under the Prevention of Food Adulteration (PFA) Act and Rules by BSI of India for caraway are defined by Pruthi (2001).

11.6.2 Caraway powder

Caraway of *Siah Jira* powder means the powder obtained from the dried seed of *C. carvi* (L). It may be in the form of small pieces of the seeds or in finely ground form. It should be free from added colouring matter. The ground product should be uniform, allowing a minimum of 95 % by weight to pass through a US Standard No. 30 sieve. In addition, it shall conform to the following standards:

- moisture: Not more than 13 % by weight
- total ash: Not more than 8 % by weight
- ash insoluble in dilute HCI: Not more than 1.5 %

11.6.3 Essential oil and fixed oil

The essential oil content of caraway seed generally ranges from 2–5 % and it primarily contains carvone (47–81 %), limonene (9–48 %) and fixed oil (15 %). The caraway oil is a mobile liquid, almost colourless to pale yellow, although it may become brownish to dark brown depending upon time. The physicochemical properties of caraway seed oil are given in Table 11.6. The quality standards as prescribed by the American Spices Trade Association (ASTA) and ISO are given in Tables 11.7 and 11.8.

11.6.4 Adulteration

Caraway seed is available in both whole and ground form and is subject to adulteration by addition of exhausted or spent seed (from which oil has been extracted), excess stems, chaff and earth or dust. Caraway essential oil is also adulterated with

Table 11.6 Physicochemical properties of caraway seed

S. No.	Characters	Requirement
1.	Appearance	Pale yellow
2.	Odour	Strong spicy
3.	Specific gravity at 15 °C	0.907–0.919
4.	Refractive index 20 °C	1.484–1.488
5.	Optical rotation	+70°0' to +80°0'
6.	Carvone contents	50–60 %
7.	Limonene	20–30 %
8.	Solubility	Seldom soluble in 70 % alcohol, soluble in 2–10 vol. of 80 % alcohol clearly soluble in equal volumes of 90 % alcohol.

Source: Singhal *et al*. (1997).

Table 11.7 Cleanliness specifications for caraway seed as per ASTA

Crop	Whole insects dead by count	Excreta mammalian by mg/lb	Excreta other by mg/lb	Mould % by weight	Insect defiled/ infested % by weight	Extraneous/ foreign matter % by weight
Caraway seed	4	3.00	10.00	1.00	1.00	0.50

Source: Muggeridge *et al*. (2001).

Table 11.8 Quality standards for caraway seed as per ISO

Commodity	Ash % w/w max.	A/A % w/s max.	H_2o % w/w max	V/O % w/w min.
Dutch caraway seed	8	1.5	13	2.5

Source: Muggeridge *et al*. (2001).

caraway chaff, caraway wild types and root oil. The range of caraway essential oil is 2.5–5 % and it should preferably contain limonene and carvone at enantiomeric ratio ranging from 0.75–1.00. If chaff oil is added then the enantiomeric ratio will rise to more than 1.00, indicating the presence of more limonene and less carvone. The ratio of limonene and carvone varies with variety and geographical location, thus further study is required to standardize such quality parameters in order to assess the quality. The oleoresin may be adulterated by added synthetic saturated acid. The detection of these adulterants for oil and oleoresins can be done by using gas chromatography or high-performance liquid chromatography techniques. Adulterations at any level can be detected by using the specifications as explained separately for whole seed, powdered seed, essential oil and oleoresins.

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12

Celery

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Abstract: This chapter discusses celery (*Apium graveolens* L.), a plant which is largely grown for its bulbous roots, green leaves, petioles and seeds and is valued for its pleasant aroma and rich nutritional and medicinal properties. The aroma chemicals present in the essential oil from different parts of the celery plant are detailed. Celery cultivation practices are outlined and several processed products from celery fresh herb and seeds, which have potential in the international market, are described. The nutritional and functional properties supporting the medicinal use of celery, such as its anti-inflammatory, antispasmodic, hypotensive and antioxidant activity, are discussed. Lastly, the toxicology and allergenicity of celery are outlined and quality specifications for different celery products such as whole seed, powdered seed, volatile oil and oleoresins production are given.

Key words: celery, cultivation, chemical composition, nutritional value, functional properties, medicinal uses, toxicity, quality specifications.

12.1 Introduction

Celery, *Apium graveolens* L., belongs to the Apiaceae (carrot family). It is grown for its bulbous roots, green leaves, petioles and seeds (fruits) in different parts of the world. The dried seeds are used as spice and for medicinal purposes. The origins of celery and its allied varieties are not clear, but wild forms are reported to be found in marshy areas throughout temperate Europe and western Asia. The eastern Mediterranean region appears to be the most logical centre of domestication, but the distribution of wild types raises some doubts (Rubatzky and Yamaguchi, 1997). Celery was probably not cultivated until the Middle Ages. Celery production is thought to have developed on the low lands of Italy before spreading further to France and England. The wild plants were used for medicinal purposes for hundreds of years before celery was used as a food plant. The present cultivated celery plants are quite sweet, appetising and wholesome, but its wild ancestors were not considered fit for consumption. The early forms of celery mostly had hollow stems and petioles. During domestication, selection altered this heritable characteristic and reduced the bitter and strong flavour of the wild plant. Celery seeds have been used in the traditional system of medicine in the Middle East since ancient times. However, the use of celery seed oil has come about with the development of

the processed food industry, as the oil is widely used as food flavourant in the USA and Europe. Celery was introduced to India from France in around 1930 AD by a trading company in Amritsar in Punjab and is now commercially grown in that area on a large scale for its seeds.

12.1.1 Classification

Celery can be classified as an aromatic vegetable because it is mainly grown as a fresh herb, i.e. for the leaves and petioles. It has also been categorized as a seed spice because the seeds are used for this purpose in whole or powdered form, or in the form of seed oil or oleoresins. Three morphotypes of celery have been distinguished, based on the part of the plant used (Orton, 1984). These are *Apium graveolens* var. *dulce* (celery; blanched celery, stalk celery), *Apium graveolens* var. *rapaceum* (edible rooted celery, celery root or celeriac) and *Apium graveolens* var. *secalinum* (leaf celery or smallage type). Rubatzky and Yamaguchi (1997) have reported that *A. graveolens* var. *secalinum* is the most popular celery in Asian and Mediterranean regions. *A. graveolens* var. *dulce* is the most popular type in the UK. The smallage type is annual in habit and is cultivated for seed for use as a spice in India (Malhotra, 2006). Celery cultivars are generally also classified according to the following characteristic features: foliage colour (green or yellow/golden), blanching habit (early or late), bolting behaviour (slow or quick), climate (temperate or subtropical), life-cycle (annual or biennial), height (tall, intermediate or dwarf) and season (autumn or winter). Celery has a somatic chromosome number of 2n = 22 and is considered a cross-pollinated crop.

12.1.2 Chemical structure

The chemical composition of celery differs considerably depending upon the plant part (leaves, stalks or seeds), geographical region of production, stage of harvesting and type and method of production of essential oil. The major constituents reported in celery seed are limonene, coumarines, bergapten and fatty acids. Limonene, phthalides, β-salinene, salinene, apiol, santalol, sedanolide, isedanic acid, citric, isocitric, fumaric, malic and tartaric acids have been reported in celery seed essential oil and oleic, palmitic, palililoleic, petroselinic, petriselaidic, stearic, myristic, myristoleic acids have been reported in fixed oils. According to Chevallier (2001), the key constituent of celery seed is volatile oil (1.5–3.0 %) which contains 60–70 % limonene, phthalides and β-salinene, coumarins, furanocoumarins (bergapten) and flavonoids (apiin). According to Farrell (1999), celery seed volatile oil primarily consists of 60 % δ-limonene, 10–20 % selinene, 2.5–3.0 % sedanolid and 0.5 % sedanonic anhydride. The aroma chemicals present in celery seed as analysed through gas chromatography–mass spectrometry (GC–MS) analysis (Cu *et al.*, 1990) are given in Table 12.1. Chowdhury and Gupta (2000) found that celery seed oil contained 28 compounds belonging to different categories such as terpenes, sesquiterpens and their derivatives (Table 12.2). The compound β-selinene was the major constituent (29.23 %), whereas most of the other workers have reported lilmonene and selinene as the major constituents. The chemical structures of both of compounds are given in Fig. 12.1.

Table 12.1 Profile of aroma-chemicals in celery seed oil

Compound	Percentage
α-Pinene	1.05
Camphene	Traces
β-Pinene	Traces
Sabenene	0.76
Myrcene	0.95
-3-Carene	Traces
α-Phellandrene	Traces
Limonene	72.16
β-Phellandrene	0.02
cis β-Ocimene	Traces
trans-β-Ocimene	Traces
ρ-Cymene	0.74
Pentyl benzene	0.02
Linalool	1.48
Isopulegone	0.16
Caryophyllene	0.17
Carvone	0.09
Geranyl acetate	0.04
α-Lonone	0.05
Cinnamic aldehyde	0.15
Thymol	0.17
β-Selinene	12.17
α-Selinene	2.05
Epoxycaryophyllene	0.55
n-butyl phthalide	2.56
Eudesmol	0.29
Lingustilide	2.41

Source: Cu *et al*. (1990).

The composition of the oil from the fresh aerial parts of celery (at flowering stage) are α- and β-pinene, myrcene, transfarnesene, humulene, limonene, *cis*-β-ocimene, G-terpenene, *trans*-β-ocimene, apiol, β-selinene, senkyuonlide and neocnidilide (Sahel *et al*., 1985). Choline ascorbate and enzyme inositol trisphosphate were isolated from the celery leaves by Kavalali and Akcasu (1985), and McMurry and Irvine (1988), respectively. The chemical constituents extracted from the roots of *A. graveolens* var. *dulce* were 4-phthalides butylphthalide, neocnidilide, cnidilide, *z*-lingustilide and senkyonolide and *A. graveolens* var, *rapaceum* contained butylphthalide and *z*-butylide nephthalide, cnidilide, *E*- and *Z*-ligustilide, neocnidilide and senkyonolide (Gijbels *et al*., 1985).

12.2 Production and international trade

Celery is reported to be grown widely in Europe, America and Asia for leaves and petioles. In the USA, the major growing states are California, Florida, Michigan and New York, and in Europe major producing countries are France, Germany, the UK, Hungary, Italy, Belgium and Holland. Celery is predominantly cultivated for its seed

Table 12.2 Constituents (%) in fruits of *Apium graveolens*

Constituent	Percentage
Terpenes	
Limonene	0.22
β-Phellandrene	0.38
α-pinene	0.98
β-pinene	1.02
Sesquiterpenes	
β-Elemene	1.30
α-Humulene	1.90
Patchoulene	0.78
β-Selinene	29.23
Aromatics	
Pentyl benzene	6.81
Alcohols	
Benzyl alcohol	1.02
Carveol	1.74
Eudesmol	3.00
Geraniol	0.46
Limonene glycol	6.19
Linalool	0.81
Menthol	1.90
Terpineol	1.62
Thujol	0.28
Oxide	
Caryophyllene oxide	3.77
Aldehydes	
Citral	2.88
Methyl heptanal	1.05
Ketones	
Carvone	5.93
Dihydrocarvone	3.49
Menthone	0.60
Phenyl ethyl ketone	1.89
Esters	
Butyl phthalide	10.56
Geranyl acetate	0.85
Exobornyl acetate	0.28

Source: Chowdhury and Gupta (2000).

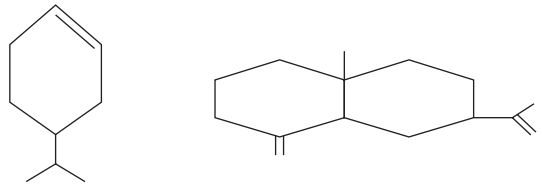

Fig. 12.1 Chemical structures of limonene and β-selinene.

in India, southern France, China and Egypt. India is the major producer and exporter of celery seed in the world market. In India, it is cultivated mainly in Amritsar, Gurdaspur, Jalandhar and Ludhiana in Punjab, Panipat in Haryana and Saharanpur in Uttar Pradesh (Malhotra, 2006). The production area is around 5800 ha and about 5900 tonnes are produced annually. Part of the annual crop is used for extraction of seed oil and oleoresins. Indian celery seed and its extractives are exported to the USA, Canada, the UK, Kuwait, the Netherlands, Singapore, South Africa, Japan and Germany. During 2009–10, India exported 5000 tonnes celery seed of worth Rs. 186 million, meeting 62 % of the world demand for celery seed. About 312 metric tonnes of celery spice powder worth Rs. 15 million, 25 metric tonnes of celery essential oil worth Rs. 38 million and 190 metric tonnes of celery oleoresins worth Rs. 48 million were also exported from India during 2009–10. The total world production of celery seed oil is about 51 tonnes, of which 25 tonnes is produced in India. The remaining amount is produced in Egypt, China, France, the UK and the USA.

12.2.1 Cultivation

Celery thrives best in climates with long, cool growing seasons where rainfall is well distributed or irrigation is assured. Optimum production now occurs when mean temperatures are between 16 °C and 21 °C as cultivars that can tolerate upper temperature ranges have been introduced. Celery can only be grown in a few subtropical regions. Celery is also sensitive to freezing temperatures but, on acclimatization, can tolerate a light frost for a short time. The leaf celery type has been reported to be more heat tolerant than the root celery or stalk celery type. As mentioned previously, cultivars of celery are generally classified according to certain characteristics, particularly as yellow/golden varieties, called 'self-blanching' varieties, or green varieties with dark green foliage. The green varieties can be further divided into two groups – early and easy to blanch; late and slower to blanch. The most important varieties are mentioned in Table 12.3. The different cultivars of celery with improved characteristics according to Tigchelaar cited by Desai *et al*. (1997) are Clean Cut, Florigreen and Transgreen. Farooqui and Sreeramu (2001) mentioned EC 99249–1 and RRL 85–1 as good varieties for cultivation under Indian conditions for high essential oil content. The National Research Centre on Seed Spices in India has developed a variety of celery – NRCSS-A Cel –1 – suitable for cultivation under semi-arid conditions for high yield and essential oil content (Malhotra and Vashishtha, 2008).

A celery crop may be raised from transplants or by direct seeding in the field. Seed germination and emergence of celery are slow even when conditions are favourable. Celery seeds are reported to possess thermodormancy resulting in no or slow germination at temperatures greater then 25 °C. A seed soaking treatment at 10 °C using growth regulators GA 4/7 and ethephon at 1000 ppm can overcome this dormancy (Thomas, 1990). Sunlight exposure also improves germination percentages when the seeds are very dormant; therefore, it is advisable to sow seeds shallow to enhance light exposure. The time of sowing varies depending on whether the crop is to be raised for fresh herb or seeds. In California, seed beds are sown in July or sometimes from December–January. The 8–12 weeks old seedlings are transplanted in to the well-prepared field. In other parts of the USA, with

Table 12.3 Types and cultivars of celery

Golden cultivars	Green cultivars
Golden Plume	**Utah type**: Utah 52–70 R, Utah 52–70 HK, Florida 683, Utah 52–70, Tall Green Light, Tender crisp
Golden Self-Blanching	**Summer Pascal type**: Summer Pascal, Giant Pascal
Michigan Improved Golden	**Slow Bolting type**: Slow Bolting Green No. 96, Slow Bolting Green No. 12
Cornell 619	
Golden Detroit	

Source: Swaider *et al*. (1992).

more severe winters, crops are started in the early spring by sowing in greenhouse or hot beds and seedlings are dug in the fall, and cold stored until planting time in spring. In India, celery is grown during September–October and transplanted from mid-December to the first week of January (Randhawa and Kaur, 1995). The row spacing of 40 cm gave a significantly greater seed yield. Celery crop yields about 60–70 t/ha of the fresh herb whereas seed yield of 2–4 q/ha can be obtained from the crops grown exclusively for the purpose (Malhotra and Vashishtha, 2008). The fresh herb crop of celery is harvested when plants are fully grown. The plants are either pulled off or cut below the soil surface along with petioles attached to the base. Normally, the salad crop is cut, trimmed and packed in the field. Mechanical harvesters are also used for harvesting of celery petioles (Swaider *et al*., 1992).

12.2.2 Post-harvest handling

Celery is mostly used as a vegetable or for seasoning and is valued for its rich nutritional properties, and pleasant aroma. To preserve its inherent properties preparation for market includes post-harvest operations such as removal of suckers, small lateral branches and damaged leaves, packaging and pre-cooling. All operations except the last may be done in the field or the packaging plant. The fresh herbs are stored mainly for short periods to increase availability and to avoid a glut in the market. Optimum storage conditions for celery fresh herb are 0 °C and a high RH (95 %). Controlled atmospheric storage can be used to maintain marketable quality for a relatively long period. Such storage requires a temperature of 0 °C and high RH in an atmosphere of 1–2 % O_2, 4 % CO_2 and with ethylene removal (Kadam and Salunkhe,1998; Malhotra and Vashishtha, 2008).

The celery seed crop is collected after harvest, allowed to cure and is then transported to the threshing floor, where it is dried in a thin layer for 1 or 2 days before carrying out light threshing to separate the seeds. The celery seeds are small in size, and separation of seed, when the whole plant is harvested causes difficulties. Therefore, individual umbel picking has been found to be more convenient. Shade-dried seeds have a higher oil content than the sun-dried seeds. The seed can be cleaned easily with a screening mill followed by a gravity separator. They are then graded by sieving and stored in gunny bags in a cool dry place (Malhotra, 2006).

12.3 Main products and uses in food

Several processed products made from fresh celery herb and celery seeds are popular in the markets of the USA and Europe and in Asian countries (Malhotra, 2010). The main uses of celery in the food processing industry are described below.

12.3.1 Products from celery leaves and petioles

Dehydrated celery

Dehydrated celery stalk and leaves are commercially marketed in the USA and the UK. The dehydrated products include celery stalk dice, leaf and stalk flakes, leaf and stalk granules and powder. The stalk of celery is valued more when it retains a deep green colour; a small quantity of sodium bisulphite or sodium sulphite is usually added for this purpose. Celery flakes are used in dry soup mixes, canned soups, sauces, stuffings, casserole products and vegetable specialities. Powdered or granulated celery is a good choice for canned and frozen sauces and dry mixes for breading and soups. Cross-cut and diced celery is used in canned and frozen soups, relishes, vegetable specialities and salad mixes.

Freeze-dried celery

Cross-cut slices of stalks of celery are also available in freeze-dried form. Freeze drying is effective in retaining the original shape and crispness of celery. This product makes a crisp garnish for potato salad, casseroles, pickles and relishes. Overall, the retention of nutrients is better in freeze-dried celery petioles than in those dehydrated by thermal methods.

Celery juice blends

Celery juice blends containing a mixture of celery and other vegetables such as carrot and tomato are becoming popular. They are marketed as healthy, cleansing beverages.

Blanched celery

Blanching removes the green colour in the petioles and is accomplished by excluding light from leafstalks while plants are still growing in the field. This process makes the leafstalks more tender but reduces the strong flavour and nutrients, particularly vitamin A. A small segment of customers still demand blanched celery. In the past, blanched celery was more popular, but currently there is more demand for green celery owing to its greater level of nutrients.

Pickled celery

Pickled celery made from both stalk and root celery has a ready market in the USA and other European countries. The tender petioles of celery are cured in dry brine and subsequently preserved using spices and condiments or in vinegar.

Canned celery

Tender celery petioles, both blanched and green, are ideal for canning. The unit operations include sorting and grading, washing, peeling (if required), coring and

pitting, blanching (if required), cane filling and brining. Usually canned celery is processed at high temperature (115–121°C) and high pressure (10–15 lb/inch^2) in an autoclave. The temperature and time of processing vary with the size of the can. Celery petioles are usually canned for later use in the off-season.

12.3.2 Products from celery fruits (seeds)

The whole seed is the basic material for preparation of various value-added items, viz. oils, oleoresins for flavouring purpose in foods, beverages and perfumery and for medicinal purpose in the pharmaceutical industry (Clevely *et al*., 1997).

Whole seed

Celery seeds are very small, dark brown, emit a characteristic odour, and induce a burning sensation. They may be used as a spice for seasoning practically any dish that calls for the flavour of celery, and they are particularly useful where fresh celery stalks would be impractical. Celery seeds may be used in tomato and other vegetable juices, bouillons, pea, chicken and turkey soups, coleslaw, pickles, scrambled eggs and omelettes, chicken and tuna casseroles, salads and salad dressing, seafood chowders, sandwich spreads and on cucumber, cabbage and beets. They have importance in the food processing industry the world over and are used in many Balkan, French, English, American and Asiatic recipes.

Celery essential oil

The volatile oil of celery is the most functionally important constituent of the seed. The oil can also be extracted from herbs and chaff, but the quality of seed oil is superior and it is commercially more important. The aroma of celery seed oil is warm, spicy, fruity and persistent. Celery chaff oil, however, has a somewhat harsher odour. Celery seed oil finds its major use in the flavouring of all kinds of prepared foods, such as soups, meats, pickles and vegetable juices. The oils also find use in perfumery and the pharmaceutical industry. Celery seed oil is produced by steam distillation. The seed should be crushed and immediately sent for distillation to avoid evaporation losses. Care should be taken during steam distillation to avoid channelling of steam. It takes 10–12 hours for distillation of one batch. Average oil yield under Indian condition has been reported to be 2–2.5 % depending upon the quality and quantity of seed and approximately 20–30 kg of celery oil is extracted from 1 ha (Farooqui and Sreeramu, 2001). The distillation wastes are usually re-distilled. Indian seeds give better yield of oil as compared to French seeds.

Celery oleoresin

Celery oleoresin is a very valuable flavouring agent as it imparts a warm, aromatic and pleasing flavour to food products. Considered 'liquid celery seed', it is much easier to handle in the preparation of tinctures and extracts than celery seeds themselves. It is a free-flowing green liquid with a herbal, slightly lemony and bitter flavour and consists of essential oil (about 9 ml/100 g), organically soluble resins and other related materials present in the original spice. The Indian types of celery oleoresin have been reported to have a pleasant lemon-like aroma and tenacious herbal undertones (Pruthi, 2001). Celery seed oleoresin is prepared by extraction

of crushed dried celery seeds with suitable volatile solvents like food-grade hexane ethanol, ethyl acetate or ethylene dichloride, filtration and desolventization under vacuum. The organic solvent should be recovered completely from the oleoresin. Fixed maximum permissible limits for the approved solvents can be found in the ISO standard, as well as those of the importing countries. Celery oleoresins are extensively used in processed foods, snacks, sauces, sausages, sea food, vegetable preparations and alcoholic/non-alcoholic beverages.

Celery powder

Celery powder is produced by milling or grinding the dried seeds. Coarsely ground material is accepted for extraction and distillation of oil and oleoresins, whereas for direct use in food seasoning, a finer product is required. Loss of volatiles and therefore characteristic aroma occurs in the grinding process. To keep this to a minimum, the seeds should be pre-chilled and ground at a reduced temperature (Anon., 1975). Freeze-grinding at −70 °C also has many advantages including increased retention of volatiles and improved dispersability of the fine ground material in food preparations (Russo, 1976). The aroma quality of ground spice deteriorates rapidly through loss of volatiles, but can be controlled by careful selection of packaging material. Celery seed powder is mainly used in food items such as salad dressings, soups, sausages, vegetable juices and pickles for flavouring purposes. It can also be sprinkled over salads, eggs and fish dishes.

Celery salt

Commercial celery salt is prepared by mixing finely ground table salt with ground celery seed or celery seed oleoresin or ground dried celery stems. According to Canadian standards, celery salt should be a combination of 25 % celery seed powder or ground celery and 75 % table salt (Pruthi, 2001).

12.4 Nutritional value and functional properties

Celery leaf petioles and seeds are valued for their bulk vitamin and mineral contents. Celery is an excellent source of vitamin C and a very good source of dietary fibre, potassium, folate, molybdenum, manganese and vitamin B6. Celery is also a good source of calcium, vitamin B1, vitamin B2, magnesium, vitamin A, phosphorus and iron. Celery also contains approximately 35 mg sodium per stalk, so salt-sensitive individuals can enjoy celery, but should keep track of this amount when monitoring their daily sodium intake. The nutritional composition of leaves, petioles and seeds is shown in Table 12.4. The composition varies with variety, region, part of the plant and age of the product.

Celery seed is not well-known in western herbal medicine, although it has been used medicinally for thousands of years in other parts of the world. During ancient times, Ayurvedic medicine used celery seed to treat colds, flu, water retention, poor digestion, various types of arthritis and certain diseases of the liver and spleen. Some medicinal uses of celery seed according to tradition and in modern times are listed in Table 12.5 (Sayre, 2001). Popular key preparations from celery are given in Table 12.6.

Table 12.4 Nutritional constituents of celery (per 100 g)

Constituents	Self blanching (petiole)	Green (petiole)	Leaves	Seeds
Energy (K cal)	29	34	64	392
Water (g)	96	95	81.3	6.0
Protein (g)	0.7	0.9	6.0	18.1
Fat (g)	0.1	0.1	0.6	25.3
Carbohydrate (g)	1.2	1.2	8.6	41.4
Vitamin A (IU)	90	120	80	52
Thiamine (mg)	00.03	0.03	Trace	–
Riboflavin (mg)	0.02	0.04	Trace	–
Niacin (mg)	0.3	0.3	Trace	–
Vitamin C (mg)	7	10	6.2	17
Ca (mg)	25	70	23	1767
Fe (mg)	0.3	0.5	6	45
Mg (mg)	10	14	–	440
P (mg)	27	34	14	547
K (mg)	–	–	–	1400
Na (mg)	–	–		160
Zn (mg)	–	–		7
Sources	Gupta *et al.* (1993)		Bahl *et al.*(1977)	Farrell (1999)

Table 12.5 Some medicinal uses of celery seed

Traditional medicinal use		Modern medicinal use	
Uses in Europe, America and Asia	Leaves, stalks, root stalks as source of nutrition	American use	Stalks as a remedy for high blood pressure and prevention of heart disease
European use	Roots as an aphrodisiac	European use	Fresh herbs and seeds as folk remedy for weight loss, lowering blood pressure, relief of anxiety insomnia, and reducing blood sugar
Ancient Egyptian use	Seeds as a medicine	Western use	Seeds and extractives as a remedy for arthritis, gout, rheumatism and urinary tract problems
Chinese use	Seeds as a remedy for arthritis, dizziness, gout	Chinese use	Seeds as a remedy for arthritis, dizziness, gout, high blood pressure, insomnia, nervousness and rheumatism
Indian use	Seeds as a diuretic and appetiser	Indian use	Seeds and extractives as a remedy for arthritis, urine problems and for liver protection

Source: Sayre (2001).

Celery seeds and extractives are known to contain several active substances, including flavonoids (plant pigments with antioxidant effects that may protect cells from damage), coumarins (chemical compounds that help thin the blood) and linoleic acid (an omega-6 fatty acid). According to a report from the University of Maryland Medical Center (Anon., 2011), celery is available in the market in

Table 12.6 Key preparations from celery and uses in medicine

Preparation	Dose formulation	Properties as medicine	Dose	Reference
1. Celery seed in buttermilk	One teaspoon of seeds soaked in a glass of buttermilk for 5–6 hours	For improving digestion	Take a glass	Anon. (2012)
2. Celery juice	Juice prepared from celery, leaf and petioles	Hypotensive and lowering blood pressure	40 ml orally 3 times a day with honey or soup	Duke (1983), Kaufman *et al*. (1999)
3. Celery and carrot juice	Juice of organic celery fresh herb and carrot	Cleansing drink	1 cup of juice daily	Chevallier (2001)
4. Infusion of seed	Infusion of seed	Gout and arthritis	1 cup daily	Chevallier (2001)
5. Tincture of seeds	Tincture from seed	Rheumatism	30 drops 3 times a day	Chevallier (2001)
6. Celery seeds	Whole seed	Chest problems, asthma and bronchitis, and urinary problems	Mix 1 tsp 3 times a day with food	Chevallier (2001)
7. Powder of seeds	Powered seed	Arthritis	Mix 1 tsp 3 times a day with food	Chevallier (2001)
8. Celery salt	25 % celery seed powder + 75 % salt	Appetizer and improves digestion	1/2 tsp daily	Pruthi (2001)
9. Celery pepper	30 % Celery seed powder + 70 % pepper powder	Appetizer and improves digestion	1/2 tsp daily	
10. Fresh herb	Fresh herbs	Antigout	4 celery stalks a day for more than 8 months	Kaufman *et al*. (1999)

Source: Pruthi (2001).

various commercial formulations as a protective medicine including as fresh or dried seeds, tablets and capsules filled with celery seed oil or celery seed extract. Celery has been reported to demonstrate a number of functional properties are as described below.

12.4.1 Anti-inflammatory

Kaufman *et al*. (1999) have reported that celery contains more than two dozen anti inflammatory compounds, including: α-pinene, apigenin, ascorbic acid, bergapten, butylidene-phthalide, caffeic acid, chlorogenic acid, cnidilide, copper, coumarin, eugenol, ferulic acid, gentisic acid, isopimpinellin, linoleic acid, luteolin, magnesium, mannitol, myristicin, protocatechuic acid, quercetin-3-galactoside, rutin, scopoletin, thymol, umbelliferone and xanthotoxin. Thus celery seed might prove useful in gout and other types of arthritis problems.

Celery seed is most often taken to aid the maintenance of healthy joints. It has been found to have anti-inflammatory properties that reduce swelling and pain around the joints. It also helps to detoxify the body and improve the circulation of blood to the muscles and joints. It is suitable for anyone who wishes to reduce the degeneration of body joints that commonly occurs with age. Due to its sedative and nerve stimulant properties, celery has been successfully employed in curing rheumatoid arthritis (Guenther, 1950). Prajapati *et al*. (2003) have advocated the use of celery for curing rheumatic pain in muscles of neck and sacrum. The phthalides present in celery seed and oil are said to have antirheumatic properties and the coumarins (furanocoumarian, fergapten) are thought to be muscle relaxants. Minerals such as calcium, iron, magnesium, phosphorus, potash, sodium and zinc present in celery also support the repair of connective tissue, thus making celery useful in slowing the progression of arthritis.

12.4.2 Antispasmodic

Celery seed oil has been reported to possess antispasmodic qualities. An emulsion of the oil is useful in relieving flatulence, colic pain and vomiting, calming the digestive system and enhancing appetite. It is a household remedy for gastric disorders. The presence of δ-limonene and β-selinene probably contributes towards the antispasmodic action of celery. Celery is a natural source of organic sodium which is needed for the lining of the stomach, as well as the joints and is a major mineral in the bloodstream.

12.4.3 Hypotensive and related effects

As reviewed by Chevallier (2001), a study in India found that extracts of seeds may lower blood fat levels. Chinese research indicates that celery seed oil lowers blood pressure. One phthalide in celery, 3-*n*-butyl-phthalide, is said to relax the smooth muscle linings of the blood vessels, thereby lowering blood pressure. The phthalide works directly by dilating vessels and is also a natural sedative. Perhaps this sedative activity could translate into reduced stress further translating into reduced cardiopathy. In addition to phthalides, celery is fairly well endowed with a

few other hypotensive compounds including ascorbic acid, bergapten (sometimes phototoxic), fibre, magnesium and rutin. However, celery also contains hypercholesterolaemic and calcium blocker phytochemicals (Duke, 1983; Kaufman *et al.*, 1999;).

12.4.4 Diuretic

Celery stems and seeds have long been taken for treatment of urinary problems. Their use helps the kidneys to dispose of urates and other waste products and works to reduce the acidity in the body as a whole. Due to diuretic properties, celery herb and seed is helpful in curing obstinate retention of urine (Prajapati *et al.*, 2003) and excessive retention of water. They are an effective treatment for cystitis, helping to disinfect the bladder and urinary tubules.

12.4.5 Antioxidant, anticarcinogenic and other properties

Celery contains compounds called *coumarins* that help prevent free radicals from damaging cells, thus decreasing the mutations that increase the potential for cells to become cancerous. Coumarins also enhance the activity of certain white blood cells, immune defenders that target and eliminate potentially harmful cells, including cancer cells (Cheun *et al.*, 2008). In addition, compounds in celery called *acetylenics* have been shown to stop the growth of tumour cells. Preliminary animal studies also show that celery seed may help prevent the formation of cancerous tumours in mice. In humans, researchers have found that people who eat a diet rich in lutein (from celery, spinach, broccoli, lettuce, tomatoes, oranges, carrots, and greens) were significantly less likely to develop colorectal cancer. However, celery was just one part of their diet, and no one knows whether the effect is due to celery, another food, or some combination of foods (Sultana *et al.*, 2005).

The major functional properties have been already discussed. Other authors have considered celery to be a stimulant, carminative and emmenagogue and able to cure headache. Studies also show that celery seeds act as a mosquito repellent (Choochote *et al.*, 2004; Tuetun *et al.*, 2004). The hepatoprotective properties of celery seed have also been reported (Ahmed *et al.*, 2002).

12.4.6 Toxicity

Celery has been identified as one of the plants known to cause dermatitis due to phototoxic reactions. Rubatzky and Yamaguchi (1997) discussed phototoxic activity in detail in their treatise and have reported that celery foliage and seed contain phthalides, terpenes, psoralen, xanthotoxin, bergapten and isopimpinellin. Out of these compounds, psoralen, xanthotoxin and bergapten are phototoxic causing dermatitis in human and animals after the skin is in contact with sunlight. McGuffin *et al.* (1997) associated the phototoxic reaction with the presence of phenolic compounds such as furocoumarins or psoralens. Some individuals exhibit much greater sensitivity to psoralens than others. Normally, the concentrations of these compounds in celery, parsley and other umbellifers do not pose a health threat for consumption or to field workers handling these plants. The concentration of these

compounds has been found to increase in response to pollutants, cold temperature, fungal infections, mechanical damage and the ultraviolet spectrum of sunlight.

It has been further cautioned that celery and celery products are not to be used during pregnancy or if suffering from a kidney disorder unless otherwise directed by an expert qualified in the appropriate use of the substance (Chevallier, 2001). In one compilation, Sayre (2001) mentioned that celery seeds lower potassium levels in the body. Celery seeds have, therefore, been suggested to be toxic if taken in excess. If a large amount of celery seed is consumed, therefore, its potassium-lowering effects need to be counterbalanced by consumption of bananas and other fresh vegetables containing a high amount of potassium. According to Kaufman *et al*. (1999), drowsiness might also be a side-effect of celery due to presence of phthalides, which are natural sedatives. Celery has also been reported to be antagonistic against calcium due to the presence of coumarins. Celery is reported to contain calcium blocker phytochemical coumarins such as bergapten, 1–520 ppm, isopimpinellin, 4–122 ppm and xanthotoxin, 6–183 ppm (Kaufman *et al*. 1999). It is also advised not to take celery without talking to your doctor first if you are taking any blood-thinning medicine, i.e. warfarin (Coumadin®), clopidogrel (Plavix®), aspirin, enoxaparin (Lovenox®), dalteparin (Fragmin®).

Lastly, Wuthrich *et al*. (1990) reported that celery contains a partly thermostable allergen, and a relatively high number of cases of severe anaphylactic reactions due to indigestion of celery have been reported in Switzerland. Celery-sensitive individuals also seem to show co-sensitization to mugwort pollen. Breitender *et al*. (1995) identified *APig1* as the gene responsible for the allergen in celery.

12.5 Quality specifications

12.5.1 Specifications for whole seeds

The quality of celery seed depends mainly on external appearance. Qualities such as colour, uniformity of size, shape and texture can be visually perceived. Celery seeds are minute, globular and light brown in colour with paler ridges; they seldom exceed 1 mm in diameter. Quality also depends on flavour, which is influenced by the composition of aromatic compounds. The intense flavouring qualities are due to presence of phthalides and terpenes (Rubatzky and Yamaguchi, 1997). Agmark of India provides three grades of celery seeds, viz. special, good and fair. The Bureau of Indian Standards has laid down Indian standards for various spices but, under the Protection of Food Act (PFA), specifications have yet been provided for celery seed. The grade designations and definitions of quality of celery seed are given in Table 12.7 (Pruthi, 2001).

The minimum specific quality indices as per Farrell (1999) are given below:

- seed moisture: 10 %
- total ash: 14 %
- acid insoluble ash: 2 %
- volatile oil: 2 %
- non-volatile ether extract: 12 %
- foreign organic matter: 2 %

Table 12.7 Grade designations and definitions of quality of celery seeds

Grade designation	Special quality characteristics		General characteristics
	Extraneous matter* (% by weight, max.)	Moisture (% by weight max.)	
Special	1.0	10.0	(a) Celery seed shall be the dried mature fruits of the botanically known *Apium graveolens* Linn
Good	2.0	10.0	(b) Free from visible moulds, live insects, any harmful foreign matter and musty odour
Fair	5.0	10.0	(c) Generally conform to the characteristic size, colour, taste and aroma of the variety type

*Extraneous matter means dust, dirt, stones, earth chaff, stalks, stems, straw or any other foreign matter.

Table 12.8 ESA – individual product specifications for celery seed

Celery Seed (ISO)	Ash % w/w(maximum)	A/A % w/w(maximum)	H_2O %w/w(maximum)	V/O % v/w(minimum)
	12	3	11	1.5

Source: ESA (2007).

The International Standard Organization (ISO) has also laid down standards. Production specifications for celery seed as per the European Spice Association (ESA) are given in Tables 12.8 and 12.9. The most recent contaminant tolerance limits for celery seed as prescribed by the American Spice Trade Association (ASTA) are:

- whole insects; dead by count 4
- excreta, mammalian; 3 mg/ef
- excreta other; 3 mg/ef
- infestion by weight; 1 %
- extraneous foreign matter by weight; 0.5 %.

12.5.2 Specifications for powdered celery seeds

Celery powder is produced by grinding dried, cleaned and sterilized celery seed. According to Farrell (1999), the powder should be ground until at least 95 % of it passes through a US Standard No. 55 sieve. After sieving through the required mesh size, the powder should be packed in airtight containers. Celery seed is ground to release the flavour: the finer the powder, the more readily available the flavour and the more readily dispersible it is in the matrix. Some flavour may be lost by heat development during grinding. The loss can be minimized by using cryo-milling or freeze-grinding instead. Celery powder is yellowish brown with an aromatic slightly

Table 12.9 Cleanliness specifications for celery in Germany, the Netherlands, UK and ESA (maximum limits)

Specifications for celery	Extraneous matter (%/weight)	Moisture (%/weight)	Total ash (%/weight)	Acid insoluble (ash %/weight)
Germany	–	10.0	12.0	2.5
Netherlands	–	12.0	10.0	2.5
UK	1.0	14.0	11.0	2.0
ESA	1.0	11.0	12.0	3.0

Source: ESA (2007).

camphoraceous odour and taste. Important precautions that need to be considered for the production of quality celery powder are:

- to maintain the moisture level at a minimum, which helps to increase the storage life;
- to use cryo-milling or freeze grinding to minimize volatile oil and flavour loss;
- to ensure the particle size is as specified by running it through a mesh;
- to make certain that further packaging is airtight;
- to guarantee the microbiological cleanliness of the powder;
- to ensure that the raw materials selected for grinding adhere to the specifications for whole celery seed mentioned above.

12.5.3 Volatile oil specification

The volatile oil content of celery seeds averages 2.5–3.0 %. The volatile oil consists primarily of 60 % *d*-limonence, 10 % *d*-selinence, 2.5–3.0 % sedanolide, 0.5 % sedanomic anhydride, and it has a fixed oil content of 15 % (Farrell, 1999). The aroma of celery seed oil is warm, spicy, slightly fatty, fruity, penetrating and very persisting. On tasting, it provokes a burning sensation and is very bitter. The physiochemical properties of celery volatile oil are given in Table 12.10.

12.5.4 Celery oleoresin specification

Celery seed oleoresin should be a green liquid with a volatile content of at least 9 ml/100 gm. It should have a lemon-like aroma and a tenacious and sweet herbal tone. The oleoresin should be prepared using the recommended organic solvents, which should then subsequently be removed as per the specifications of the importing country.

12.5.5 Adulteration

Celery seed is available in both whole and ground form. It is subject to adulteration by addition of exhausted or spent seed (from which oil or oleoresin has been extracted), excess stems, chaff and earth or dust, etc. Samples of celery seed are also sometimes adulterated with ajowan seeds and, because of the similarity in seed shape, this is difficult to detect. Ground celery is sometimes adulterated with

Table 12.10 Physiological properties of volatile oil of celery

Properties	Specification values		
	Singhal *et al*. (1997)	Bahl *et al*. (1977)	ISI specification
Colour and appearance	Pale yellow	Pale yellow	Pale yellow to light brown liquid, sometimes pale green
Specific gravity	0.872–0.891 (15 °C)	0.850–0.895 (20 °C)	0.8710–0.9100 (27 °C)
Refractive index (at 20 °C)	1.480–1.484	1.478–1.486	1.4765–1.4865
Optical rotation (at 20 °C)	+655°3 to 76°5	+65°82	+50°80
Solubility characteristic	Saponification number 25.1–47.6	–	–
Acid value	–	15–40	3.5 (max.)
Odour	–	Spicy	Persistent, spicy and typical of celery seed

farinaceous products, linseed meal, worthless vegetable seeds or at times even with weed seeds. Celery seed oil is also frequently adulterated with celery chaff oil or with *d*-limonene, the addition of which is difficult to detect. The celery seed oil contains β-selinene as one of the important component, and a good-quality oil should contain minimum 7–7.5 % β-selinene (Straus and Wolstromer, 1979). Adulteration should be suspected if the oil contains less than 7.0 % β-selinene. The oleoresin may be adulterated by added synthetic saturated acid. Adulterants can be detected by a sophisticated form of gas chromatography of the saponified extract or by thin-layer chromatography coupled with high-performance liquid chromatography (HPLC). The adulteration level can be calculated by reference to the specifications for whole seed, powdered seed, volatile oil and oleoresins outlined earlier in this section. Filth, such as insect fragments, rodent droppings and fungal spores, is an indication of poor handling and storage. Heavy metals and chemical residues from pesticides represent another adulteration problem but are generally found in very low levels in celery and its extractives.

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13

Chervil

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Abstract: Chervil (*Anthriscus cerefolium* L.) is a warmth-giving herb of the family Apiaceae (Umbelliferae), popular especially in France for culinary purposes. In the past, it was called *myrhis* on account of its volatile oil which has an aroma similar to the resinous substance of myrrh. The chapter gives a description of the plant and its chemical composition. Production and cultivation – soil, climate, propagation, cultivars, fertilizing, weeding, irrigation, intercultural operations, intercropping and pest control – are described together with harvesting and post-harvest care. The chapter details the medicinal and culinary uses of chervil.

Key words: chervil, *Anthriscus cerefolium*, volatile oil, cultivation, propagation, herbalist, seasoning.

13.1 Introduction

Chervil (*Anthriscus cerefolium* L. Hosffm.), also called as Garden Chervil or French Parsley, is a warmth-giving herb belonging to the family Apiaceae (Umbelliferae). It is a popular herb used in France for decoration of cold and warm dishes. This herb is native to the Middle East, southern Russia, and the Caucasus, and was probably introduced to Europe by the Romans. It is found in Europe and Asia.

Chervil was once called *myrhis* for its volatile oil, which has an aroma similar to the resinous substance of myrrh. One of the traditional fine aromas with a hint of myrrh, chervil is noticed even when in the background, because of its warm and cheering flavour and fragrance. The herb is called by many different names in different countries: *Maqdunis afranji* in Arabic, *San lo po* in Chinese, *Kervel* in Dutch, garden chervil or French parsley in English, Cerefolio in Esperanto, *Maustekirveli* in Finnish, *Cerfenil* in French, *Aed-harakputk* or *Harakputk* in Estonian, *Kerbel, Gartenkerbel*, *Franzosiche* or *Petersilic* in German, *Tamcha* in Hebrew, *Turboloyo* or *zamatos turbolya* in Hungarian, *Kerfill* in Icelandic, *Cerfoglio* in Italian, *Kjorvel* or *Hagekjorvel* in Norwegian, *Trybula Ogrodowda* in Polish, *Certolho* in Portuguese, *Kervel* in Russian, *Perifollo* or *Certafolia* in Spanish and *Korvel, Dansk Korvel* or *Tradgardskorvel* in Swedish.

Chervil is a hardy annual which grows to a height of 25–70 cm and width of 30 cm. The lacy, light green leaves are opposite, compound and bi-pinnate; they are subdivided again into opposite and deeply cut leaflets. The lower leaves are pointed and the upper leaves are sessile with stem sheaths. The stems are finely grooved, round, much branched, light green and hairy. The white flowers are arranged in tiny umbels and grow into compound umbels. The whole plant smells of anise and tastes a little of pepper and of anise; it blooms during May–August. Chervil has a white, thin and single tapering root. The oblong fruit is 0.5–0.75 cm long, segmented and beaked. The seeds are long, pointed with a conspicuous furrow from end to end.

Chervil is available in two distinct types, salad chervil (*Myrrhis odorata*) and turnip-rooted chervil (*Chaerophyllum bulbosum*). Salad chervil is grown in a similar way to parsley. Both have a fern-like leaf structure as delicate and dainty as the flower. The stems are branched and finely grooved and the root is thin and white. Another type of chervil, known as wild chervil/woodland chervil (*Anthriscus sylvestris)*, is also used in some parts of Europe.

13.1.1 Chemical composition

Chervil contains essential oil which occurs in a duct accompanying each of the veins in the leaf and rachis to the extent of 0.03 % comprising methyl chavicol, fatty oil, bitter substances, vitamins B and C, mineral salts, etc.

13.2 Production and cultivation of chervil

Chervil probably originated in Southern Europe or the Caucasus region. It is found in Europe and Asia. It has been cultivated in England since 1597 and in America since 1806. However, it can also be found growing in other places where the right conditions prevail.

13.2.1 Soil

Chervil grows well in any good garden soil with high fertility. However, moist, humus-rich soils with good drainage are most suitable. It can be successfully grown in soils with a pH of 6.5, especially turnip-rooted chervil, which has a wider adaptability and grows in all parts of the chervil-growing world where the soil is fertile and has sufficient moisture.

13.2.2 Climate

Chervil is a hardy plant and may thrive in much cooler climates provided it finds a warm location but, as a cold weather crop, chervil is susceptible to frost and should be planted in a sheltered area. In temperate climates, it can also be grown as summer season crop. Under such conditions, it prefers partial shade. It is helped by having the leaves cut off, so they can shoot up again. The plants are not robust and soon wither and die. In other parts of the world, it is mainly grown as a cold season crop.

13.2.3 Propagation

Chervil can be propagated only through seeds. For this purpose, the seeds must be bedded in damp sand for a few weeks before being sown, otherwise their germination is slow. In temperate regions the seeds are usually sown in March–April, whereas in tropical or subtropical parts they are sown during October by drill or scattered in well-prepared land and mixed with well-decomposed farmyard manure. The recommended seed rate is roughly 3 kg/ha which is sown in rows. Seeds should be grown in the spring in shallow drills 30 cm apart. When the seedlings are about 7–8 cm high, the plants should be thinned to 8–10 cm apart. The seedlings are too fragile to be transplanted. In the south, the seeds are usually sown in the autumn, but they may not germinate until spring. In the north, the seeds may be sown in the

Fig. 13.1 (a) Dried chervil leaves; (b) chervil flower cluster and unripe fruits; (c) chervil plants in flower; (d) chervil leaf.

autumn to germinate in the spring; or the plant may be started indoors in later winter and transplanted to open ground later on.

Chervil seed has a maximum life expectancy of one year. Seed vernalization induces rapid bolting and flowering under long days; without vernalization, bolting is very slow under all conditions. Vernalization also decreases yield, but higher yields were obtained when vernalized seeds were germinated at 20 °C. Later adjustments in sowing dates in field resulted in higher yields.

13.2.4 Cultivars

The variety 'Brussels Winter' has recently gained in popularity, as it is slow in bolting compared to other cultivars. This variety is a flat-leaf type. Most people prefer flat-leaf chervils over the curly-leafed types, but there is no flavour difference between the two types.

13.2.5 Manure and fertilizers

Chervil prefers to be grown organically with the application of well-decomposed farmyard manure or leaf mould at about at 8–10 t/ha. However, to obtain higher yields, its inorganic fertilizer requirement needs to be assessed.

13.2.6 Weeding and irrigation

The 'wild chervil' (*A. sylvestris*) is a highly invasive weed and almost impossible to eradicate. Thus, hand weeding in the initial stages is recommended. Since chervil is a herbaceous crop, it requires frequent irrigation. It grows poorly in hot, dry conditions. Regular watering is therefore essential. Chervil should be protected from summer sun, wherever it is grown as a summer season crop.

13.2.7 Intercultural operations

Soil should be earthed up to loosen it and to enhance aeration for better growth. Once the plants are established, they will self-seed. The flowers should be picked as soon as they appear as this encourages the stalks to shoot rapidly. It is better to follow the practice of cutting flower stems before they bloom in order to get denser foliage. Chervil bolts quickly, especially in the warmer months, so pinching is a very important operation in such months.

13.2.8 Intercropping

Chervil and radishes planted together produce hotter radishes, since chervil prefers light shade. Chervil can be intercropped with *Rauvolfia serpentina* or *Mentha arvensis* or *Salvia scalrea.*

13.2.9 Pests and diseases

Among pests, aphids occasionally cause damage and are generally controlled by spraying malathion (0.5 %) two or three times during the infestation. There are a

few botanical insecticides such as rotenone that are sold as 1 or 2 % dust and which controls aphids, thrips and some soft-bodied sucking insects. Rotenone is available at 40 % liquid concentrate, which is diluted in water and sprayed. Other botanicals such as pyrethrum, ryania sulphur, etc. are also recommended. *Trichoderma* spp. are used to control diseases such as root rot.

Pyrethrum is a botanical obtained from the dried flower of *Chrysanthemum cinerarifolium* and is used as an insect control agent. It provides a rapid knockdown of a wide range of insects. Pyrethrum is very expensive and has a very short residual effect. Therefore, it is usually used in combination with other insecticides such as rotenone and with an activator or synergist such as piperonyl cyclonene or piperonyl butoxide.

Among the diseases, powdery mildew can be noticed at the flowering and early seedling stages. It can be controlled by spraying wettable sulphur (0.2 %) two or three times at weekly intervals. *Fusarium* sps cause root rot disease which can be controlled by following phytosanitary measures, seed treatment with Agroson (@3 g/kg of seed) and by foliar spray of Bavistin (0.1 %). Chervil may be infected with the virus for anthriscus yellows and has also been reported to exhibit mottling, leaf necrosis, dwarfing and malformation due to viral infections.

13.2.10 Harvesting

In general, chervil matures in 6 weeks if conditions are ideal. Harvesting should be properly timed, and this depends mainly on the purpose of the crop, whether for salad or vegetable or for obtaining the seeds. If the chervil is being harvested for salad or vegetable, the flowers should be removed well before harvesting to obtain maximum shoots. The leaves can generally be cut 6–8 weeks after sowing. After the required leaves have been harvested, the plant should be cut down to the ground to allow more growth to occur.

After picking, the leaves and stems can be dried on wire racks in a cool, ventilated, shady place. Once the leaves are dried, they become brittle (either whole or crumbled) and can be stored in an airtight container. Fresh chervil may be chopped and frozen with water in ice cube trays.

If the plants are to be harvested for seed purposes, they should be allowed to mature until there are seeds in the field. Then the harvested material is dried in the field until the fruits are easily threshed. The threshed fruits should be spread in a thin layer and frequently turned over until they are thoroughly dry.

A herbage yield of about 30–35 q/ha can be obtained in case of leaf crops and 500–700 kg of seeds per hectare can be obtained.

13.3 Main uses of chervil

Chervil has been used by herbalists for several medicinal purposes throughout history. Chervil has been used in the past as a diuretic, expectorant, digestive aid and skin freshener. It was also thought to relieve symptoms of eczema, gout, kidney stones, and pleurisy. Today, it is most widely known as a remedy for high blood pressure.

The tender young leaves of chervil have been used in spring tonics for thousands of years, dating back to the ancient Greeks. A combination of chervil, dandelion and watercress rejuvenates the body from vitamin and mineral deficiencies brought on by winter and lack of fresh greens. Even today European herbalists recommend this tonic.

Both leaves and root are used in cookery. The sprigs of chervil make an excellent garnish. In French cookbooks chervil is called 'pluches de cerfeuille' or blanched sprig of chervil. These are used in soups. The French also use chervil in their traditional 'fines herbes' along with tarragon, parsley and chives.

Chervil is a traditional remedy for bad dreams, burns and stomach upsets. It is an excellent source of antioxidants that stabilize cell membranes and reduce inflammation associated with headache, sinusitis, peptic ulcer and infections. Chervil is used as an eyewash to refresh the eyes. Chervil was also made into a tea and ingested to reduce blood pressure.

Chervil is nutritious, being a good source of vitamin C, carotene, iron and magnesium. Chervil is also a rich source of bioflavonoids, which aid the body in many ways, including vitamin C absorption. It is an aid to sluggish digestion. When brewed as a tea, it can be used as a soothing eyewash. The whole plant reportedly relieves hiccoughs, a practice still tried by some people.

Chervil's flavour is lost very easily, either by drying the herb, or from too much heat, so it should be added at the end of cooking or sprinkled on food in its fresh, raw state. One way to keep chervil's flavour is to preserve it in white wine vinegar. Because its flavour is so potent, little else is needed as flavouring when it is added to foods. This makes it a low-calorie way to add interest to meals. Chervil's delicate leaves make it an attractive herb to use for garnishes. Despite its fragile appearance, it keeps well. Chervil will last up to a week in the refrigerator. Chervil has been overlooked in American cooking until recently, because most people have tasted only dried chervil, which is basically tasteless and musty and, at best, tastes sweet and grassy with a touch of liquorice. It is also linked to the Easter celebration in parts of Europe, where it is eaten as part of the ceremony for Holy Thursday. Chervil is associated with Easter because its aroma is similar to that of myrrh (one of the gifts to the baby Jesus from the three wise men) and because of its early spring sprouting symbolizes renewal.

Chervil is one of the staples of classic French cooking. Along with chives, tarragon and parsley, it is used as an aromatic seasoning blend called "Fines Herbs." It is most frequently used to flavour eggs, fish, chicken and light sauces and dressings. It also combines well with mild cheeses and is a tasty addition to herb butters. This blend is the basis for ravigote sauce, a warm herbed veluté served over fish or poultry. Being a spring herb, it has a natural affinity for other spring time foods: salmon, trout, young asparagus, potatoes, baby green beans and carrots, salads of spring greens.

Chervil's flavour is lost very easily, either by drying the herb, or too much heat. That is why it should be added at the end of cooking or sprinkled on in its fresh, raw state. One way to keep chervil's flavour is to preserve it in white wine vinegar. Because its flavour is so potent, little else is needed as flavouring when added to foods. This makes it a low calorie way to add interest to meals. Chervil's delicate leaves make it an attractive herb to use for garnishes. Despite its fragile

appearance, it keeps well. Kept in a zip lock bag, chervil will last up to a week in the refrigerator.

Chervil is an effective seasoning to foods. Both the leaves and the stems can be used for cooking and whole sprigs make a delicate and decorative garnish. Blanched sprigs of chervil are occasionally used in soups.

13.4 Sources of further information

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14

Fennel and fennel seed

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Abstract: This chapter opens with the description and classification of fennel (*Foeniculum vulgare* L.), a plant largely grown as a herb or for its fruits and valued for its pleasant aroma, abundant nutritional and medicinal properties. The chapter details the aroma chemicals present in essential oil from herb and seeds. The principal constituents from volatile oil from fruits are 50–60 % anethole and 15–20 % fenchone. Several processed products from fennel fresh herb and fruits for which there is demand in the international market are given. The nutritional and functional properties to which the medicinal uses of fennel are attributed–antimicrobial, antiflatulent, stimulant, carminative and expectorant and a few others – are depicted. Toxicity, allergenicity and adulteration are also mentioned. The quality specifications for different fennel products such as whole seed, powdered seed, volatile oil and oleoresins production are given.

Key words: fennel, cultivation, chemical composition, main products, nutritional value, functional properties, medicinal uses, toxicity, adulteration, quality specifications.

14.1 Introduction and description

Fennel is traditionally used for medicinal and culinary purposes. The entire plant is valuable in the medicinal industry; its enlarged base is used as a vegetable; its leaves are used for culinary purposes and its seeds as a spice and for essential oil extraction. The flowers and leaves are also used to make yellow and brown dyes. Fennel pollen is the most potent form of fennel, but it is extremely expensive.

In early Sanskrit writings, fennel was known as *madhurika* and its cultivation in India is thought to date back at least to 2000 BC. To the ancient Greeks, fennel represented success and was called 'marathon', after which the battle of Marathon (490 BC) was named when it was fought in a field of fennel (Chadwick, 1976). Fennel was also a symbol of success to the Romans and fennel leaves were used to crown victors in games. The English name fennel comes from Old English fenol, or finol, and fennel is one of the nine plants invoked in the pagan Anglo-Saxon Nine Herbs Charm recorded in a tenth century manuscript. During the thirteenth century in England, fennel was considered a royal spice and was served to kings with fruit, bread and in dishes such as pickled fish seasoned with fennel seeds.

A native of southern Europe and the Mediterranean region (Clevely *et al*., 1997), fennel has become naturalized along roadsides, in pastures and in other open sites

in many regions, including northern Europe, Cyprus, the USA, southern Canada and in much of Asia, the Far East and Australia. Introduced to North America by Spanish missionaries for cultivation in their medicinal gardens, it is now known as wild anise in California (and often mislabelled as anise in American supermarkets), where it can be found growing in San Francisco and on the Pacific coast. English settlers also took the herb with them to the New England colonies, where it became part of their kitchen gardens. It is now considered a weed in the USA, as well as in Australia (Bown, 2001).

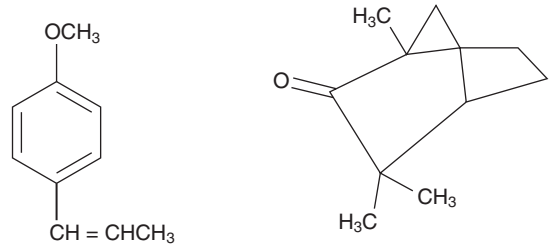
14.1.1 Classification

The genus *Foeniculum* (fennel) belongs to the family Apiaceae and the order Apiales. Three main varieties have been described: *F. vulgare* Mill. var. *piperitum* (Ucria) Cout. (bitter fennel), *F. vulgare* Mill. var. *dulce* DC Batt. et Trab. (sweet fennel) and *F. vulgare* Mill. var. *azoricum* Thell. (Florence fennel, or finocchio) (Seidemann, 2005). Bitter fennel is grown for its fruits and essential oil, whilst Florence fennel is cultivated for its fruits, essential oil, leaves (used for culinary purposes) and enlarged leaf base (eaten as a vegetable). Sweet fennel is cultivated for its enlarged leaf base, for its fruits and for the essential oil taken from its fruits. Weiss (2002) describes fennel varieties as biennial or perennial aromatic herbs, whilst other authors detail annual, biennial and perennial types. *Foeniculum* is a cross-pollinated crop and has the somatic chromosome number 2n = 22.

14.2 Chemical composition

The chemical composition of fennel varies with morphotype, source, climate and harvesting stage. Every 100 g edible portion of fennel seeds contain on average: 8.8 g water; 15.8 g protein; 14.9 g fat; 36.6 g carbohydrate; 15.7 g fibre; and 8.2 g ash (containing 1.2 g Ca, 19 mg Fe, 1.7 g K, 385 mg Mg, 88 mg Na, 487 mg P and 28 mg Zn). Every 100 g contains: vitamin A (135 IU); niacin (6 mg); thiamine (0.41 mg); and riboflavin (0.35 mg); with an energy value of about 1440 kJ. The seeds contain mucilage, sugars, starch, tannin, essential oil and fixed oil (the main components of the fixed oil being petroselenic, oleic, linoleic and palmitic acids (Bernath *et al*., 1994)). The variety and quantity of vitamins contained is variable: folates, 270 mg/kg; vitamin B3, 6.4 mg/kg; vitamin C, 8.7–340 mg/kg. Fennel contains potassium (4.24–5.85 g/kg), the most abundant mineral by far, with low amounts of phosphorus (500 mg/kg), calcium (5.6–363 mg/kg), magnesium (8.2–389 mg/k) and sodium (7.7–512 mg/kg) (Koudela and Petrikova, 2008).

The principal constituents of the essential oil extracted are anethole (50–60 %) and fenchone (15–20 %) (Fig. 14.1). The essential oil extracted is mainly composed of (E)-anethole, (Z)-anethole and α-thujone (Mata *et al*., 2007). Singh *et al*. (1990) reported 20 compounds in fennel essential oil of which 18 constituted 96.04 % of the total essential oil, the major components being anethole (68 %), limonene (11 %), fenchone (3.7 %) and a few others. Approximately 45 constituents have been determined from fennel seed oil, the main constituents being *trans*-anethole (60–65 %, but up to 90 %), fenchone (2–20 %), estragole (methyl chavicol), limonene,



t-Anethole Fenchone

Fig. 14.1 Chemical structure of anethol and fenchone (Shamina, 2008).

Table 14.1 Composition of sweet and bitter fennel oil

Component	Fennel oil (%)	
	Sweet fennel	Bitter fennel
α-Phellandrene	–	12.98
α-Pinene	4.03	18.10
Anethole	52.03	47.97
Estragole	2.53	8.31
Fenchol	3.18	–
Fenchone	2.67	2.84
Limonene	28.92	–

Source: Karlsen *et al*. (1969).

camphene, α-pinene and other monoterpenes, fenchyl alcohol and anisaldehyde. Small quantities of α-pinene, camphene, δ-α-phellandrene, dipentene, methyl chavicol and ρ-hydroxy phenyl acetone are also present. The main components of the fixed oil are petroselenic, oleic, linoleic and palmitic acids (Farrell, 1999). Dried fennel seeds contain 0.6–6 % volatile oil.

The composition of sweet and bitter fennel oil is given in Table 14.1. Analysis of essential oils obtained from the seeds and leaves of *F. vulgare* has revealed that anethole is the major constituent (58.5 % in seed oil and 51.1 % in leaf oil) followed by limonene (19.6 % in seed oil and 22 % in leaf oil), in addition to the list of other components presented in Table 14.2 (Chowdhury *et al*., 2009). When the fruits (seeds) are mature, up to 95 % of the essential oil is located in the seeds themselves. Hydrodistillation yields 1.5–35.0 %, with the largest quantity of herbal essential oil being obtained by hydrodistilling fresh or slightly wilted foliage just before flowering (Bellomaria *et al*., 1999).

Recently, El-Awadi and Hassan (2010) reported that fennel seeds contain 0.79 % essential oil, 5.82 % fixed oil and total phenolic compounds 1.17 mg/g dry weight. According to their analysis, the major constituents of essential oil are α-pinene (0.37 %), δ-limonene (0.07 %), 1,8-cineole (5.09 %), fenchone (4.13 %), anethone (86.11 %) and estragole (methyl chavicol) (0.05 %). Brender *et al*. (1997), however, reported that the major constituents were *trans*-anethole (50–70 %), fenchone (12–33 %), methyl chavicol (estragole) (2–5 %), α-pinene, camphene, ρ-cymene,

Table 14.2 Essential oil composition of *Foeniculum vulgare* Mill. cultivated in Bangladesh

Seed oil		Leaf oil	
Compounds	Percent	Compounds	Percent
γ-Terpinene	1.10	γ-Terpinene	0.06
3-Methoxycinamaldehyde	0.27	2-Methoxybenzeneethanol	0.10
4-Terpinolene	0.28	3-Methoxycinamaldehyde	0.14
Anethole	58.54	4-Hexen-1-ol, acetate	0.22
Anisaldehyde	0.72	Allyl-3-methoxybenzoate	0.06
Apiol	0.27	Anethole	51.08
Camphene	0.08	Anisaldehyde	7.55
Camphor	0.63	Apiol	0.63
Caryophyllene	0.10	Camphene	0.07
cis-Sabinenehydrate	0.09	Camphor	0.04
Ethenyl)-2-cyclohexeneone	1.19	*cis*-Verbenol	0.18
Eugenol	0.08	Fenchone	1.65
Fenchyl acetate	1.20	Fenchyl acetate	5.34
Germacrene	0.47	Limonene	22.90
Isopinocampheol	0.11	Limonene-1,2-epoxide	0.11
l-Fenchone	7.72	Methyleugenol	0.07
Limonene	19.63	Methylisoeugenol	0.12
Ocimene	0.09	Myristicin	0.08
Sabinene	0.69	N-amyl isovalerate	0.01
Terpinolene	0.12	Octahydro-1-benzothipene	0.07
trans-Limonene oxide	0.83	*p*-Anisic anhydride	0.20
trans-p-Mentha-2,8-dienol	0.29	Plinol D	0.11
(S)-2-methyl-5-(1-methyl		*trans*-Carvyl acetate	0.25
trans-Verbenol	0.15	*trans*-Carvyl propionate	0.41
α-Phallandrene	0.30	*trans-p*-2,8-Menthadien-1-ol	0.15
α-Pinene oxide	0.18	α-Curcumene	0.05
β-Bisabolene	0.08	β-Bisabolene	0.03
β-Camphor	0.16	β-Myrcene	0.63
β-Pinene	1.80	β-Ocemene	0.27
β-Pinene	0.22	β-Phallandrene	0.04
β-Thujaplicine	0.04	β-Pinene	0.14
		β-Thuzaplicin	4.82

Source: Chowdhury *et al*. (2009).

myrcene, liomonene, α-and β-phellandrene, γ-terpinene, terpineol, *cis*-ocimene and γ-fenchone. The dried distillation residue of fennel seeds contains 14–22 % protein and 12–18 % fat and is suitable for use as stock feed (Weiss, 2002).

Moura *et al*. (2005) determined global yields of volatile oil for fennel fruits analysed using CO_2 super critical fluid extraction and found that yield varied from 3–12 %; the major compounds identified in the extracts were *trans*-anethole and fenchone. Fruits contained 15–30 % fixed oil and up to 12 % volatile essential oil. The fruit also contained flavonoids, iodine, kaempferols, umbelliferone and stigmasterol and ascorbic acid; traces of aluminium, barium, lithium, copper, manganese, silicon and titanium were also found.

Fatty acids (palmitic, palmitoleic, stearic, oleic, linoleic and linolenic acid) were also detected. Parejo *et al*. (2004a,b) identified caffeoylquinic and dicaffeoylquinic

acids, flavonoids and rosmarinic acid among the ten main antioxidant phenolic compounds obtained from bitter fennel, using a simple high-performance liquid chromatography (HPLC) technique. Distilled fennel was found to contain a higher proportion of antioxidant phenolic compounds than non-distilled plant material.

Gámiz-Gracia and De Castro (2000) devised a sub-critical extractor equipped with a three-way inlet valve and an on/off outlet valve to perform sub-critical water extractions in a continuous manner for the isolation of fennel essential oil. This extraction method is superior to both hydrodistillation and dichloromethane manual extraction in terms of speed, efficiency, cleanliness and the possibility of manipulating the composition of the extract. The major compounds in supercritical CO_2 and hydrodistilled extracts of ground fennel seeds are *trans*-anethole (68.6–75.0 and 62.0 %, respectively), methylchavicol (5.09–9.10 and 4.90 %, respectively) and fenchone (8.4–14.7 and 20.3 %, respectively) (Damjanović *et al*., 2005).

Muckensturm *et al*. (1997) characterized different populations of *F. vulgare* containing 10-nonacosanone as a specific chemical marker. *F. vulgare* subsp. *piperitum* (bitter fennel) is characterized by the presence of rotundifolone. *p*-Butylanisole is present in traces in fennel containing large amounts of *trans*-anethole. A chemotaxonomic classification based on the amount of estragole, *trans*-anethole, limonene and fenchone was also proposed for the different varieties and chemotypes of *F. vulgare* subsp. *vulgare*. Miraldi (1999) reported inverse proportions of *trans*-anethole and estragole, suggesting a common precursor. A chemotypic characterization of populations of fennel based on the occurrence of glycosides was attempted by Harborne and Saleh (1971) and confirmed the presence of quercetin 3-arabinoside in the leaves of fennel and three other flavonol glycosides: kaempferol 3-arabinoside, kaempferol 3-glucuronide and quercetin 3-glucuronide.

Bitter fennel contains 50 % *trans*-anethole, 10–20 % fenchone (which contributes to the bitter flavour), 10–30 % limonene, 3–11 % α-phellandrene, 12–16 % α-pinene, with α-thujene, β-pinene, estragole (methyl chavicol), myrcene, and 1,8-cineole. The sweeter variety has 50–80 % anethole, little (5 %) or no fenchone, slightly higher levels of limonene with estragole, safrole and pinene (Raghavan, 2006). Turkish bitter fennel is rich in methyl chavicol (47.09 %), as well as limonene (29.07 %), fenchone (13.43 %), α-terpinene (2.5 %), fenchyl acetate (exo) (1.95 %) and *cis*-β-ocimene (Özcan and Akgül, 2001).

The fixed oil primarily contains petroselinic acid (60 %), oleic acid (22 %), linoleic acid (14 %) and palmitic acid (4 %) (Singh *et al*., 1990). Harborne *et al*. (1969) were the first to report that the psychotropic aromatic ether myristicin occurs in the seed of cultivated fennel but is absent from wild collections of this species.

Essential oil taken from different plant parts and between different regional cultivars tends to be very variable (Akgül, 1986; Karaca and Kevseroglu, 1999; Kruger and Hammer, 1999; Piccaglia and Marotti, 2001). In European and Argentinian types of *F. vulgare*, limonene concentration in the whole plant does not exceed 10 %, but α-phellandrene is 23–25 % in leaves and 22–28 % in stems. By contrast, the limonene content in young leaves and stems of European and Indian types of *F. dulce* ranges from 37–40 % and 28–34 %, respectively, decreasing with age. The α-phellandrene content is low (1–4 %) and remains constant with age. Fruits contain condensed glucides, phytosterols (β-sitosterols, stigmasterol), coumarin, stragol (5 %) and traces of α-pinene, limonene, mircene, fenchone, canfene, sabinene,

β-mircene, β-pinene, α-feladrene and α-terpinene, whilst its leaves contain flavonoids and traces of essential oils. Notable differences have been recorded in the components of the 'vulgare' and 'dulce' strains (Kresanek 1989;Simandi *et al*., 1999).

The yield and composition of the volatile fraction of the pentane extracts of leaves, stems and seeds of *F. vulgare* Mill. were studied by Guillén and Manzanos (1996). The yield obtained from seeds was much higher than that obtained from leaves and stems. The volatile fraction of the pentane extract of the latter two has a higher concentration of terpene hydrocarbons and a smaller concentration of oxygenated terpene hydrocarbons than that of the seeds. Sesquiterpenes and the antioxidant vitamin E have been detected in the leaves and petroselinic acid in the seeds. Saturated aliphatic hydrocarbons with 25 or more carbon atoms have been found in all the plant parts.

Akgül and Bayrak (1988) reported the volatile oil composition of various parts of bitter fennel (*F. vulgare* var. *vulgare*) growing as wild Turkish plants, investigated by gas – liquid chromatography. The major component of all oil samples was *trans*-anethole (29.70, 37.07, 54.22, 61.08 and 64.71 % in leaf, stem, flowering umbel, flower and fruit, respectively). The other main components were α-pinene (in leaf, stem, flowering umbel and flower), α-phellandrene (in leaf, stem and flowering umbel) and fenchone (fruit oil). The volatile oils of flowering umbels, flower and fruit contained high amounts of oxygenated compounds, in gradually increasing percentages. The root essential oil contains (on average) α-pinene (1.0 %), *p*-cymene (0.3 %), β-fenchylacetate (1.0 %), *trans*-anethole (1.6 %), eugenol (0.2 %), myristicin (3 %) and dillapiole (87 %). By comparison, the root and bulbous stem base of Florence fennel contains less than 1 % of dillapiole but 70 % of *trans*-anethole, giving a very different taste. The herb contains 1.00–2.55 % essential oil, up to 75 % of which is *trans*-anethole.

Barros *et al*. (2009) observed different levels of antioxidant potential for shoots, leaves, stems and inflorescense, particularly composition of ascorbic acid, tocopherols and phenolics. Shoots were also found to have high radical-scavenging activity and lipid peroxidation inhibition capacity.

The synthesis of the major essential oil components, estragole and anethole, has been elucidated. Cell-free extracts from bitter fennel tissues display *O*-methyltransferase activities able to methylate chavicol and *t*-anol *in vitro* to produce estragole and *t*-anethole, respectively, using *S*-adenosyl-L-methionine as a methyl group donor (Gross *et al*., 2002). An association between estragole accumulation and chavicol *O*-methyltransferase activity during the development of different plant parts was found. Young leaves had greater *O*-methyltransferase activity than old leaves. In developing fruits, *O*-methyltransferase activity levels increased until the wasting stage and then decreased drastically. The metabolism of *l-endo*-fenchol to *d*-fenchone in fennel was studied by Croteau and Felton (1980), whilst Croteau *et al*. (1980a) reported a soluble enzyme preparation from the leaves of fennel which catalysed the cation-dependent cyclization of both geranyl pyrophosphate and neryl pyrophosphate to the bicyclic rearranged monoterpene *l-endo*-fenchol. Croteau *et al*. (1980b) found that (+)-(1*S*)-fenchone, an irregular bicyclicmonoterpene ketone thought to be derived via rearrangement of a bicyclic precursor, was one of the major terpenoids of the volatile oil of fennel. They could provide strong evidence that fenchone was derived by the cyclization of geranyl pyrophosphate or neryl

pyrophosphate to *endo*-fenchol, followed by dehydrogenation of this bicyclic alcohol, and demonstrate the biosynthesis of a rearranged monoterpene in a cell-free system. Croteau *et al*. (1989) elaborated on the biosynthesis of the monoterpene (geranyl pyrophosphate) in fennel: (–)-*endo*-fenchol cyclase catalyses the conversion of geranyl pyrophosphate to (–)-*endo*-fenchol by a process thought to involve the initial isomerization of the substrate to the tertiary allylic isomer, linalyl pyrophosphate, and the subsequent cyclization of this bound intermediate.

14.3 International trade, production and post-harvest processing

Fennel is cultivated on a large scale in Romania, Russia, Germany, France, Italy, India, Argentina and USA. It is also grown in Bulgaria, China, Denmark, Egypt, Syria, Morocco and Japan. In India, the major fennel-producing states are Gujarat, Rajasthan and Uttar Pradesh, whilst many other states grow it on a small scale, such as Punjab, Tamil Nadu, Bihar, Karnataka, Maharashtra, Jammu and Kashmir. In 2008–9, 114277 tonnes of fennel seed was produced in India from 74149 ha whilst during 2011, 7250 tonnes of fennel seed was exported (Spice Board, 2011). The worldwide production of anethole is 1000 tonnes per year, with China and Vietnam being the main producers. Fennel is the preferred source for anethole in Brazil, due to difficulties associated with anise cultivation there (Brender *et al*., 1997).

14.3.1 Cultivation and organic farming

Fennel is a cool season crop, with dry and cold weather favouring higher seed production. A temperature of 15–20 °C is the optimum for growth and high temperatures result in premature flowering and very low seed yield. The crop is susceptible to frost injury during the flowering stage. Since fennel is a long duration crop and has slow initial growth, it can be grown as a mixed or intercrop. Varieties selected should be adapted to the prevailing soil and climatic conditions and preferably have resistance/tolerance to pests and diseases. Fennel is propagated through seeds which can be sown directly in the field, or from seedlings raised in a nursery before being planted out. About 2.5 kg of seed is required to raise enough seedlings in a nursery for 1 ha but, as a main season crop, 8–10 kg seed is required for direct sowing over the same area. Plant nutritional requirements vary from region to region due to the type and fertility of the soil. Plant protection measures under an organic farming system should place emphasis on crop-management practices at the time of sowing, on balanced nutrition, crop rotation, green manuring, etc. to reduce the incidence of diseases and pests, as well as initial selection of resistant varieties and the adoption of biocontrol measures.

There is a demand for organic fennel and many spice companies offer certified organic fennel on the internet. Fennel seeds produced in India are mostly from arid and semi-arid regions, which are by default organic, since production is achieved with minimal or no chemical inputs. Such produce is termed and sold as ‘near organic’ in the market. The general and specific guidelines for organic production of seed spices including fennel have been detailed by Malhotra and Vashishtha (2008). Europe, the USA, Canada and Japan are the largest markets looking for

organic spices including fennel, with Australia and New Zealand representing new emerging markets. The future demand for organic spices appears to be bright.

14.3.2 Varieties

The two main types of fennel cultivated are sweet fennel (also known as French or Roman fennel) and bitter fennel. Bitter fennel grows wild as well as being cultivated mostly in Argentina, Czechoslovakia, France, Germany, Hungary, India, Italy, Japan, Romania and in southern Russia. Sweet fennel does not grow wild but is cultivated in Bulgaria, France, Italy and Macedonia (Shiva *et al*., 2002). Finnochio (or Florence) fennel is a sweet fennel grown for its bulbous stalk which is eaten as a vegetable (Raghavan, 2006). The young stems of the Italian carosella fennel (*F. vulgare* var. *piperitum*) are used for flavouring salads as well as a vegetable. Called variously Rubrum, Purpureum or Nigra, *F. vulgare* var. *purpureum*, a bronze leaved fennel, is grown widely in the UK as a decorative garden plant.

The Indian fennel seed is smaller and straighter than European fennel with a sweet anise flavour, whilst Persian fennel is the smallest of all with a strong anise taste. In India, about 14 varieties of bitter fennel are grown, the most popular of which are RF 101 and NRCSS AF 1 (Malhotra, 2011), the latter being suitable for cultivation under semi-arid conditions giving high seed yield and an essential oil content of up to 1.6–2.5 %, depending on the stage of harvest and season.

14.3.3 Harvesting and yield

Green fennel leaves can be harvested from time to time throughout the growing season but bulbs are only harvested in late autumn, at the same time that the stems are harvested as a vegetable. Clean, crisp bulbs with a fresh green colour are selected with no sign of browning. The time of harvesting depends upon the type of product being marketed. Usually, the crop is harvested before the fruits are fully ripe. For green fennel used for chewing purposes, umbels are harvested about 30–40 days after flowering whilst they are still green and have attained just half their final size (if left to grow). Since not all plants mature at the same time, harvesting of umbels has to be done 4–5 times, as and when they become ready. With scientific crop management, a yield of 2–2.5 tonne/ha can be achieved.

14.3.4 Post-harvest processing

Common fennel is bulbless, its stem and green leaves being used in the same way as Florence fennel. It is refrigerated, tightly wrapped in plastic bags for up to 5 days. Harvested umbles, by contrast, should be dried in the shade under well-aerated conditions, particularly for green fennel. Umbels should never be piled as this can deteriorate the quality (Singh and Malhotra, 2007). Dried umbels are separated and cleaned by winnowing to remove chaff, dust and dirt. The moisture content of the seeds should be kept to 9 %, whilst any higher seed moisture content can lead to chances of storage contamination by fungus. Dried, cleaned and graded produce is packed in standard sized packs/containers and labelled appropriately. The dried seed is packed in gunny bags lined with degradable, environment-friendly plastic

film. Waste-generating packaging material should be avoided. Each bag is sealed and stored in a clean, dry and ventilated place (Malhotra and Vashishtha, 2008).

Care should be taken to maintain the vital quality of any organic ingredient throughout each step of its processing. Processing methods should be selected in such a way that they limit the number and quantity of additives and processing aids required. Mature dried seeds are distilled to obtain essential oil. Generally, either a hydro-or steam distillation method is used for extraction. The percentage of essential oil varies depending upon variety and type of fennel: volatile oil content is lowest in Indian fennel (0.7–2.5 %) and highest in European (2–6 %). Essential oil (used as a common component in toothpaste, soaps and lotions (Muñoz, 1987)) should be kept in well-sealed bottles or aluminium containers.

Processed products include essential (volatile) oil, powder, fixed oil and oleoresins (also in demand on the international market). The oleoresins of fennel fruit are prepared by extraction of crushed dried seeds using suitable volatile oil solvents like food-grade hexane ethanol, ethyl acetate or ethylene dichloride, followed by filtration and desolventization under vacuum. Any organic solvents should be recovered completely from the oleoresin as per ISO maximum permissible limits. Fennel powder is produced by grinding dried seeds; pre-chilling and reduced temperature grinding can be used to overcome the loss of volatile oils.

14.4 Main uses of fennel in food

The bulb, foliage and seeds of the fennel plant are potential sources of different nutrients and thus all are widely used both raw and cooked in side dishes, salads, pastas, vegetable preparations, sausages, etc. Raw fennel bulb contains carbohydrates, dietary fibre, protein, vitamin B complex, vitamin C and minerals (Table 14.3). The fennel plant is aromatic and used as a pot herb. It is popularly used as a spice and as a vegetable, having many applications for flavouring and culinary purposes. The whole seed, powder and oil are used as adjuncts for flavouring foods, as antioxidants and as a preservative in confectioneries and beverages. Fennel seeds are largely used to give flavour to a number of foods such as soups, sauces, pickles, breads and cakes. In industry, fennel is used for flavouring and aromatizing, and as an organoleptic flavour corrector, in non-alcoholic beverages, baked goods, condiments, ice creams and liqueurs such as Anisette, and as a seasoning for prepared meats such as hot pepperoni and sweet Italian sausages (Farrell, 1999).

14.4.1 Fennel bulb and green herb

The bulb and green 'herb' fennel are used to flavour food during cooking, or as a garnish prior to serving, especially in the Middle East and India. The leaves of bulb fennel have a flavour similar to herb fennel, but cutting the leaves decreases the potential size of the bulb. The bulb is a crisp, hardy root vegetable and may be sautéed, stewed, braised, grilled or eaten raw. As a very good source of fibre, fennel bulb may help to reduce elevated cholesterol levels.

The common herb fennel, *F. vulgare* var. *dulce* and its colour variant Rubrum (bronze fennel) are consumed for their high antioxidant content. Common fennel

Table 14.3 Nutritional value per 100 g (3.5 oz) fennel bulb raw

Energy	130 kJ (31 kcal)
Carbohydrates	7.29 g
Dietary fibre	3.1 g
Fat	0.20 g
Protein	1.24 g
Thiamine (vitamin B1)	0.01 mg (1 %)
Riboflavin (vitamin B2)	0.032 mg (2 %)
Niacin (vitamin B3)	0.64 mg (4 %)
Pantothenic acid (B5)	0.232 mg (5 %)
Vitamin B6	0.047 mg (4 %)
Folate (vitamin B9)	27 µg (7 %)
Vitamin C	12 mg (20 %)
Calcium	49 mg (5 %)
Iron	0.73 mg (6 %)
Magnesium	17 mg (5 %)
Phosphorus	50 mg (7 %)
Potassium	414 mg (9 %)
Zinc	0.20 mg (2 %)
Manganese	0.191 mg

Note: Percentages are relative to US recommendations for adults.
Source: USDA (2010).

can also be blanched and/or the leaves marinated, or cooked in risotto, whilst its seeds and leaves (delicately flavoured and similar in shape to those of dill) can be used in salads. The thickened leaf stalks of Florence fennel are blanched and used as a vegetable (Farrell, 1999; Chevallier, 2001). Florence fennel is a key ingredient in some Italian and German salads, often tossed with chicory and avocado, or it can be braised and served as a warm side dish.

Green bulbs and the herb itself are also used for the preparation of herbal teas or juice blends with other herbs, and are a good source of calcium, iron, vitamins B and C, folic acid and carotenes. In all cases, the leaves lend their characteristically mild, anise-like flavour.

14.4.2 Whole seeds

Dried fennel seed is an aromatic, anise-flavoured spice. The seeds are brown or green in colour when fresh and turn slowly to a dull grey as the seed ages. Green seeds are best for cooking. Fennel seeds are sometimes confused with aniseed, which is very similar in taste and appearance, though smaller. Fennel seeds are well known for their distinctive pleasant flavour and are thus used for chewing alone after meals or in betel leaves; sugar-coated pelleted fennel seeds are also used as a breath-freshener. In different parts of India and Pakistan, roasted fennel is consumed as an organoleptic flavour correcter, or as an after meal digestive (hence why some Indian restaurants serve a fennel seed mix after meals). People in farming communities often chew fresh sprigs of green fennel seeds. It is an essential ingredient in the Bengali spice mixture *panch phoron* and in the Chinese five-spice powder. In the

west, fennel seed is a very common ingredient in Italian sausages and northern European rye breads. Many egg, fish and other dishes employ fresh or dried fennel leaves.

The whole seeds are used both as a spice and condiment in many countries (including in China, India and Egypt). Fennel seeds are used in India as a traditional spice in many foods including curries. A small quantity of whole fennel seeds can completely dominate the flavour of a dish, and they are used mostly to flavour soups, meat dishes and sauces, bread rolls, pastries and confectionery. Farrell (1999) reported the use of fennel seed in English-style soups, German breads, Polish borscht, and in spaghetti, salads, sweet pickles and vegetable dishes.

The seeds also have use for flavouring liquors and in the preparations of various types of pickles. Fennel seed vinegar is very popular for use in salad dressings and to sharpen herb sauces (and is easily prepared by placing 2 tbsp fennel seeds in a jar for every 600 ml of white wine vinegar used; cover the jar and leave it in a cool dark place for 2–3 weeks, shaking it occasionally, after which the vinegar should be strained into clean bottles, labelled and stored in a cool place away from direct sunlight).

14.4.3 Fennel essential oil

Essential oil extracted from fennel fruits is a rich source of bioactive compounds, thus used as a flavouring agent in various food items, in pickles and liquorice candy. It has been identified as a natural food flavourer with potential for use either individually or in admixture in beverages, bakery and other food preparations for its antimicrobial and antioxidant properties.

14.4.4 Fennel oleoresin

Fennel oleoresin prepared from seeds gives a warm, aromatic and pleasing flavour to food products. The oleoresins from fennel are used in processed foods, snacks, sauces and various vegetable preparations.

14.4.5 Fennel powder and curry powders

Finer powder products are mostly used for food seasoning, whilst coarser products are used for the extraction of oils, oleoresin and other extractives (Malhotra, 2010). A number of simple blends containing fennel can easily be prepared in the home, including:

- ***Sri Lankan curry powder***: The spices coriander (6 tbsp), cumin (3 tbsp), fennel (1 tbsp) and fenugreek (1 tbsp) are well roasted separately, then powdered with a 5 cm (2 in) piece of dry-fried cinnamon stick, cloves (1 tsp), 8 green cardamoms, 8 curry leaves and chilli powder (1–2 tsp), resulting in 12 tbsp of a gloriously rich, dark curry powder which can be used for fish, poultry, meat or vegetable curries.
- ***Singapore-style curry powder***: Made up of 3–4 dried red chillies, coriander seeds (6 tbsp), cumin seeds (1 tbsp), fennel seeds (1 tbsp), black pepper corns (2 tsp), a 2.5 cm (1 in) piece of cinnamon stick, 4 green cardamoms, 6 cloves and 2 tsp

ground turmeric, and making 10 tbsp of curry powder. The spices are dry-fried or roasted before mixing, and then ground to a fine powder for use as curry powder for a variety of dishes.

- ***Singapore-style seafood curry powder***: Made up of 2–3 dried red chillies, coriander seeds (6 tbsp), cumin seeds (1 tbsp), fennel seeds (2 tbsp), fenugreek seeds (1 tsp), black peppercorns (1 tsp) and ground turmeric (2 tsp), this seafood curry powder mixture is prepared in the same way as the Singapore-style curry powder above, and makes 8 tbsp of powder.

14.4.6 Fennel-based commercial blends

Various fennel-based commercial blends are available (see Clevely *et al*., 1997), including:

- ***Fennel tea***: Prepared from fresh leaves or dried herbs. The whole leaves are pure herbs and are less processed than herbal tea bags, so that the plant oil quality is better retained, therefore making a more concentrated tea (usually prepared by infusion). As well as fennel leaves and bulbs, fennel green seed tea is also available. Organic fennel seeds for infusion and tincture making are sold in herbal stores or online distributors. Fennel herb and seeds are often used in blends or mixes with other herbs for organic herbal tea preparations containing catnip, spearmint, lemongrass, calendula flowers, skullcap, rosemary and sage leaf.
- ***Cough syrups***: Produced from mixtures of fennel with honey and organically-grown and wild-harvested herb ingredients such as elecampane root, osha root, marshmallow root, horehound and mullein; syrups containing 10 % alcohol by volume are also available.
- ***Absinthe***: An alcoholic mixture, for which Florence fennel is one of the three main herbs used. Absinthe is an alcoholic mixture which originated as a medicinal elixir in Switzerland but, by the late nineteenth century, had become a popular alcoholic drink in France and other countries.
- ***Indian* panch phoran *(five spices)***: This spice mixture is very popular, especially for meat dishes, in the Indian union states of West Bengal and Sikkim, as well as in Bangladesh. It contains nigella, fenugreek, cumin, black mustard seed and fennel, usually in equal parts, with ajowan sometimes used instead of cumin, and black pepper sometimes added.
- ***Chinese five spice blend***: Popularly used to flavour several kinds of foods and made from organic products including anise, black pepper, fennel seeds, cinnamon and cloves.

14.5 Functional properties of fennel

Indian fennel has been reported to be a rich source of high dietary fibre (28.7 % fibre) which has a beneficial physiological effect on the digestive system (Srinivasan, 2005). The isolation and identification of the different active principles in fennel is of great therapeutic interest due to the wide spectrum of uses in traditional medicine where, for example, it has been recommended as an anti-anorexigenic. Its nutritional status indicates food value and medicinal value as a protective food for controlling

various disorders. Fennel is officially recognized in the USA and UK and many other pharmacopoeias from different countries. The key preparations using fennel for medicinal purposes are given in Table 14.4. Fennel has a long history of herbal use: its leaves, bulb, seed, essential oil and water possess a number of functional properties and thus it has been popularly used in various forms for teas, tinctures and extractives. Fennel has a powerful anise-like aroma and is used in aromatherapy for its cleansing and toning properties. In modern herbal medicine sciences, fennel is recorded as having a long list of medicinal properties, including being used to treat bruises, cellulite, flatulence, gum disease, halitosis and mouth sores, some of which are described in the sections below.

14.5.1 Antimicrobial (antifungal and antibacterial)

Fennel essential oils have an antibacterial effect (Ruberto *et al*., 2000; Singh *et al*., 2002). The bacterostatic effects of the crude extract derived from fennel has been proved against *Helicobacter pylori*, the most prevalent gastric pathogen causing gastric dysfunction, ulceration and even cancer (Sadeghian *et al*., 2005). The antibacterial effects of herbal extracts such as fennel oil have been shown to be potent when in combination with benzoic acid derivatives such as methyl paraben (methyl 4-hydroxybenzoic acid), as judged by studies on *Listeria* and *Salmonella* species (Fyfe *et al*., 1998). Fennel essential oil has an antibacterial effect against *Acinetobacter baumannii*, a gram-negative bacteria (Jazani *et al*., 2009). The antibacterial and antifungal activity has been reported equally for *azoricum* and *dulce* cultivars (Anwar *et al*., 2009) and *piperitum* cultivars (Özcan *et al*., 2006).

14.5.2 Antiflatulent and antispasmodic

Fennel is an excellent stomach and intestinal remedy for treating flatulence and colic conditions, while also stimulating healthy appetite and digestion. Fennel seeds increase gastrointestinal motility and act as an antispasmodic in high doses. Fennel extracts produce a reduction in acetylcholine-induced contraction and decrease maximum possible contractility (Vasudevan *et al*., 2000). In tests, fennel in a concentration of 10 % weight/volume increased gastric acid secretion in rats from 0.12 mL (basal level) to 0.42 mL, although the exact mechanism of increasing gastric acid secretion is unknown. Owing to this property, a fennel infusion is used domestically worldwide to stimulate gastrointestinal action.

A decoction of fennel seeds is used in Indian and Chinese medicine for abdominal pain, colic and stomach chills. The infusion is used to treat indigestion and abdominal distention (Chevallier, 2001). A fennel extractive, peppermint and ginger in an enteric-coated hard gelatin capsule are also available on the market, to treat discomfort, abdominal colic and gastrointestinal disease. There are five commercial Ayurvedic products, *Satapuspadi churana*, *Satapushpa arka*, *Satapushpadya Ghrita*, *Abhayrishta* and *Panchsakar churna*, which are prescribed by Ayurvedic practitioners to improve digestion, control colic pain and other gastrointestinal problems (Dhiman, 2006).

The by-product *ark saunf* (water of fennel), produced after the extraction of fennel essential oil, possesses good medicinal properties for curing indigestion

Table 14.4 Key preparations from fennel and their application in medicine

Preparation	Dose	Properties as medicine	References
Enteric-coated hard gelatin capsule containing 0.2 ml peppermint oil and 0.1 ml fennel oil	1 capsule to be taken 3 times per day, taken 30–60 minutes before food; dose may be increased to 2 capsules in severity	Antiflatulent, for the treatment of discomfort, abdominal colic and distension	Anon. (2012a)
1 tsp seeds in 300 ml water that is just off the boil	Take a cup of tea 2–3 times a day	Carminative, anticolic	Clevely *et al.* (1997)
1.5–4 tsp crushed fruit or seed in 1 cup water for infusion purpose and pass through tea strainer after 10 minutes	Take a cup of tea 3 times a day	For treatment of respiratory congestion and also relaxes the smooth muscle lining the digestive tract, appetite control	Anon. (2012b)
Pour 1 cup boiling water over the tea bag or dried organic herb of fennel,	Full cup of tea daily	Irritable bowel syndrome	Anon. (2012b)
Caraway seeds (1 part) Fennel seeds (1 part) Mentha leaves (1 part) Chamomile leaves (1 part) Valerian roots (1 part) Mix them all and boil in water for infusion purpose and pass through tea strainer after 10 minutes	3 times a day for 2–3 weeks	Carminative, for curing problems of gas, bloating, belching or heavy feeling, for functional liver and gall bladder problem	Bergner (2007)
Vegetable charcoal (242 g) and fennel seed (162 g) – WPC (whole phyto complex concentrate of 8 herbs) blend (this quantity is for 2 capsules)	2 capsules daily	Reduces bloating, gas and heaviness, absorbs gases and toxins	Anon. (2012c)
Fennel tincture	1–2 ml tincture 3 times a day	Antiflatulent	Anon. (2012d)

problems and is a popular home-produced remedy in India. Fennel water has properties similar to those of anise and dill water, and, when mixed with sodium bicarbonate and syrup, these waters make up the domestic gripe water used to correct flatulence in infants (Grieve, 1931) and as a folk medicine it is still popular.

Fennel water has long been used in India as a home remedy to control abdominal problems, flatulence, digestive gas and bloating.

14.5.3 Stimulant, carminative and expectorant

Fennel is known to stimulate a healthy appetite and digestion; significant shortening of food transit time when some prominent dietary spices (including fennel) were added to the diet was reported by Patel and Srinivasan (2001). In the composition of fennel, there are large amounts of anethole found throughout the plant, although mostly concentrated in the seeds. The digestive and carminative action of fennel is attributed to this substance and its pleasant taste and distinct perfume convert fennel into an appetizing vegetable to be included in meals. Vegetable charcoal has a long history of use and is known for its ability to readily adsorb gases and liquids in the intestines, also supporting healthy intestinal bacteria that promote good digestion. Vegetable-based charcoal with fennel is available in commercial encapsulated form on the market. Fennel gives a delicious flavour and aromatic lift to herbal blends and cough syrups. Additionally, fennel can help expel wind from the alimentary canal, freeing the respiratory system, rendering a calming effect on coughs and bronchitis; anethole and fenchone (the major constituents of its essential oil) have been shown to have a secretolytic effect on the respiratory tract (Brender *et al.*, 1997).

Fennel seeds are simmered in syrups for coughs, shortness of breath and wheezing. Fennel oil mixed with honey can be taken for coughs, and its tea is used as a gargle. Kloss (1994) described fennel as a 'thoroughly tried' remedy for gas, acid stomach, gout, cramps and colic. The essential oil of fennel has carminative qualities that are at least as effective as peppermint oil (Guenther, 1982), and it is described as excellent for obesity treatment (Kloss, 1994); the effects of fennel in obesity are believed to be related to an appetite suppressant effect although this area of efficacy remains under-explored. It has been hypothesized that the carminative effect of essential oils may be related to their action on intestinal foam. Peppermint, fennel, cinnamon, orange, dill and caraway oils have been shown to be highly effective in disrupting gastrointestinal foam as a consequence, perhaps, of the stimulation of gastric and intestinal secretion (Harries *et al.*, 1978).

14.5.4 Anticarcinogenic properties

Anetholes from fennel, anise and camphor are among the several dietary factors that have the potential to be used to prevent and treat cancer (Anand *et al.*, 2008). The chemopreventive potential of fennel against carcinogenesis has been shown by Singh and Kale (2008). The effects of anethole may be mediated by the modulation of the tumour necrosis factor (TNF)-induced cellular responses. Anethole may interfere with TNF signalling and lead to the activation of NF-κB, AP-1, JNK, MEK and apoptosis. Anethole may suppress NF-κB-dependent gene expression induced

by TNF (NF-κB controls the expression of some genes involved in carcinogenesis and inflammation; see Chainy *et al*., 2000).

Estragole, a constituent of fennel, is a procarcinogen but has minimal carcinogenic risk. To reach full toxicity, estragole must be activated by liver enzymes. Fortunately, other liver enzymes inactivate it, limiting liver damage (De Vincenzi *et al*., 2000; Iten and Saller, 2004; Iyer *et al*., 2003).

14.5.5 Antioxidant activity

The fennel leaf and bulb stalk, mostly consumed raw, have high antioxidant potency and are considered important in disease processes like coronary vascular disease, inflammatory disease, carcinogenesis and ageing. The anti-inflammatory, analgesic and antioxidant activities of fennel fruit have been reported by Choi and Hwang (2004). The essential oil, water and ethanol extracts from fennel fruits have a strong antioxidant effect (Oktay *et al*., 2003; Mata *et al*., 2007). One hundred grams of water and ethanol extracts exhibit 99.1 % and 77.5 % inhibition of peroxidation in the linoleic acid system, respectively, which is greater than the same dose of α-tocopherol (36.1 %), a natural antioxidant. Both extracts have effective free radical-scavenging, superoxide anion radical-scavenging, hydrogen peroxide-scavenging and metal-chelating activities, which are directly proportional to the concentration of the sample. Indications are that fennel seeds are a potential source of natural antioxidants.

The fennel herb and bulb is a good source of flavonoids, occurring as glycosides or in a free state, and known for their antioxidant effect against free radicals. Anwar *et al*. (2009) reported appreciable levels of total phenolic content, flavonoids and DPPH radical scavenging activity inhibiting peroxidation by 45–70 % (an 80 % ethanol extract exhibited the highest antioxidant activity). Parejo *et al*. (2004b) identified 42 phenolic substances, 27 of which had not previously been reported in fennel, including hydroxycinnamic acid derivatives, flavonoid glycosides and flavonoid aglycons.

14.5.6 Muscle relaxant

Essential oils, such as peppermint and fennel oil, have been demonstrated to exert a significant smooth muscle relaxant effect which is believed to relate to the inhibition of calcium channels (Taylor *et al*., 1985). Peppermint oil and menthol alone are known to block the carbachol (achetylcholine-like) induced influx of calcium ions into cells; thus, essential oils appear to be calcium channel blockers and exert pharmacological effects similar to those observed with current prescription medications such as nifedepine or diltiazem, which are calcium channel antagonists. The effect of commercial essential oils of celery, sage, dill, fennel, frankincense and nutmeg on rat skeletal muscles involved a contraction and inhibition of the twitch response to nerve stimulation, at final bath concentrations of 2×10^{-5} and 2×10^{-4} g/ml (Lis-Balchin and Hart, 1997). Fennel oil has been demonstrated to increase the resting force of guinea pig tracheal smooth muscle; anethole may be responsible for this positive inotropic effect (Reiter and Brandt, 1985). In another animal study, sweet fennel oil inhibited acetylcholine-induced contractions of ileal and bladder smooth

muscles; the mechanism of action is thought to be due to an inhibition of calcium release from intracellular stores and the binding to calcium-binding proteins by constituents in the fennel oil (Saleh *et al*., 2005).

14.5.7 Nausea and stress relaxer

In a study by Gilligan (2005), a variety of aromatherapy treatments were used on patients suffering from the symptom of nausea in a hospice and palliative care programme, using a synergistic blend of *Pimpinella anisum* (aniseed), *F. vulgare* var. *dulce* (sweet fennel), *Anthemis nobilis* (Roman chamomile) and *Mentha x piperita* (peppermint). The majority of patients who used the aromatherapy treatments reported relief, using measurements taken on the Bieri scale (a visual-numeric analogue). Since the patients were also on other treatments for their symptoms, it was impossible to establish a clear scientific link between the aromatherapy treatments and nausea relief, but the study suggested that the oils used in this aromatherapy treatment were successful complements to the relief of this symptom.

14.5.8 Hepatoprotective

As well as being a useful treatment for chest, spleen and kidney diseases (Singh and Kale, 2008), fennel fruit also has liver protection properties (Özbek *et al*., 2003). The hepatotoxicity produced by acute carbon tetrachloride-induced liver injury was found to be inhibited by essential oil from fennel, as evidenced by decreased levels of serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and bilirubin (Özbek *et al*., 2003). An increase in biliary solids and a pronounced higher rate of secretion of bile acids were caused by various spices including fennel, probably contributing to the digestive stimulant action of the test spices (Patel and Srinivasan, 2000). Gershbein (1977) reported increases in liver increment (the amount of tissue regenerated) in partially hepatectomized rats, by subcutaneous injection of oils of anise, fennel, tarragon, parsley seed, celery seed and oleoresin, nutmeg, mace, cumin and sassafras, and of the aromatic principles, 4-allylanisole, 4-propenylanisole, ρ-isopropylbenzaldehyde, safrole and isosafrole. Many of the agents effective by the subcutaneous route were also active when added directly to the diet.

14.5.9 Antidysmenorrheal

In a study comparing the efficacy of the drug mefenamic acid against that of the essence of fennel seeds, Jahromi *et al*. (2003) found that the latter could be used as a safe and effective herbal drug for primary dysmenorrhoea; however, it may have a lower potency than mefenamic acid in the dosages used for this study (2 % concentration). Both drugs relieved menstrual pain effectively; the mean duration of initiation of action was 67.5 ± 46.06 minutes for mefenamic acid and 75 ± 48.9 minutes for fennel. Increased ectopic uterine motility is the major reason for primary dysmenorrhoea and its associated symptoms (including pain). Treatments include long-term therapy, where a combination of oestrogens and progestins is used; in short-term therapy, non-steroidal anti-inflammatory drugs (NSAIDs) are

sometimes used. Most NSAIDs in long-term therapy show severe adverse effects. Ostad *et al*. (2001) used fennel essential oil (FEO) in an attempt to find agents with less adverse effect. Administration of different doses of FEO reduced the intensity of oxytocin- and PGE2-induced contractions significantly (25 and 50 g/ml for oxytocin and 10 and 20 g/ml for PGE2, respectively). FEO also reduced the frequency of contractions induced by PGE2 but not with oxytocin. The estimated LD50 was 1326 mg/kg. No obvious damage was observed in the vital organs of the rat.

14.5.10 Antihirsutism

Idiopathic hirsutism is the occurrence of excessive male-pattern hair growth in women who have a normal ovulatory menstrual cycle and normal levels of serum androgens, and may be caused by a peripheral androgen metabolism disorder. Javidnia *et al*. (2003) evaluated the clinical response of idiopathic hirsutism to topical application of creams containing 1 % and 2 % fennel extract, which had been used as an oestrogenic agent, by measuring hair diameter and rate of growth. The efficacy of the cream containing 2 % fennel extract was better than the cream containing 1 % and these two were more potent than the placebo used (the mean values of hair diameter reduction were 7.8 %, 18.3 % and –0.5 % for patients receiving the creams containing 1 %, 2 % and 0 % (placebo) fennel extract, respectively).

14.5.11 Antiparasitic

Powdered fennel seeds are used to keep fleas and other parasites away. The acaricidal activity of components derived from fennel seed oils against *Tyrophagus putrescentiae* adults using direct contact application, and compared with compounds such as benzyl benzoate, dibutyl phthalate and *N*,*N*-diethyl-*m*-toluamide, was reported (Lee *et al*., 2006). The bioactive constituent of the fennel seeds was characterized as (+)-carvone by spectroscopic analyses. The most toxic compound to *T. putrescentiae* was naphthalene, followed by dihydrocarvone, (+)-carvone, (–)-carvone, eugenol, benzyl benzoate, thymol, dibutyl phthalate, *N*,*N*-diethyl-*m*-toluamide, methyl eugenol, myrcene and acetyleugenol, on the basis of LD50 values, and reviewed by Shamina (2008).

14.6 Toxicity and allergenicity

Fennel herb, bulb, seeds and extractives do not appear to have any significant toxicity. The amount of fennel normally consumed in food is non-toxic. Fennel herbal tea and other preparations have a broad profile and have almost no adverse reaction in therapeutic doses. Excess amounts of fennel oil may cause nausea, vomiting and seizures. Some fennel products contain a naturally-occurring cancer-causing substance known as estragole, and it has therefore been suggested that large quantities of fennel be avoided and that it be used only according to the advice of a herbal health practitioner. Bergapten (furanocoumarin) compounds found in fennel essential oil may be carcinogenic during exposure to sun. In rare cases, allergic reactions

have been noted of the skin and respiratory tract. Pregnant women should not use the herb, seeds, tincture or essential oil of fennel in medicinal remedies, due to their oestrogenic effects; small amounts used as a culinary spice are considered safe, though in large doses fennel acts as a uterine stimulant. The essential oil of fennel is toxic in doses as small as 5 ml, and may cause skin irritation, vomiting, seizure and respiratory problems. The volatile oil should not be ingested. The herb and seed oil may cause contact dermatitis in sensitive individuals (Hanrahan, 2005).

The need to clarify the safety of the use of FEO was addressed by Ostad *et al*. (2004), since its use as a remedy for the control of primary dysmenorrhoea increased concerns about its potential teratogenicity due to its oestrogen-like activity. The results showed that FEO at concentrations as low as 0.93 mg/ml was cytotoxic. However, this reduction was due to cell loss, determined by neutral red cell viability assay, rather than due to a decrease in cell differentiation. These findings suggest that FEO at the studied concentrations may have a toxic effect on foetal cells, but there was no evidence of teratogenicity.

Estragole, a natural constituent of tarragon, sweet basil and sweet fennel, is used widely in foodstuffs as a flavouring agent. Several studies, as detailed in the review by De Vincenzi *et al*. (2000), have shown the carcinogenicity of estragole. Sekizawa and Shibamoto (1982) reported the mutagenicity of anethole present in fennel. Stich *et al*. (1981) examined the clastogenic activities (substances or processes which cause breaks in chromosomes) of quercetin from fennel seeds and the ubiquitous transition metal Mn2+, both individually and in various combinations (the clastogenic effects of the simultaneous application of arecoline from betel nut plus quercetin were greater than the action of quercetin alone).

14.6.1 Fennel as a food allergen

Changes in dietary habits and the internationalization of foods have led to the increasingly frequent use of spices. Children with allergy symptoms to spices were evaluated by prick tests, using the basic foodstuffs, crushed or diluted in saline, for aniseed, cinnamon, coriander, cumin, curry, fennel, nutmeg, paprika, sesame and vanilla; labial and/or challenge tests were performed for certain spices (mustard, fennel) by Rancé *et al*. (1994). The spices responsible for sensitization (found in 46 % of cases) were mustard, fennel, coriander, cumin and curry. Fennel was responsible for a case of recurrent angio-oedema (positive labial challenge test). Mustard and fennel are incriminated most frequently and are also responsible for clinical manifestations. Avoidance of these allergens in the diet is made difficult by masking in mixtures of spices or in prepared dishes.

14.7 Quality issues

14.7.1 Specifications for whole seed

Quality specifications for fennel were outlined in detail by Malhotra (2009) and can be classified into three main categories: commercial requirements, cleanliness and health specifications, each of which are discussed below.

Table 14.5 Quality specifications for whole and ground fennel

Parameter	Specification
Odour	It should have a warm, agreeable, sweet odour
Volatile oil	A minimum value of 1 % in Germany, 3 % in the Netherlands, 2 % in the UK
Appearance	It should be a free-flowing seed
Colour	In Germany, the colour should be light green and light brownish-green
Aroma	Sweet aroma combined with a herby camphoraceous note
Packing	Whole seed is packed in jute bags; fennel powder is packed either in polywoven or jute bags with inner polylining

Source: Potty and Krishnakumar (2001).

Commercial requirements

These specifications vary from country to country and crop to crop, and depend on many factors such as customer needs, acceptability and country specifications. The commercial specifications of seed spices crops are colour, appearance, taste, pungency, texture, shape, volatile oil and packaging. In the first instance, the quality of fennel seeds (botanically speaking, the fruit) depends mainly on their external appearance, which provides a visual perception of quality such as colour, uniformity of size, shape and texture. The size and colour of fruits will depend upon the variety and stage of harvesting. The fennel fruits are normally light green to grey in colour and fully grown fruits are 4–10 mm long, straight or slightly curved, oval in shape, mesocarp is 5 ridged and contains agreeable, aromatic and sweet aromas. Agmark of India provides three grades (special, good and fair) for fennel seed, based on size and shape. The general attributes described for the quality of fennel are that the seeds, or dried fruits (*F. vulgare*), have the characteristic size, shape, colour, taste and aroma normal to the variety and are free from visible mould, musty odour or signs of insects (living or dead). The general commercial requirements for fennel seed (Table 14.5) are that they should be light green and light brownish green, bitter or sweet, small or large seeds, aromatic, free-flowing and with high volatile oil content (minimum 1 % in Germany, 3 % in the Netherlands, and 2 % in the UK).

Cleanliness

The permittable cleanliness specifications (as per ASTA, ESA and ISO) are given in Tables 14.6 and 14.7.

Health specifications

Health specifications include limitations on microcontaminants such as pesticide residues, microbial counts, aflatoxins and heavy metals, which make food unsafe. Buyers in the international market have limited the maximum levels of these contaminants (see Table 14.8).

14.7.2 Fennel powder

Fennel seed powder, or herbal powder, is produced by grinding dried, cleaned and sterilized raw material, and is a greenish yellow powder with an aroma similar to

Table 14.6 ASTA cleanliness specifications for fennel

Crop	Whole insect dead (by count)	Excrets mammalian (mg/lb)	Excreta other (mg/lb)	Mould (% wt)	Insect defiled/ infected (%/wt)	Extraneous foreign matter (%/wt)
Fennel	2	2	2	2	1	0.5

Source: Asta (2007).

Table 14.7 ESA and ISO cleanliness specifications for fennel

Crop	Ash level % w/w (min.)	Acid insoluble ash % w/w (max.)	Moisture content % (max.)	Volatile oil % (min.)
Fennel	9	2	12	1.5

Source: ESA (2007).

Table 14.8 Maximum permitted levels of contaminants in imported fennel

Maximum residue level	0.05–0.2 %
Microbial counts	1×10^2 / g to 1×10^6 /g (*Salmonella*, *E. coli*, yeast, moulds)
Aflatoxin	5–10 ppb (max.) aflatoxin, mycotoxins
Heavy metals	Arsenic (5 mg/kg), copper (20 mg/kg) lead (10 mg/kg), zinc (50 mg/kg)

anise. After sieving through the required mesh size – at least 95 % of the ground product should pass through a US Standard No. 30 sieve (Farrell, 1999) – the powder should be packed in airtight containers. Flavour can be lost by heat produced during grinding and consequently freeze-grinding techniques are often used. The full whole seed specifications should also be strictly followed in addition to seed powder quality specifications.

14.7.3 Essential oil

Fennel seed oil yields 1–6 % oil depending upon variety and method of distillation (steam distillation being preferred). Generally, there is more oil in European varieties and less in Asian varieties. The main constituents of the sweet fennel oil distilled from the fruit of *F. vulgare* var. *dulce* are limonene (20–25 %), fenchone (7–10 %) and *trans*-anethole (4–6 %). Arctander (1960) placed this oil in the sweet, non-floral, candy-flavoured group. In the USA, both fennel oil (GRAS 2481) and sweet fennel oil (GRAS 2483) are generally regarded as having a 'safe' regulatory status.

Fennel oil, star anise and anise are natural sources of anethole, although synthetic substitutes are also readily available. In many countries, the use of synthetic anethole in food products is illegal. Anethole can also be synthesized from estragole extracted from pine (*Pinus*) oil (Weiss, 2002). Anethole is almost colourless to pale yellow, and crystallizes on standing, so may require warming before use (the congealing temperature should not fall below 3 °C). It has a pleasant, aromatic, anise

Table 14.9 Physicochemical constants of volatile oil from fresh herb of bitter fennel

Properties	Value of fresh herb oil	Fennel seed oil	Fennel seed oil
Specific gravity	0.873–0.925 (15 °C)	0.965–0.977 (20 °C)	0.889–0.921
Refractive index	1.484–1.508 (20 °C)	1.528 to 1.539 (20 °C)	1.484–1.568
Optical rotation	+40° to 68°	+11° to +24°	+20° to +58°
Solubility	Soluble in 0.5–1.0 vol. of 90 % alcohol	Soluble in 5–8 vol. of 80 % ethanol	–
Anethol	60–70 %	50–80 %	–
Congealing point	–	Not below 5° and as high as 10° in good oils	–
Reference	Singh *et al*. (1990)	Singhal *et al*. (1997	Agrawal (2001)

odour and a characteristic camphor-like taste, which is spicy and mildly bitter. Arctander (1960) placed it in the warm phenolic, fresh herbaceous group. The maximum permitted level in food is about 0.3 %, but usually less than 0.1 % (in perfumery and cosmetics, the maximum permitted is 0.4 %). The physiological constants of volatile oil from fresh herb and fennel fruit oil are given in Table 14.9. In India, small seeds generally have a higher essential oil content than larger seeds, and the essential oil's main characteristics are:

- specific gravity (at 15 °C): 0.9304
- refractive index (at 15 °C): 1.4795
- optical rotation: +35°
- saponification value: 181.2
- iodine value (Wijs): 99
- unsaponified material: 3.7 %.

Expressed oil is classified as semi-drying and is a source of lauric and adipic acids (Weiss, 2002).

14.7.4 Fennel oleoresin

Fennel oleoresin is a brownish green liquid with a minimal volatile oil content of 50 ml per 100 g. Fennel oleoresin should be prepared with recommended organic solvents followed by the subsequent removal of the solvent (as per importing countries' specifications). Approximately 2.95 kg of fennel oleoresin is equivalent to 45.45 kg of freshly ground fennel seed in flavour and aroma characteristics (Farrell, 1999).

14.7.5 Fixed oil

Fennel seeds have 9–13 % fixed oil and its physicochemical constants are given in Table 14.10. Essential oils and fixed oils share a similar chemical foundation: their structures are based on the linking of carbon and hydrogen atoms in various configurations. Fixed oils are made up of molecules comprising three long chains of carbon atoms bound together at one end, called a triglyceride. The fixed oils are greasy, whereas the essential oils easily evaporate and do not feel greasy.

Table 14.10 Fennel fixed oil physicochemical values

Properties	Value
Specific gravity at 15 °C	0.9304
Refractive index at 35 °C	1.4795
Saponification value	181.2
Iodine value	99.0
Unsaponification value	3.68
Soluble point	2
Fatty acids	–
Palmitic acid	4 %
Oleic acid	22 %
Linoleic acid	14 %
Petroselinic acid	60 %

Source: Singh *et al*. (1990).

14.7.6 Adulteration

Fennel traded on the market varies greatly in quality, either due to a lack of care in harvesting or as a result of deliberate adulteration. Adulterants are intentionally added to more expensive substances to increase visible quantities and reduce manufacturing costs, or added for some other malicious purpose. Adulterants can also be accidentally or unknowingly introduced into substances. Consignments of fennel may contain sand, dirt, stem tissues, weed seeds or other material, which amounts to adulteration and makes it unfit for medicinal use. Fennel may even already have had some of its oil removed by distillation. Seeds already exhausted by water or steam are darker, contain less oil and sink at once in water; those exhausted by alcohol, however, retain 1–2 % of essential oil content and are only slightly altered in appearance, although they do acquire a peculiar fusel oil odour. Exhausted or otherwise inferior fennel is occasionally improved in appearance by the use of a colouring, but old exhausted seeds that have been re-coloured can be detected by rubbing the seed between the hands (and the colour comes off). Ground seeds are subjected to adulteration by the addition of exhausted or spent seeds (from which oil or oleoresins have already been extracted), excess stems, chaff and plant waste. Fennel essential oil is also adulterated with fennel chaff, fennel wild plant and bulb oil. It is more difficult to judge the quality of fennel essential oil: it should preferably contain more anethole than fenchone, but the ratio varies with variety and geographical location, and further study is thus required to standardize such quality parameters. Adulterants in oil and oleoresins can be detected using gas chromatography or HPLC techniques (Križman *et al*., 2006). The adulterants range from synthetic chemicals and earthy materials to products of plant origin. Although conventional analytical tools are used to detect the synthetic adulterants of food and agricultural commodities, these methods are not always good enough to identify biological adulterants. DNA-based molecular marker methods (RAPD and SCAR) have application in biological adulterant detection and authentication of a wide range of food and agricultural commodities (Dhanya and Sasikumar, 2010).

14.8 References

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15

Galangal

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Abstract: This chapter discusses briefly the spice *Alpinia galanga* (L.) Sw. (Zingiberaceae) known commonly as galangal, greater galangal or Siamese ginger. This spice plays an important role in the cuisines of South East Asian Countries and of Indonesia. It is a very important medicinal plant used widely in traditional medicines of many countries, especially in the Ayurvedic system of medicine that is prevalent in India. It is also a garden plant, especially the variegated variety that is grown as a foliage plant mainly along the fence lines. Inflorescence is also attractive. This chapter describes the botany, production, chemical composition, pharmacological properties and uses of galangal in food and in medicine before finishing with a discussion of quality issues and adulteration.

Key words: galangal, greater galangal, Siamese ginger, Chinese ginger, *Alpinia galanga*, *A. officinarum* and *A. calcarata.*

15.1 Introduction

Alpinia galanga (L.) Sw. (Zingiberaceae) is known by various names – galangal, galanga, greater galangal, Java galangal and Siamese ginger (English). Two closely related species are *A. officinarum* Hance and *A. calcarata* Rosc., both of which are known as lesser galangal. All three species have more or less similar properties and are used in similar ways in culinary art as well as in medicine. Indeed, production, consumption and trade data on each species are not reliable because traders as well as consumers make no distinction among them. All three are used as the source plants for the Ayurvedic raw drug '*raasna*' (Ravindran *et al*., 2004). India is one of the major suppliers, the others being Thailand and Indonesia (Scheffer and Jansen, 1999). The volatile oil attracts more international interest than the spice because of its high medicinal value (Charles *et al*., 1992).

Galangal is a native of Indonesia although its exact origin is not known, but it has become naturalized in many parts of South and South East Asia. The oldest reports about its use and existence are from southern China and Java. The sub-Himalayan zone is also believed to be a natural home of galangal, and large populations are found in northern West Bengal, Bihar, Assam and other north eastern regions. At present, *A. galanga* is cultivated in all South East Asian countries, India,

Bangladesh, China and Surinam (Scheffer and Jansen, 1999). It shows exuberant growth along the eastern Himalayas and in south west India, and is cultivated throughout the Western Ghats (Warrier *et al*., 1994). Production in South East Asia must be considerable as it is a common spice used daily by millions of people; however, no reliable data are available. It is mostly cultivated in home gardens and organized plantations do not exist. The Netherlands imports yearly over 100 tons of fresh rhizomes and about 30 tonnes of dried rhizomes. The main suppliers are Thailand, Indonesia and India (Scheffer and Jansen, 1999). Recent reviews on *Alpinia* can be found in Tewari *et al*. (1999), Gupta and Tandon (2004), Chudiwal *et al*. (2010) and Verma *et al*. (2011).

15.1.1 Description

The species epithet *galanga* (and similar forms of Persian/Arabic name *qulanjan* or *khalanjan*) is probably an adaption of Chinese *gao liang jiang*, which means high, good ginger. The names in Northern Indian languages have the same source: *kulanja* in Sanskrit, *kulanjan* in Hindi and *kholinjan* in Urdu. The Latin generic name 'Alpinia' was given to commemorate Prospero Alpini (1553–1617), an Italian botanist who catalogued and described exotic plants.

A. galanga (Fig. 15.1) is a perennial, robust, tillering, rhizomatous herb, growing up to 3.5 m tall, with a subterranean, creeping, copiously branched aromatic rhizome. The rhizomes are 2.5–10.0 cm thick, reddish brown externally, and light orange–brown internally. The aerial leafy stem (pseudo-stem) is erect, formed by the rolled leaf sheaths. Leaves are 23–45 by 3.8–11.5 cm, alternate, distichous, oblong–lanceolate, acute and glabrous. The inflorescence is a terminal many-flowered

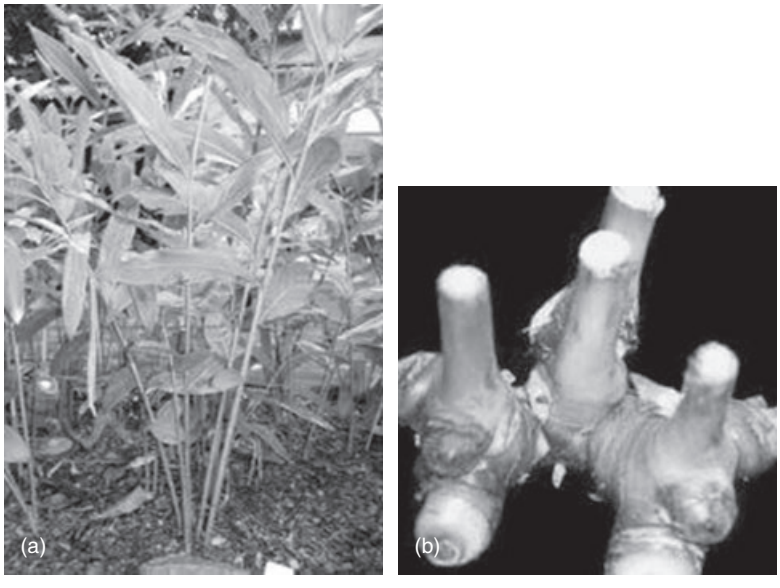

Fig. 15.1 (a) Galangal plant; (b) young rhizome used as a spice.

raceme; the flowers are fragrant, 3–4 cm long, yellow–white; the fruit is a globose to ellipsoidal capsule, 1–1.5 cm in diameter, orange–red to wine red. Rhizome anatomy shows a central stele surrounded by an outer cortical zone. Fibrovascular bundles are distributed throughout the cortex and stele. Numerous resin canals are also present. Its chromosome number is 2n = 48 (Ravindran *et al*., 2007). Much variability may exist as the species occurs naturally in many countries under varying agro-ecological situations; however, information is lacking. Cultivars with pink to red rhizomes and with yellow–white rhizomes are known. The pseudo-stems of white cultivars reach about 3 m in height, and the rhizomes 8–10 cm in diameter. The red cultivars, which are more common and widely used, reach 1–1.5 m in height and the rhizomes 1–2 cm in diameter. Plants with broad leaves that are tomentose beneath are distinguished as var. *pyramidata* (Blume) Schuman. This occurs wild and under cultivation in Java, Borneo and the Philippines (Scheffer and Janson, 1999).

15.1.2 Production

Galangal is found in wild/semi-wild and cultivated states. The plant requires sunny or moderately shady locations. Soil should be fertile, moist but not swampy; sandy or clayey soil rich in organic matter and with good drainage is ideal. Wild or semi-wild types occur in old clearings, thickets and forests. In the tropics, galangal occurs up to an altitude of 1200 m. Rhizome pieces (a rhizome piece with an aerial shoot, known as a slip) are used for propagation. Soil should be well tilled before planting. Alternatively, holes, 35 cm × 35 cm and 15–20 cm deep, are dug, filled with manure mixed with soil, inorganic fertilizers and lime (for acid soils). One slip is planted per hole, and covered with mulch. New shoots from pieces of galangal rhizome emerge about one week after planting. About four weeks after planting three to four leaves develop. Rhizomes develop quickly and reach best harvest quality in three months after planting. Galangal is usually planted along the borders of gardens, in rows at distances of 0.5–1 m square.

Harvesting for use as spice is usually done three months after planting (during late summer or early autumn) for market purposes. Rhizomes more than four months old turn fibrous and spongy and lose their value as spice (Ravindran *et al*., 2006). For essential oil extraction, rhizomes are harvested when plants are about seven months old. However, for use in Ayurvedic and other traditional medicinal preparations, rhizomes are harvested after 15 months, when the rhizomes become woody and fibrous (Scheffer and Jansen, 1999). Harvested rhizomes are washed, trimmed, dried and marketed fresh or dried after packing. Dried product is ground before use. Ground rhizomes are not traded in bulk as they may be adulterated. Essential oil is also a product (Scheffer and Jansen, 1999).

15.1.3 Chemical composition

Many investigators have studied the chemistry of galangal rhizome (Scheffer *et al*., 1981; Janssen and Scheffer, 1985; Charles *et al*., 1992; Raina *et al*., 2002; Jirovetz *et al*., 2003; Ibrahim *et al*., 2004; Chudiwal *et al*., 2010; Padilla *et al*., 2010; Verma *et al*., 2011). Scheffer *et al.* (1981) analysed a rhizome sample from Indonesia and reported 1,8-cineole (47.3 %), α-pinene (11.5 %), β-pinene (7.1 %), α-thujene

Table 15.1 Comparative percentage composition of the rhizome and leaf oils of *A. galanga* of Malaysian origin

Compound	Rhizome oil	Leaf oil
α-Pinene	1.16	9.00
β-Pinene	0.04	1.69
Myrcene	94.51	52.34
Limonene	0.35	0.52
1,8-Cineole	0.13	0.12
(*Z*)-β-ocimene	2.05	17.06
Borneol	–	4.13
Bornyl acetate	–	1.38
β-Caryophyllene	0.11	3.53
β-Bisabolene	0.57	3.04
(*E*)-β-farnesene	0.94	1.31
Caryophyllene oxide	–	1.04

(6.2 %), terpinen-4-ol (6.0 %), α-terpineol, limonene (4.3 % each) and many other compounds in lesser concentrations. De Pooter *et al.* (1985) analysed a sample from Malaysia and reported (*E*)-farnasene (18.2 %), β-bisabolene (16.2 %), α-bergamontene (10.7 %) and α-pinene (10.2 %) as the important components. Janssen and Scheffer (1985) determined that the oil of *A. galanga* contained 1′-acetoxychavicol acetate, 1′-acetoxyeugenol acetate and 1′hydroxychavicol acetate as trace constituents. They further determined that 1′-acetoxychavicol acetate showed moderate antimicrobial activity against gram-positive bacteria, yeast and some dermatophytes.

The volatile constituents of the rhizomes and leaves of galangal from the lower Himalayan region of India were analysed by GC and GC–MS. The main constituents identified in the rhizome were 1, 8-cineole, fenchyl acetate and α-pinene. The leaf oil contained 1, 8-cineole, α-pinene and camphor as major constituents (Raina *et al.,* 2002). Jirovetz *et al.* (2003) investigated the essential oils of the leaves, stems, rhizomes and roots of galangal from southern India by GC–FID, GC–MS and olfactometry. Mono- and sesquiterpenes and (*E*)-methyl cinnamate could be identified in all the four samples and these are responsible for the characteristic odour and the reported use in (folk) medicine as well as in food products. Main constituents of the rhizome essential oil are 1, 8-cineole (28.4 %), α-fenchyl acetate (18.4 %), camphor (7.7 %), (*E*)-methyl cinnamate (4.2 %) and guaiol (3.3 %). Three new 8–9′ linked neolignans, galanganal, galanganols A and B and a sesquineolignan, galanganol C, have been isolated and their structure determined (Morikawa *et al.*, 2005). The results of this study are shown in Table 15.1.

Yang and Eilerman (1999) determined that the pungent principle of galangal was 1′2′-acetoxy-chavicol acetate (which they renamed galangal acetate). Galangal acetate or the oleoresin of galangal possesses a capsaicin-like pungency; the hot sensation was perceived initially on the tip of the tongue before spreading throughout the oral cavity. Yang and Eilerman (1999) determined that galangal acetate was not stable in aqueous solution where it undergoes hydrolysis/isomerization reactions to 1′-hydroxy-chavicol acetate, *p*-acetoxycinnamyl alcohol and *p*-coumaryl diacetate.

15.2 Functional properties

Galangal rhizome is bitter, acrid, thermogenic and aromatic. It is considered a nervine tonic, stimulant, carminative, stomachic, disinfectant, aphrodisiac, expectorant, bronchodilator, febrifuge, anti-inflammatory and tonic (Warrier *et al*., 1994). Dried rhizomes of galangal are an important drug in traditional medicine systems of India and China. Many chemical components of galangal have potent biological properties. Such molecular pharmacological properties contribute to its therapeutic effectiveness. The USDA phytochemical and ethnobotanical database (Duke, 2011) lists 387 distinct activities for *A. galanga.* Some of the important constituents and their biological properties are provided in Table 15.2.

Galangin (3, 5, 7-trihydroxyflavone) is a flavonoid with multiple biological activities. It was originally found and characterized from galangal rhizome in 1881. Several recent studies with this flavonoid suggest that it may have a potent anticancer effect, specifically through inhibition of the detoxification enzyme CYP1A1 and modulation of the aryl hydrocarbon receptor (Ciolino and Yeh, 1999; Quadri *et al*., 2000). The implication with this type of research is that this flavonoid exerts a protective effect against the carcinogenic potential of overcooked, char-grilled foods (Chudiwal *et al*., 2010). Galangin has also been shown to be a potent preserver of the endogenous free radical scavenger glutathione, thereby playing another anticarcinogenic role. Galangin has a proven antioxidative effect on endothelial tissues and acts to help preserve other protective antioxidants such as vitamin E, vitamin C and other flavonoids; in this function, it also serves to prevent lipid peroxidation. An aromatase inhibitor, it specifically prevents the conversion of testosterone to oestrogen in both men and women thus providing yet another mechanism for preventing cancer (especially of the breast and prostate) and heart disease (Heo *et a*l., 2001).

15.2.1 Antiallergic activity

Aqueous-acetone extract (80 %) of rhizome inhibits release of β-hexosaminidase, a marker of antigen IgE-mediated degranulation in RBL-2h3 cells. Nine known phenylpropanoids and *p*-hydroxybezaldehyde were isolated from the extract. Among them, I′-S-I′-acetoxy-eugenol acetate exhibited potent inhibitory activity with IC(50) values of 15 and 19 mM. The above compounds also inhibited passive cutaneous anaphylaxis reactions in mice and the antigen IgE-mediated TNF-α and IL-4 production, both of which participate in the late phase of type 1 allergic reaction in RBL-2H3 cells (Matsuda *et al*., 2003a, 2004). Galangal rhizome extract effectively suppressed hypersensitivity reactions. This explains the usefulness of galangal in the treatment or prevention of allergic reactions and such conditions as asthma, allergic rhinitis, anaphylaxis and autoimmune disorders like ulcerative colitis and rheumatoid arthritis, as well as for the alleviation of pain (Weidner *et al*., 2002).

15.2.2 Antidermatophytic, antimicrobial and antiviral activites

Alcoholic extract of galangal was reported to be effective in suppressing zoonotic dermatophytes such as *Microsporium cannis*, *M. gypseum* and *Trichophyton* spp. Galanagal oil exhibits antimicrobial activity against gram-positive bacteria, yeast

Table 15.2 Attributed biological properties of the chemical components of galangal

Compound	Attributed properties
1′ Acetoxy-eugenol acetate	Anticarcinoma, antitumorous, antiulcer
1′-acetoxy chavicol acetate	Antitumour, antiulcer, nematicide
Borneol	Analgesic, antibronchitic, acetylcholine anatagonist, anti-inflammatory, antipyretic, antispasmodic, CNS-stimulant, CNS-toxic (at high doses), hepatoprotectant, myorelaxant, sedative
1,8-Cineole	Anaesthetic, antiacetylcholinesterase, antiallergic, antibacterial, antibrochitic, anticarcenogenic, anti-inflammatory, antirheumatic, antirhinitic, antiseptic, antispasmodic, antitussive, candidicide, ascaricide, carcinogenic (high and constant use), CNS-stimulant, convulsant, decongestant, expectorant, myorelaxant, P-450 inducer, neurotoxic, rubifacient, sedative, testosterone-hydroxylase inducer
Eugenol	Analgesic, anticancer, anticonvulsant, antioedemic, antiarchidonate, anti-inflammatory, antimutagenic, prostaglandin inhibitor, antipyretic, antiseptic, antispasmodic, antitumour necrosis factor, CNS-depressant, COX-1 and COX-2 inhibitor, cytochrome P450 inhibitor, hepatoprotective, larvicide, irritant, insecticide, motor depressant, neurotoxic, sedative, trypsin enhancer, ulcerogenic, vasodilator, vermifuge
Galangin	Antiaflatoxic, anti-inflammatory, antimutagenic, anticancer, antioxidant, antiviral, aromatase inhibitor, cycloxygenase inhibitor, COX-2 inhibitor, hepatoprotective, NO inhibitor, quinone reductase inducer, topoisomerase I inhibitor, tyrosinase inhibitor
Camphor	Analgesic, anaesthetic, antiseptic, antispasmodic, fungicide, anticancer, decongestant, expectorant, antiemetic, carminative
β-Bisabolene	Abortifacient, antirhinoviral, antiulcer, stomachic
Myrcene	Analgesic, anaesthetic, antibacterial, anticonvulsant, antimutagenic, antinitrosaminic, antioxidant, antipyretic, antispasmodic, irritant, aldose-reductase inhibitor
Quercetin	Analgesic, antiaggregant, anti-inflammatory, antileukaemic, antileukotriene, antilipoperoxidant, antimelanomic, antimutagenic, antinitrosaminic, antioxidant, antiperoxidant, antitumour, apoptotic, COX-2 inhibitor, cyclooxygenase inhibitor, hepatoprotective, lipoxygenase inhibitor, mastcell stabilizer, ornithine decarboxylase inhibitor, P-450 inhibitor, protein kinase-C inhibitor, topoisomerase I and II inhibitor, tyrosine kinase inhibitor, NADH-oxidase inhibitor, hypoglycaemic, quinone reductase inhibitor
Terpinene-4-ol.	Antiacetylcholineesterase, antiallergic, antiasthmatic, antispasmodic, antitussive, antiulcer, spermicide

Sources: Duke (2003); *Martindale: The Complete Drug Reference* (Sweetman, 2011).

and some dermatophytes. The main component exerting such activity was terpene-4-ol. 1′*S*-1′-acetoxychavicol acetate (ACA), a small molecular compound isolated from the rhizomes of galangal oil, is active against seven dermatophytic fungi at a concentration of 14 mg/ml and has shown significant activity against *Trichophyton* spp. Reports also indicated that the chloroform extract of galangal has potent inhibitory effect on *Giardia intestinalis* (Janssen and Scheffer, 1985; Sawangjaroen *et al*.,

2005). Tewtrakul *et al*. (2003) reported that methanolic extract of galangal rhizome showed potent inhibitory effect on human immunodeficiency virus 1 and human cytomegalovirus and hepatitis C virus. ACA inhibited Rev (a protein involved in the transport of RNAs) transport at a low concentration by binding to chromosomal region maintenance 1 and accumulating full-length HIV-1 RNA in the nucleus, resulting in a block in HIV-1 replication in peripheral blood mononuclear cells. Additionally, ACA and didanosine acted synergistically to inhibit HIV-1 replication. Thus, ACA may represent a novel treatment for HIV-1 infection, especially in combination with other anti-HIV drugs (Ye and Li, 2006). Many reports are also available on the antifungal and antibacterial properties of galangal rhizome extract and its components such as ACA (Chudiwal *et al*., 2010; Verma *et al*., 2011).

15.2.3 Anticancer activity

ACA occurring in the rhizome extract has shown significant activity against lung cancer cell lines and breast cancer cell lines with IC50 of 7.8 and 23.9 μM, respectively. Cytotoxicity assays and bioassays of oil fraction and compounds showed that the most potent cytotoxic compound present in galangal is ACA. This chemical component has also been reported to act as an antiulcer and antitumour agent as well as an inhibitor of chemically-induced carcinogenesis (Mitsui *et al*., 1976a, b; Murakami *et al*., 2000; Chudiwal *et al*., 2010; Verma *et al*. (2011). Itokawa *et al*. (1987), who isolated the phenylpropanoids, 1-acetoxychavicol acetate and 1-acetoxyeugenol acetate, also reported that both showed antitumour activity against sarcoma 180 ascites in mice. Zhang *et al*. (2010) demonstrated that galangin induced apoptosis in hepatocellular carcinoma cells through the mitochondrial pathway, leading various effects including inhibition of cell growth, nuclear fragmentation mitochondrial membrane potential collapse, etc.

15.2.4 Gastroprotective effect

Antisecretory and cytoprotective action of galangal is responsible for its antiulcer activity. Ethanolic extract of galangal has gastric antisecretory, antiulcer and cytoprotective properties in rats; at a dose of 500 mg/kg it significantly reduced the intensity of gastric mucosal damage induced by pyloric ligation and hypothermic restraint stress in rats (Al-Yahya *et al*., 1990). These workers reported that rhizomes of galangal are used widely in Arabian and Unani systems of medicine to treat stomach disorders. They found that the ethanolic extract significantly reduced gastric secretion and showed marked cytoprotective activity; it is suggested that these properties may be responsible for the antiulcer activity of galangal. Qureshi *et al*. (1994) reported treatment of cytological and biochemical changes induced by cyclophosphamide in mice by treatment with the ethanolic extract of galangal rhizome. The rhizome of galangal is used in traditional medicine to treat dyspepsia, gastralgia, sea sickness and abdominal colic, and as an anti-inflammatory, anti-neoplastic, digestive and tonic, because of its effect on the gastrointestinal system.

ACA present in the rhizome markedly inhibited the ethanol-induced gastric mucosal lesions. This compound also inhibited HCl and aspirin-induced mucosal

lesions but not indomethacin-induced gastric lesions. ACA significantly enhanced glutathione levels of gastric mucosa in rats. Studies led to the conclusion that endogenous prostaglandins and sulphydryl compounds are involved in the protective effects of ACA (Matsuda *et al*., 2003b).

15.2.5 Hypolipidaemic activity

The ethanolic extract of galangal rhizome exhibited hypolipidaemic activity *in vitro*. Oral administration of the extracts (20 mg/day) effectively lowered the serum and tissue levels of total cholesterol, triglycerides and phospholipids and significantly increased the serum levels of high-density lipoproteins (HDL) in high cholesterol fed white wistar rats over a period of four weeks (Achuthan and Padikkala, 1997). The extract inhibits fatty acid synthase; galangin, quercetin and kaempferol present in the extract have potent reversible inhibitory activity on this enzyme. Studies have led to the conclusion that the inhibitory mechanism of galangal extract is different from that of some other previously reported inhibitors of fatty acid synthase (Li and Tian, 2003).

15.2.6 Anti-inflammatory activity

Anti-inflammatory effects of galangal in a variety of rheumatologic conditions have been studied by several authors. Sharma and Sharma (1977, 1978) found that water-soluble fraction of the alcoholic extract of the plant was active in chronic arthritis in albino rats. Its anti-inflammatory activity was similar to that of β-methazone. Concentrated galangal rhizome extract has been reported to be effective in reducing substantially pain due to rheumatic knee inflammation (Chudiwal *et al*., 2010).

Yu *et al.* (2009) isolated certain components from rhizome extract that selectively suppressed T-helper cell activity which is responsible for the inflammatory response. Isolated chavicol analogues (ACA and hydroxy-chavicol acetate–HCA) exhibited potent antioxidant activity, increased cell apoptosis and decreased cytokine production by T-helper cells. HCA has suppressive effect on the inflammatory immune disorders caused by over-activation of cytokine production (Min *et al*., 2009). Anti-inflammatory potential of rhizome of galangal was reported by Satish and Dhananjayan (2003), Nagashekhar *et al*. (2005) and Jaju *et al*. (2009).

15.2.7 Cardiovascular and related effects

Galangal rhizome extract acts as a potential source of platelet activating factor antagonists. In rabbit platelets, methanolic extract showed significant inhibitory effect on PAF with IC50 value of 5.5 μg/ml. This effect, in turn, contributes to the atherosclerotic property of galangal and subsequently helps in cardiac protection (Jantan *et al*., 2005). Intravenous treatment of rats with galangal ethanolic extract induced a dose-dependent decrease in aortic pressure. The action is attributed to terpine-4-ol. This compound has a direct vasorelaxant effect, independent of the sympathetic nervous system. Morello *et al*. (2006) reported the vasodilator effect of

galangin on rat thoracic aorta through an endothelium-dependent mechanism involving nitric oxide and also through an endothelium-independent mechanism, inhibiting calcium movement through cell membrane.

Oral administration of galangal rhizome extract induced significant reduction in systolic and diastolic arterial pressure in rats. The vasodilator effect of galangal extract seems to be dependent on the activation of the NO-cGMP pathway and independent activation of adenosine triphosphate (ATP)-dependent, voltage-dependent and calcium-dependent K+ channels (de Moura *et al*., 2005). Intravenous injection of rhizome extract induced an immediate and significant hypotension in rats. This effect is mainly due to the essential oil component terpine-4-ol, and it is reported to be independent of the sympathetic nervous system, suggesting that galangal extract could have produced a direct vasorelaxant effect (Lahlou *et al*., 2002, 2003).

15.2.8 Nitric oxide (NO) inhibition

An 80 % acetone–water extract of galangal showed NO inhibitory action in mouse peritoneal macrophages. Three neolignans have been isolated from the acetone extract (galanganal, galanganol A and B) and a seaquineolignan, galanganol-C). The NO-inhibition activity has at least partly been attributed to the neolignans. Six diarylheptanoids of galangal are reported to be inhibitors of NO production in the lipopolysaccharide-activated macrophage cell line RAW 264 (Itokawa *et al*., 1985; Ohata *et al*., 1998). Active diarylheptanoids suppressed the expression of the inducible NO synthase protein and mRNA. Such results at least partly explain the use of galangal for inflammation reduction. ACA has been shown to have potent NO-inhibitory activity in lipopolysaccharide-activated mouse peritoneal macrophages.

15.2.9 Other activities

Galangal extract has immune-stimulating activity. The extract induced a marked stimulating effect on the reticulo-endothelial system and increased the number of peritoneal exudates cells and spleen cells in mice. The effect is due to the polysaccharide fraction of the extract (Bendjeddou *et al*., 2003). Galangin and kaempferol, the flavanols present in the rhizome, are known to possess tyrosinase-inhibitory activity as well as COX-(cyclo-oxygenase) inhibitory activity, probably due to the ability of these compounds to chelate copper (and also other divalent cations) in the enzymes. Galangin inhibits monophenolase activity, and both galangin and kaempferol inhibit diphenolase. Galangin and quercetin present in the rhizome possess antioxidant and radical-scavenging activities, and hence can modulate enzyme activities and suppress the genotoxicity of chemicals (Duke, 2003). The oil also shows a potential insecticide property (Abeywickrama *et al*., 2006). Sadique *et al*. (1989) reported that galangal extract demonstrated sheep RBC membrane stabilizing activity. Guo *et al.* (2010) reported recently that galangin significantly inhibits acetyl cholinesterase activity at an IC50 of 120 μM, and the result indicates that galangin can be a potential candidate for managing Alzheimer's disease.

15.3 Main uses of galangal

15.3.1 Uses in traditional medicine

This plant is used to treat loss of appetite, upper abdominal pain and sluggish digestion. It relieves spasms, combats inflammation and has stress-reducing properties. In Asia, this herb is also used for arthritis, diabetes, stomach problems and difficulty in swallowing. It is especially useful in flatulence, dyspepsia, nausea, vomiting and sickness of the stomach, being recommended as a remedy for seasickness. It tones up the tissues and is sometimes prescribed in fever. Galangal is used in cattle medicine, and the Arabs use it to make their horses fiery (Grieve, 1931). It is included in several compound preparations. The reddish-brown powder is used as a snuff in catarrh.

In the Indian traditional medicine, Ayurveda, *A. galanga* is used as the raw drug *raasna*, especially in the South Indian stream of Ayurvedic medicine. According to Khare (2007), galangal rhizome is carminative, stomachic, circulatory stimulant, diaphoretic and anti-inflammatory. It is used in over 62 formulations intended to cure a variety of ailments. Unani medicine practitioners consider galangal rhizomes and seeds as a good remedy for impotence and debility (Khare, 2007). It is a popular remedy for many respiratory ailments. Administration of a paste of galangal rhizome powder in honey lessened the paroxysm of cough in children suffering from whooping cough; it is also useful in bronchitis. Two of the related species, *A. calcarata* and *A. officinarum*, have properties similar to galangal and are used as sources of the raw drug *raasna*.

15.3.2 Uses in food

The use of galangal is now predominantly confined to local cuisines of Indonesia, Thailand, Cambodia, China, Malaysia and other South East Asian countries (Farnsworth and Bunyapraphatsara, 1992; Uhl, 1996). Galangal rhizome is sold fresh, frozen, dried or in powdered forms; however, the best flavour and taste is when used fresh. The rhizome and its powder are used in fish as well as in a wide variety of dishes such as sauces, soups and sambas, chicken, meat and vegetable curries. Galangal rhizome enhances the palatability and flavour of dishes such as chicken that are delicately spiced with fennel and lemon grass and gently cooked in coconut milk. However, these mild dishes are usually accompanied by vegetable or fish sambas fiery with chilli and spiced with galangal powder. Galangal is an essential ingredient in shellfish recipes in combination with garlic, ginger, chilli and lemon. Galangal is widely used in various fish preparations as this spice can mask the disagreeable smell of many fishes. Galangal is not commonly used in Indian, European or American cuisines. Some of the dishes in which galangal is an essential ingredient are given in Table 15.3.

The principles of galangal, galangin and its derivatives exhibit a unique pungent sensation, which is less intense than that of capsaicin and without a lingering effect. Isolated galangin is used in beverages, sweet goods, dressings and personal care products. In many applications, galangin is preferred to other pungent ingredients because of the mild sensation that it elicits. It can be used as an alcohol enhancer or an alcohol replacer in alcohol as well as in alcohol-free beverages (Yang and

Table 15.3 Some important dishes in which galangal is a major constituent

- acar
- aroma blended tom yam goong
- aromatic black tiger shrimp
- Assam laksa stock
- bbq duck and ramen soup
- bajak chili sauce
- basic lemon grass curry sauce
- Bruneian rendang
- Cambodian lemon grass soup
- cambogee beef-chicken soup with coconut milk
- chicken soup with potato patties
- Thai green curry chicken with basil
- tom yum gai-chicken in lemon grass soup
- chicken soup with galangal (kai tom kha)
- coconut fish curry parcels
- coconut tofu soup
- gaeng ki lek
- gulai daun singkong tumbuk
- Indonesian liver
- lemongrass curry (Cambodian)
- Malay beef rantang
- mee rebus
- nasi koening – spiced yellow rice
- hot and sour shrimp soup
- Thai green curry paste
- spicy seafood soup
- lab nuea (ground beef salad)
- Singapore – May laksa
- masaman curry with beef (gaeng masaman nua)
- Indonesian spiced coconut beef (rendang)
- peanut sauce (sambal kacang)
- rawon daging sapi
- salor kor-ko sap
- sayur lodeh
- somlah machoo soup
- sousi pa
- tea-crusted tofu over greens
- Thai noodles with chicken patties (soup or salad)
- tom kha gai
- tom yam goong
- Thai beef and noodle stir fry
- tomkha gai (chicken coconut soup)
- grilled beef Cambodian style
- gaeng gai (Thai chicken curry)
- nam prik kaeng matsaman (massaman curry paste)

Sources: Anon 2012a, b, c, d.

Eilerman, 1999). Recently galangal oil has also been made into microcapsules for convenient use in cooking (Jiamrungraksa and Charuchinda, 2010).

15.4 Quality issues and adulteration

Little is known about the quality issues pertaining to galangal. The major point to be remembered is that three species of *Alpinia* – *A. galanga, A. calcarata* and *A. officinarum* – are all treated as galangal in the world market. No differentiation is made either by the exporting countries or by the importing countries; quality and flavour-wise, they are similar and their medicinal properties are also similar. In the traditional Indian Ayurvedic medicine, all three species are used as the source for the raw drug *raasna*. Akhtar *et al.* (2010) reported the pharmacognostical standardization and evaluation of galangal rhizome as a valuable tool for identification, authentication and detection of adulterants and quality control of the drug. Dried, powdered rhizome is sometimes adulterated with other species such as *A. calcarata*, *A. conchigera*, *A. mutica*, *A. nigra*, *A. rafflesiana* and *A. scabra.* The fruits of *A. galanga* are used in traditional Chinese medicine, but the dry fruits are easy to adulterate with other species that are used as medicine in local areas. The dry fruits of the adulterants are very similar in odour, morphology, chemical constituents and anatomical characters and they are difficult to distinguish. Zhao *et al.* (2001) characterized *A. galanga* and the species used as adulterant using nuclear ribosomal

DNA internal transcribed spacer (nrDNA ITS) region sequences, and the molecular markers are used to distinguish the drug at DNA level.

Galangal rhizome, extracted oil and oleoresin are given the regulatory status 'generally regarded as safe' (GRAS) in the USA (Scheffer and Jansen, 1999). Duke (2003) has listed the application of *A. galangal* and *A. officinarum* as herbal medicines in the treatment of various ailments.

15.4.1 *Alpinia officinarum* Hance (lesser galangal, Chinese ginger)

A. officinarum looks similar to *A. galanga*, but it is smaller in stature. The immature rhizome of this plant is a favourite spice in East and Central Asian countries, and it is known to have been in use for over 1000 years in these regions. The young rhizome has a unique taste that is said to be in between pepper and ginger (Duke, 2003). The rhizomes have been in use in cooking, for adding flavour to vinegar and local liquors ('*nastoika*'). Rhizomes are popularly used in the preparation of tea (similar to ginger tea) (Watt, 1972). The emerging shoots are used as a vegetable in north east India. Alcoholic extract of the rhizome contains tannins, phlobaphenes; chloroform extract showed the presence of flavones such as kaempferide, galangin and alpinin (Sastry, 1961). Its essential oil is warm and spicy. Ray and Majumdar (1975) reported the isolation of a flavonoid possessing antifungal activity. The decoction of the rhizome revealed anti-inflammatory activity against carrageenin-induced rat paw oedema (Sharma and Singh, 1980). Kaleysa Raj (1975) reported anthelmintic activity against human *Ascaris lumbricoides*. *A. officinarum* is a very valued medicinal plant and has been in use traditionally. It has been in use in cases of chronic enteritis and gastralgia, and the decoction is a folk remedy for cancer in Louisiana and Oklahoma (Duke, 2003). The rhizomes are considered aphrodisiac, aromatic, carminative, stimulant and stomachic. It is useful in dyspepsia and in preventing fermentation and flatulence. It is considered a nervine tonic (Duke, 2003). The properties are more or less similar to those of *A. galanga.* The therapeutic effects when used in traditional medicines might be mainly due to the contents of quercetin, galangin and kaempferol (Lawrence, 2005).

15.4.2 *Alpinia calcarata* (lesser galangal)

A. calcarata is also known as lesser galangal and its properties and uses are similar to those of *A. galangal* and *A. officinarum.* In the Ayurvedic medicines, *A. calcarata* has virtually taken the place of *A. galanga*, mainly due to the non-availability of the latter. The essential oil content of the plant is reported to be 0.07–0.10 % in the leaves, 0.17–0.25 % in the rhizomes, and 0.25–0.28 % in the root. The essential oil of rhizome and leaves revealed about 31 and 28 compounds, respectively. The major constituent is 1,8-cineole (Tewari *et al*., 1999).

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16

Kaffir lime leaf

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Abstract: The kaffir lime leaf, or *Citrus hystrix*, is part of the Rutaceae family. It is known by various different names, the etymology of which is discussed in this chapter. The areas and systems of cultivation, diseases and pest control, production technology and marketing of kaffir lime leaves are also introduced in this chapter. Research methods for the analysis of the chemical composition of essential oils or extracts from the kaffir lime leaf are reviewed and discussed together with their biological activities. Finally, the uses of fresh leaves and their essential oils in the food and cosmetics industries are considered.

Key words: kaffir lime leaves, food flavorings, cosmetic, therapeutic activity.

16.1 Introduction

Kaffir lime is a member of the genus *Citrus*, family Rutaceae and order Sapindales. This genus comprises about 16 species of evergreen trees and shrubs that have perfumed flowers and characteristic aromatic fruits (Bown, 2002). The scientific name of kaffir lime is *Citrus hystrix* DC. A few other common names are 'kieffer lime', 'leech lime' and 'makrut' (Thai). Several other vernacular names are given to the fruit throughout South East Asia, the region to which the plant is said to be native. These are 'jeruk purut' (Indonesia), 'juuk purut' (Bali) and 'limau purut' (Malaysia). The term 'kaffir' is believed to have originated hundreds of years ago and is found in several languages. In Arabic, it means 'infidel', coming from the word 'kafara'. It is stated in an Anglo-Indian Dictionary (Whitworth, 1885) that not only was the term applied by Muslims to unbelievers, but that it is a common term of abuse in Western India. In South Africa, it was once used by the white immigrants to refer to the native Africans. Due to these derogatory meanings, the term 'kaffir' was probably assigned to this species of citrus lime because it was considered inferior to other limes before the unique flavour of its oil in the leaves and zest was discovered. Later in 1824, the name 'mauritius papeda' was introduced to kaffir lime by De Candolle (DC), who brought the seeds from Mauritius to his botanical garden in Montpellier in southern France. Before that time, De Candolle had studied and classified *Citrus hystrix* as the first species of the papeda sub-genus (Swingle and Reece, 1967) and his description of the fruit can be found in the 'Catalogus Horti

Fig. 16.1 Sizes of kaffir lime leaves at different ages.

Botanici Monspeliensis' (De Candolle, 1813). The name Mauritius papeda is widely used in the Netherlands and Germany while in France, Italy and Spain, the lime is often called 'combava'.

The kaffir lime tree is generally small, having a height of 3–5 m and a width of 2.5–3 m. Its small white flowers are followed by small, dark green fruits approximately 4 cm wide with warty skins (Bown, 2002). Leaves of kaffir lime are unique among the citrus varieties and, for some particular applications, are more important than the fruits. These leaves are dark green and have a glossy sheen. Each leaf comes in two parts, seemingly a double leaf. The top leaflet is lightly pointed at its tip and is attached to another leaflet beneath that is broader on its upper edge (Fig. 16.1). The size of the leaves can vary quite a bit and the larger leaves are usually darker in colour.

16.2 Cultivation and production

Like other citrus plants that grow well in a warm subtropical or tropical climate, kaffir lime prefers well-drained, neutral to slightly acid soil and direct sunlight with ample moisture during the growing season (Bown, 2002). Despite its huge distribution in South East Asia, this plant grows worldwide as a backyard shrub due to its main use as cooking ingredient. A variety of well-known Southeast Asian recipes call for kaffir lime leaves as an important food flavouring because they possess a unique and strong aroma that cannot be substituted by other kinds of citrus leaves. The popularity of these Southeast Asian foods in many parts of the world has, thus, led to the increasing production of not only kaffir lime leaves but also related products such as essential oil. Kaffir lime leaf products can be fresh, frozen or dried. The leaves can be harvested all year round, especially when the trees are small. The essential oil is normally produced from fresh leaves by steam distillation and serves as a source of kaffir lime leaf flavours and essences in a large variety of internationally marketed products. The main producers of kaffir lime leaves are Thailand, Indonesia, Malaysia and India. Recently, Thai growers have developed and started

growing a kaffir lime without wrinkles that is easier to pack and ship around the world.

Although propagation by grafted varieties is the preferred choice, and these grafted trees tend to bear fruit earlier than seedlings, grafted varieties are not as available as the ripened seeds. In seed propagation, the seeds of kaffir lime tree should be buried 2.5–3.8 cm in the soil. They take about 15 days to germinate at a constant temperature of 15.5 °C. Once the trees begin to grow, the young trees need regular pruning to encourage branching and a more bushy plant. Then, it can be easily propagated using the branches. A healthy kaffir lime tree will resist attacks of insects and fungal diseases. Nonetheless, there is evidence of invasion by a range of viral diseases and a number of pests, including scale, leaf miner, bronze orange bug, spined citrus bug and fruit fly, if situated near infected or infested plants. It is probably better to graft cuttings of the kaffir lime tree onto a robust citrus, orange tree or rootstock. This will yield faster growth and improved disease resistance.

Since kaffir lime is mainly used as a food flavouring in the form of fresh leaves (either whole, ripped or thinly sliced, according to demand), clean fresh leaves are packed in sealed plastic bags and kept in cool containers prior to export to other parts of the world. Sometimes the leaves are exported in frozen form to retain their freshness. Despite having less aroma and flavour, dried leaves are often used as an alternative because they are more easily obtainable. In Europe, North America and Australia, dried kaffir lime leaves are available in most of the Asian marts. Usually, a bag of dried leaves can be stored in a sealed airtight container for a couple of years with little physical change. However, the quality of the dried kaffir lime leaf product depends mostly on the drying methods that are industrially employed. A heat pump-dehumidified dryer has been developed and is widely utilized for drying a range of different biomaterials such as holy basil leaves (Phoungchandang *et al*., 2003) white mulberry leaves (Phoungchandang, 2009), ginger (Phoungchandang *et al*., 2009) and garlic (Phoungchandang, 2009). A comparative study on drying of kaffir lime leaves (Phoungchandang *et al*., 2008) and sliced carrot (Phoungchandang and Wongwatanyoo, 2010) by heat pump-dehumidified drying and conventional tray drying revealed that the heat pump-dehumidified dryer could reduce drying time and the resulting dried kaffir lime leaves contained a higher amount of the aroma compound citronellal. Apart from optimizing industrial processing conditions in order to decrease decomposition of the impact aroma of the kaffir lime leaf product, improvement of the aroma quality of the plant material itself through a plant breeding programme should further be considered and investigated using recent advances in genetics and biotechnological research (Khan, 2007).

16.3 Chemical composition

Although the aroma of kaffir lime leaves is unique and can be distinguished from other citrus leaves, not much attention has been paid to the chemicals that are responsible for it. Few reports have focused on the analysis of volatile constituents of kaffir lime leaves, either those contained in essential oils obtained by hydrodistillation or those obtained by other volatile extraction techniques. Jabalpurwala *et al*. (2009) studied the chemical composition of kaffir lime blossom volatiles and a few

others have reported the identification of those volatiles in peel extracts along with their medicinal properties. Data presented by some unpublished sources showed that the main chemical constituents of kaffir lime leaf essential oil are citronellal, β-myrcene, limonene, terpinen-4-ol, citronellol, citronellyl acetate, geranial, geranial acetate, β-pinene and neral, all of which are aroma volatiles in the monoterpenoids group. Recently, the essential oils from the leaves of kaffir lime (*C. hystrix*) collected in New Caledonia were extracted by hydrodistillation, analysed by gas chromatography–mass spectrometry (GC–MS) and evaluated for their antimicrobial activity (Waikedre *et al.*, 2010). Thirty-eight constituents were identified, representing 89.0 % of the total constituents in the essential oil. Among these identified volatiles of kaffir lime leaf essential oil, 87.0 % were from the monoterpene group, the major components of which were terpinen-4-ol (13.0 %), followed by β-pinene (10.9 %), α-terpineol (7.6 %), 1,8-cineole (6.4 %) and citronellol (6.0 %).

In order to verify volatile constituents that are responsible for the distinct aroma of fresh kaffir lime leaves, an analysis employing headspace extraction has been performed by our research group. The headspace extraction method employed is known as SPME since it utilizes a device called 'solid-phase microextractor' to absorb headspace volatiles of the plant material of interest that are confined in a closed sample vial for a time period sufficient to allow the distribution of volatile components to the headspace above the sample (Pawliszyn, 1997). The main advantage of headspace SPME extraction is that degradation or oxidation by boiling of volatile constituents can be avoided due to the low temperature used. Thus, the extraction method allows the identification of the true plant volatiles responsible for the scent of plant parts (Wongpornchai *et al.*, 2003). Analysis of fresh mature kaffir lime leaf volatiles by GC–MS was performed after desorption of the extracted volatiles on the SPME fibre into the injection port of the GC–MS instrument (Fig. 16.2). Table 16.1 summarizes structural assignments, retention indices and relative

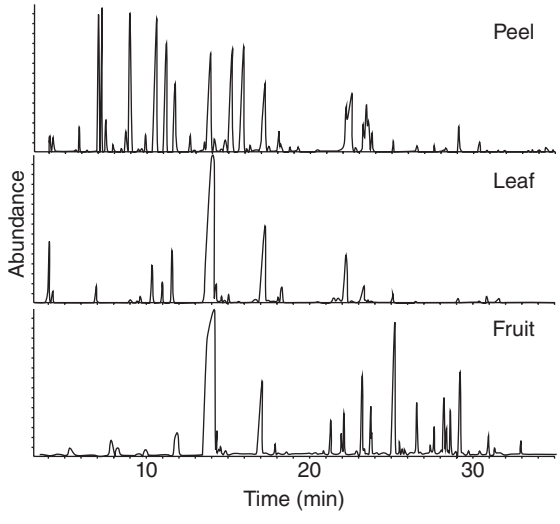

Fig. 16.2 SPME–GC–MS chromatogram of volatile constituents from various parts of leech lime at room temperature condition.

Table 16.1 Volatile components of fresh kaffir lime leaves and peel quantified by headspace SPME–GC–MS technique

No.	Component	Tr	*LTPRI*	% Relative content	
				Leaf	Peel
1	2*E*-hexenal	4.02	867	0.56	0.37
2	3*Z*-hexenol	4.07	869	2.34	0.29
3	2*E*-hexenol	4.25	876	0.26	0.37
4	Hexanol	4.31	878	0.36	0.26
5	α-Thujene	5.64	924	0.01	0.15
6	α-Pinene	5.86	932		0.55
7	Camphene	6.33	948	0.05	0.14
8	Benzaldehyde	6.78	964	0.03	0.11
9	Sabinene	7.01	972	0.58	5.38
10	β-Pinene	7.20	979	0.01	5.72
11	Myrcene	7.45	987	0.13	0.72
12	Octanal	7.91	1003		0.26
13	Degydroxy-*cis*-linalool oxide	7.99	1005		0.14
14	α-Terpinene	8.42	1016		0.17
15	*p*-Cymene	8.72	1024	0.04	0.81
16	Limonene**	8.96	1031	0.04	7.41
17	β-Phellandrene	9.01	1032	0.12	0.18
18	Satolina alcohol	9.08	1034	0.17	
19	*trans*-β-Ocimene	9.43	1043	0.10	
20	Benzene acetaldehyde	9.46	1044		0.20
21	Bergamal	9.68	1050	0.34	0.20
22	γ-Terpinene	9.91	1056	0.03	0.49
23	*trans*-Linalool oxide (furanoid)**	10.60	1074	2.20	10.90
24	*cis*-Linalool oxide (furanoid)	11.17	1090	1.22	5.96
25	Linalool*	11.72	1104	5.13	3.45
26	*trans*-Sabinene hydrate	11.79	1105		0.33
27	*trans*-*p*-Menth-2-en-1-ol	12.65	1126		0.68
28	*cis*-*p*-Menth-2-en-1-ol	13.50	1146		0.55
29	Citronellal*/**	13.84	1154	48.20	8.03
30	Iso-isopulegol	14.12	1161		0.43
31	Pinocarvone	14.21	1163		0.16
32	Neoiso-isopulegol	14.51	1170	0.86	0.21
33	Borneol	14.79	1176		0.86
34	Terpinen-4-ol**	15.19	1186	0.46	8.36
35	β-Fenchyl alcohol**	15.90	1203	0.16	9.58
36	*trans*-Piperitol	16.30	1212		0.31
37	Citronellol*	17.20	1232	14.25	5.44
38	*cis*-Citral	17.45	1238	0.43	0.36
39	Geraniol	18.06	1252	0.35	0.86
40	Piperitone	18.16	1254	0.35	0.52
41	Geranial	18.69	1266	0.06	0.27
42	Citronellyl acetate*	22.18	1347	7.78	3.24
43	α-Ylangene	23.23	1371	1.86	1.29
44	β-Maaliene	23.73	1383	0.13	1.58
45	*trans*-Caryophyllene	25.03	1413	0.61	0.45
46	*E*-β-Farnesene	26.49	1448	0.20	0.34
47	α-Humulene	26.55	1449		
48	*trans*-Cadina-1(16),4-diene	27.29	1467		0.17
49	γ-Muurolene	27.55	1473		0.36
50	Bicyclogermacrene	28.10	1486		0.17

Table 16.1 *Continued*

No.	Component	Tr	*LTPRI*	% Relative content	
				Leaf	Peel
51	α-Amorphene	28.27	1490	0.05	0.24
52	β-Selinene	28.37	1493		0.15
53	γ-Cadinene	28.87	1505		
54	δ-Cadinene	29.06	1510	0.29	1.01
55	Elemol	30.32	1541	0.11	0.40
56	*E*-nerolidol	30.78	1553	0.34	
57	*Z*-dihydro apofarnesol	31.22	1564		
58	Spathulenol	31.38	1568	0.11	
59	Caryophyllene oxide	31.51	1571	0.37	

Tr = retention time (min) in GC–MS chromatograms, LTPRI = linear temperature programme retention index.
* Major component in leaves.
** Major component in peel.

percentages of the major volatile components of fresh kaffir lime leaves compared with those of the peel. These identified volatiles were mainly monoterpenes and sesquiterpenes and their derivatives. The dominant volatiles of fresh kaffir lime leaves were citronellal (48.2 %), citronellol (14.3 %), and citronelly acetate (7.8 %), whereas *trans*-linalool oxide (furanoid) (10.9 %), followed by β-fenchyl alcohol (9.6 %), terpinen-4-ol (8.4 %), citronellal (8.0 %) and limonene (7.4 %) were the major constituents of the peel. Although most of the volatile components identified in the leaves were similar to those in the peel, their quantities were significantly different. These differences in chemical composition certainly reflect the distinct individual aroma of kaffir lime leaves and peel as well as their functional properties. The major volatiles of fresh kaffir lime leaves obtained by SPME were completely different from those found in the hydrodistilled essential oil by Waikedre and coworkers (2010). An experiment conducted a few years ago comparing the extraction efficiencies of the two essential oil extraction techniques in question – controlled steam distillation and hydrodistillation – showed that different percentage yields of essential oils, 0.43 and 0.18 %, respectively, were obtained. Additionally, oil extracted by controlled steam distillation contained a higher percentage of limonene (27.97 %) and α- and β-pinene (9.82 %) compared to that extracted by hydrodistillation (Kasuan *et al*., 2009).

Among 62 Southeast Asian edible plants, the content of α-tocopherol in leaves of kaffir lime was ranked second, with a value of 398.3 mg/kg edible portion, while the highest content was found in *Sauropus androgynus* leaves (426.8 mg/kg) and the lowest content (7.7 mg/kg) was contained in root of *Allium sativum* (Ching and Mohamed, 2001). Being one of the important ingredients in the well-known Thai seasoning, 'Tom Yum', a kaffir lime leaf sample was subjected to analysis for its β-carotene contents by high-performance liquid chromatography (HPLC) (Siripongvutikorn *et al*., 2005). The results revealed kaffir lime leaves as one of the

main sources of β-carotene in the fresh Tom-Yum formulation. Contents of β-carotene in medium size leaves were more than those found in young leaves. The contents were 173.60 ± 61.45 and 78.80 ± 34.06 μg per gram of the leaf samples, respectively. It is noteworthy that some of these experiments showed that percentages of the chemical constituents in kaffir lime leaf extracts or essential oils depend not only upon the techniques employed for their extraction but also on the growth state of the leaf source.

16.4 Main uses and functional properties

16.4.1 Uses

The main use of kaffir lime leaves is as a flavouring, especially in Southeast Asian cuisines. The leaves are precious in a wide array of Thai dishes, from soups, such as Tom Yum, and salads to curries and stir-fried dishes. They blend well with lemon grass and lime juice in Tom Yum to give the soup its wholesome lemony essence. For this kind of soup, the leaves can be added as whole or torn into smaller pieces to release more aroma. In salads or garnishes, the fresh leaves are required, and dried leaves are not a suitable substitute. The finely shredded fresh leaves are sprinkled over salads for a burst of flavour. Being rather thick, they must be cut very fine, like threads, and the thick mid-rib removed. Finely chopped fresh kaffir lime leaves are also added to some Thai curry dishes, especially those containing coconut cream such as 'Panang'. For watery simmered dishes, the leaves are bruised and added whole. Also, when a stock is made, whole fresh or dried leaves can be added to get rid of any undesirable smell and are then removed after cooking. Dried kaffir lime leaves should be green, not yellow. The flavour of kaffir lime leaves also combines well with other culinary herbs such as basil, cardamom, chillies, cilantro, cumin, curry leaves, lemon grass, galangal, ginger, mint, tamarind and turmeric. Some Asian garnished bouquets are make up with kaffir lime leaves, lemongrass and ginger as the ingredients and are used to flavour stock. Other Southeast Asian cuisines utilizing the unique flavour of keffir lime leaves are Laotian dishes such as 'sousi pa gnon', Cambodian paste or 'krueng', Indonesian dishes, especially Balinese and Javanese, such as 'savar asam' and some cuisines of Malaysia and Myanmar.

Kaffir lime leaves are also used as natural deodorizer by bruising a few leaves and adding them to an outdoor citrus-scented potpourri. The scent that lingers in the air can act as insect-repellent for people while they are eating outdoors. In Thailand, people use bruised kaffir lime leaves to rub over hands to refresh them, especially after cooking. The leaves also find their roles in some skin care products such as soaps, lotions and insect repellent roll-ons. Additionally, kaffir lime leaves are included in the ingredients of Thai herbal compresses that are normally used in traditional Thai massage.

16.4.2 Functional properties

Leaves of kaffir lime play an important role in Thai folk medicine as a digestive aid with many known health benefits. The leaves are thought to cleanse blood while helping to maintain healthy teeth and gums. They are also used for the treatment

of colds, congestion and cough. There have been a number of scientific reports showing that extracts from kaffir lime leaves possess antioxidant and cancer-preventative properties. Murakami and co-workers screened edible plants from Thailand for *in vitro* anti-tumour activities and found that methanol extracts from a number of them possessed these properties (Murakami *et al*., 1995a). Among these plants, roots of *Boesenbergia pandurata* (Zingiberaceae), *Languas galanga* (Zingiberaceae) and *Citrus hystrix* (Rutaceae) were found to contain strongly active chemicals. The plant parts used in this study were root, rhizome and leaves, respectively. A glyceroglycolipid, namely 1,2-di-*O*-α-linolenoyl-3-*O*-β-galactopyranosyl-*sn*-glycerol (DLGG) isolated from methanol extract of kaffir lime leaves, was found to inhibit the tumour-promoting activity of 12-*O*-tetradecanoylphorbol 13-acetate, a tumour promoter, in mouse skin (Murakami *et al*., 1995b). Later, the modifying effects of these Thai herb extracts on chemical-induced hepatocarcinogenesis were reported in rats (Tiwawech *et al*., 2000). This research group found that kaffir lime leaf extract significantly enhanced 2-amino-3,8-dimethylimidazo(4,5-*f*)quinoxaline-associated preneoplastic liver cell focus development. Results suggested that the extract from kaffir lime leaves may contain agents augmenting the hepatocarcinogenicity of the food mutagen, 2-amino-3,8-dimethylimidazo(4,5-*f*)quinoxaline. Thus, it is necessary to avoid consuming them with heavily cooked meats and fish that contain 2-amino-3,8-dimethylimidazo(4,5-*f*) quinoxaline.

The study of Hutadilok-Towatana and co-workers (Hutadilok-Towatana *et al*., 2006) revealed that the extract from leaves of kaffir lime exerted the strongest effect on production of the hydroxyl radical ($OH^{\bullet}$) among those obtained from seven medicinal plants, which included *Angiopteris evecta* Hoffm. (Marattiaceae), *Laurentia longiflora* (L.) Peterm. (Campanulaceae), *Nelumbo nucifera* Gaertn. (Nelumbonaceae), *Piper sarmentosum* Roxb. (Piperaceae), *Portulaca oleracea* Linn. (Portulacaceae) and *Stachytarphera indica* (L.) Vahl (Verbenaceae). Also, the kaffir lime leaf extract conferred a twice greater protection on deoxyribose from $OH^{\bullet}$ than did tannin. A study performed by Siripongvutikorn and co-workers aimed to evaluate the total phenolic content and antioxidant activity of herbs and spices used in an experimental garcinia Tom-Yum mix and a commercial one (Siripongvutikorn *et al*., 2009). The antioxidant property of kaffir lime leaves was evident from the increase in total phenolic content and antioxidant activity of the extract from Tom-Yum ingredients when dried kaffir lime leaves were added.

A recent report on antileukemic activity of ethanolic extracts from 13 Thai tropical fruits showed that only three out of 20 extracts, those of kaffir lime leaves, mangosteen peels and wampee leaves, possessed strong cytotoxic effects on K562, U937 and Molt4 cells. The IC_{50} values of kaffir lime leaf extract on those cells were 26.1, 9.0 and 11.9 μg/ml, respectively (Ampasavate *et al*., 2010). However, it has been shown by a few recent reports that antimicrobial activities of the extracts from kaffir lime leaves against *Salmonella* spp. and some other enterobacteria using disc diffusion tests were not as high as those obtained from some other herbs such as leaves of *Citrus macroptera* against *Trichophyton mentagrophytes* var. *interdigitale* (Waikedre *et al*., 2010), seeds of *Amomum krervanh* Pierre, barks of *Cinnamomum verum* J. S. Presel and flower buds of *Eugenia caryophyllus* (Sprengel) Bullock and Harrison (Nanasombat and Lohasupthawee, 2005). Both the essential oil and

ethanolic extract of kaffir lime peels showed greater antibacterial action compared to the extracts of kaffir lime leaves (Nanasombat and Lohasupthawee, 2005).

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17

Lavender

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Abstract: This chapter looks at the genus *Lavandula* (lavender), a plant for which 32 species have been described. The chapter describes the phytochemistry of the genus and the chemistry of the essential oils of different lavenders. Production methods for lavender are discussed including production of lavender oil and organic lavender oil and also lavenders grown for drying and pot pourri. The uses of lavender in the food processing, perfumery and paramedical spheres are covered, together with the functional properties, pharmacological, physiological, psychological and antimicrobial effects, and toxicity. The chapter concludes with coverage of quality issues and adulteration.

Key words: lavender, *Lavandula*, phytochemistry, essential oil, functional properties, toxicity, quality.

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17.1 Introduction

A total of 32 species of *Lavandula* have been described in the literature, plus a number of infraspecific taxa and hybrids (Upson, 2002). They are distributed from the Canary Islands, Madeira, Mediterranean Basin, North Africa, South West Asia, Arabian Peninsula, and tropical NE Africa and India. Chaytor (1937) had classified the genus into five sections: all the common commercial plants belong to two main sections: *Stoechas (Lavandula stoechas*, *L. dentata*, *L. viridis* and *L. pedunculata*) and *Spica (L. officinalis*, syn. *L. angustifolia*, *L. latifolia* and *L. lanata);* most are probably hybrids between *L. angustifolia* and spike, *L. latifolia;* there is confusion with the naming of lavenders round the world, owing to differences in their appearance under different climatic and/or husbandry conditions (Lis-Balchin, 2002a).

17.1.1 Chemical composition

Phytochemistry of the genus Lavandula

This genus *Lavandula* is relatively rich in phenolic constituents, with 19 flavones and 8 anthocyanins (Harbourne and Williams, 2002). Characteristic of the family are various glycosides of hypolaetin and scutellarein. Triterpenoids include ursolic acid (Le Men and Pourrat, 1953). Leaf flavonoids are mainly flavone glycosides and their individual distribution among the taxa shows some taxonomic significance.

In a survey of anthocyanin pigments in flowers, Saito and Harborne (1992) showed that *L. dentata* and *L. stoechas* were characterized by eight floral pigments, e.g. delphinidin and malvidin (purplish). Two hydroxycinnamic acid esters, rosmarinic acid and chlorogenic acid, are regularly present in the leaves. Coumarins and 7-methoxycoumarin (herniarin) have been detected in the volatile oil fractions.

Chemistry of the essential oils of different lavenders

The commercial hybrids, lavandins, have variable concentrations of 1,8-cineole and camphor, absent from *Lavandula angustifolia* P. Miller, which provide the harsher notes. The 'rhodinol content', consisting of citronellol, geraniol, nerol, neryl acetate and geranyl acetate, which amounts to a very small percentage of the total composition, gives a sweet, rose-like odour to the lavandin oils, with small differences between the cultivars (Lis-Balchin, 2002b). The chemical composition of *L.* 'Grosso' varies with the method of extraction: steam-distilled and CO_2-extracted samples showed differences in linalool and linalyl acetate compared to an absolute.

Lavandula latifolia Medicus, the spike oil of commerce, with a high yield, 0. 8–1.2 % has variable compositions (Lawrence, 1976–1978; 1979–1980; 1981–1987; 1988–1991; 1994–1995). More than 300 components have been identified and the main ones are linalool (19–48 %), 1,8-cineole (21–42 %) and camphor (5–17 %).

Lavandula angustifolia of commerce (Naef and Morris, 1992), whose main components are linalool (25–38 %) and linalyl acetate (25–45 %) shows some considerable differences between the subspecies *L. angustifolia* ssp. *pyrenaica* (DC), growing wild in NE Spain (Garcia-Vallejo *et al*., 1989), whose three main components were: linalool (20–66 %), borneol (6–32 %) and camphor (2–14 %), making it unacceptable as normal lavender oil.

Lavandula lanata Boisse is morphologically similar to *L. latifolia* but has a very high concentration of camphor (43–59 %) and variable amounts of lavandulol (3–27 %); *L. dentata* L. grows wild along the Mediterranean coast of Spain (Garcia-Vallejo *et al*., 1989) and has two chemotypes: 1,8-cineole/β-pinene and β-pinene/α-pinene; *L. multifida* has carvacrol and β-bisabolene. *Lavandula stoechas* L. ssp. *pedunculata* (Miller) Samp. ex Roziera (*L. pedunculata* Cavanilles) and ssp. *sampaioana* (*L. stoechas* L. ssp. *sampaioana* Roziera) had two chemotypes: camphor/fenchone and β-pinene/camphor/fenchone; *L. stoechas* L. ssp. *stoechas* has camphor and fenchone (with 1,8-cineole). Four wild populations of *Lavandula stoechas* L. ssp. *stoechas* in Crete had different percentages of α-pinene, 1,8-cineole, fenchone, camphor and myrtenyl acetate (Skoula *et al*., 1996). *Lavandula lusieri* (Rozeira) Rivas-Martinez (*L. stoechas* ssp. *luisieri* (Rozeira) Rozeira) has two chemotypes with an unidentified ester as their main component; *L. viridis* has a high concentration of 1,8-cineole, camphor and α-pinene (Garcia-Vallejo *et al*., 1989). *Lavandula pinnata* L. il. *var. pinnata* grown on Madeira (Figuereido *et al*., 1995) has a high percentage

of monoterpenes (37–80 %) and a relatively small proportion of sesquiterpenes (13–22 %). A further comparison of the composition of essential oils from leaves of different species is given by Wiesenfeld (1999) and Lis-Balchin (2002g).

17.2 Production

17.2.1 Lavender grown for oil production

Lavandula angustifolia is mainly propagated by seed, sown in spring or autumn, depending on the severity of the winters in the region (Weiss, 1997). Sowing can be directly into fields but more often is in nursery beds, where the plants remain for about a year. Clonal plants are made via cuttings. Healthy mother plants are cut down near ground level and the branches can be stored for months before preparing the cuttings of 10–15 cm with one or two branchlets. These are also planted in a nursery, usually in the spring, for a year. Green cuttings can be used but these require tender care, growth hormones and misting. The plants are planted out in rows 1.5 m apart with 0.4–0.4 m between rows; giving 10 000 plants per ha for *L. angustifolia* and about half for the hybrids (Weiss, 1997). Husbandry has now improved the lavender crops (Lis-Balchin, 2002c) and include fertilizers, often as ash (Chaisse and Blanc, 1990). The soil is loosened superficially two or three times a year to remove weeds, or else weedkillers are used.

There are many lavender pests and diseases and this reduces a possible 15–20 year life span to 3 years. Root rot due to *Armillaria mellea* is a very serious fungal disease; *Thomasiniana lavandulae* (Diptera) is the most serious insect as its larvae feed under the bark, causing damage to the tops of branches. Other diseases are due to the fungus *Rosellinia necatrix*, the Homoptera *Hyalesthes obsoletus, Cechenotettix martini, Eucarazza elegans*; Coleoptera include *Arima marginata*, *Chrysolina americana* and *Meligethes subfumatus*; Lepidoptera include *Sophronia humerella*; *Argyrotaenia pulchellana, Pyterophorus spicidactyla* and many others (Chaisse and Blanc, 1990).

Harvesting was done by hand, especially in the mountains, using a sickle, but mechanical harvesters are now fully developed, cutting 7500 kg per day compared with hand harvesters cutting 500 kg. The yield of lavender oil is 40 kg/ha and lavandin is up to 120 kg. Spike lavender yields 50 kg/ha. The harvested lavender is left in the fields for a few days then steam distilled (Denny, 2002) or extracted with CO_2 or other solvent.

17.2.2 Production of lavender oils

The recent primary sources of lavender oil include: France, Bulgaria, China and Spain. Most of the lavender plants were originally grown and distilled in the higher areas of Mediterranean France (600–1500 m). Exact figures for the production of the oil are difficult to obtain owing to the immense amount of adulteration, mixing, cutting and addition of synthetics or simply synthetic lavender oil itself. In 1984, world production of lavender oil was 200 tonnes; Bulgaria produced 100–129 tonnes; France 55, USSR 35, Australia 5. Recently US imports varied from 303 to 555 tonnes per year, peaking in 2000. US exports varied from 52 tonnes from 1996 to 2001,

peaking at 121 in 2000. More than 30 different types of lavender oils and blends are traded on world markets, but there are only a few that are sold in bulk, mainly *L. angustifolia* oil and a few lavandins.

17.2.3 Organic lavender oil

Organic essential oils, especially lavender oil, are produced in various parts of the world, including the UK, Australia and the USA; a comparative study of the essential oil quantity and quality of ten cultivars of organically grown lavender and lavandin is provided by Charles *et al*. (2002). There does not seem to be any great difference in the essential oil composition of organic compared with conventionally grown lavender, except for some percentages of enantiomers; however, the absence of pesticides would be welcomed. Farmers in the UK must comply with European Council Regulation (EEC) No. 2092/91, enforced 22 July 1991, regarding organic production and the rules governing the processing and sale of organic products. Land must be put into conversion prior to full-scale organic production and then applications must be made for status with the Soil Association, which inspects the sites. This takes around three years, and this, together with a lower yield, due to loss by natural predation, increases the cost. The premium charged, however, is often treble that of normal produce and reflects the gross over-commercialization of the produce. Organic essential oils have not been widely accepted by the main dealers for the food and cosmetics industry and the market is small, reserved mainly for aromatherapists. In France the certification is ECO-Cert, Qualité France, SOCOTEC, brought in recently to control the expanding organic market.

17.2.4 Lavenders grown for gardens, pot-pourri and drying

There are hundreds of different lavenders grown for the garden, perfumes, aromatherapy, pot-pourri and for drying on the stalk, etc. Different types require different conditions, especially regarding the temperature they are grown at throughout the year (Charlesworth, 2002). Lavenders can be grown in Australia, Europe and the USA, but require sunshine and dryness for maximum growth and perennial habit.

Very hardy lavenders are traditional lavenders: 'true lavender' (*L. angustifolia*) and lavandin (*L.* × *intermedia*). *Lavandula angustifolia* is the most popular species grown in England for oil extraction, yielding high-quality oils used for perfumes, aromatherapy, pot-pourri and drying on the stalk. Cultivars include: 'Ashdown Forest', 'Compacta', 'Folgate', 'Loddon Blue', 'Munstead', 'Nana Alba', 'Royal Purple'.

Lavendula × *intermedia* (Lavandin) is a sterile hybrid of *L. angustifolia* and *L. latifolia* (spike Lavender). Its camphoraceous oils are used in soaps, cosmetics and detergents and also for drying off the stalk and for pot-pourri, e.g. 'Vera', 'Grappenhall', 'Grosso', 'Hidcote Giant', 'Old English'. They can withstand –10 °C.

Frost-hardy lavenders all have 'ears' on top, which are sterile bracts (coma). They will survive to –5 °C and often lower. Most have a camphoraceous foliage, but no appreciable scent to the flowers. They include some of the subspecies of *L. stoechas* and the species *L. viridis* and their hybrids, e.g. *L. stoechas* subsp. *pedunculata* (Spanish lavender), also known as 'Papillon'; *L. stoechas* subsp. *stoechas* (French

lavender); 'Kew Red', 'Fathead', 'Helmsdale', 'Marshwood'. Half-hardy lavenders will thrive above 0 °C and include: *L. dentata* (fringed lavender) and *L. lanata* (woolly lavender), and one hybrid *L. lanata* with *L. dentata,* 'Goodwin Creek Gray'.

Tender lavenders need to be brought in before the first frosts and kept warm at around 5 °C. All have spiralling triple flower spikes in a trident formation but no scent, e.g. *L. buchii* varietas *buchii*, *L.* × *christiana* (a sterile hybrid of *L. canariensis* and *L. pinnata*), *L. minutolii* and *L. pinnata*.

17.3 Main uses in food processing, perfumery and paramedical spheres

17.3.1 Natural food flavours

Lavandin oil, lavender oil, spike lavender oil and lavender absolute and even concrete are used as natural food flavours. Reported uses in the food industry (Fenaroli, 1998) include: baked goods, frozen dairy, soft candy, gelatin, pudding, non-alcoholic and alcoholic beverages from 4 to 44 ppm. Lavenders are also included in tissanes or teas; booklets of Norfolk Lavender, UK, suggest many recipes for cooking with lavender at home, e.g. herring or trout stuffed with lavender sprigs, and Vickers (1991) offers further recipes for cooking and garnishing foods and use as crystallized flowers.

17.3.2 Perfumery and cosmetic uses

Lavender and lavandin oils are used in colognes, lavender-waters, *fougères*, *chypres*, *abres*, floral and non-floral perfumes. They blend well with bergamot and other citrus oils, clove, patchouli, rosemary, etc. (Wells and Lis-Balchin, 2002). Lavandin is used in cheaper products, such as soaps.

17.3.3 Paramedical uses

Lavender drops were used for fainting, and red lavender (lavender mixed with rosemary and cinnamon bark, nutmeg and sandalwood and macerated in spirit of wine for several days) was used for indigestion (Grieve, 1937). The *British Pharmacopoeia* (BPC) officially recognized red lavender 200 years ago. In the 18th century it was known as palsy drops and red hartshorn. BPC products included: Compound Lavender Tincture BPC 1949 (dose: 2–4 ml) and Lavender Spirit BPC 1934 (dose: 0.3–1.2 ml).

Paramedical uses appear in many modern books, e.g. Potter (1988), where *Lavandula angustifolia* is stated to be a carminative, spasmolytic, tonic and antidepressant. Bertram (1995) suggests numerous uses for *L. angustifolia*, which are identical to those suggested both by Culpeper (1653) and Gerard (1633), both of whom were referring to a different species! These include: nervous headache, neuralgia, rheumatism, depression, insomnia, windy colic, fainting, toothache, sprains, sinusitis, stress and migraine. The use of *Lavandula latifolia*, with its high camphoric content was recently suggested as an expectorant by Charron (1997). Aromatherapy should be defined as 'treatment with odours' (Buchbauer, 1992) but different definitions

abound. Many of the attributes of lavender oil were mistakenly taken from herbals, e.g. Culpeper (1653), who used alcoholic extracts or teas, not distilled essential oils; there was also an interest in astrology, hence every plant had an assigned planet: *Lavandula angustifolia* has Mercury and now also a 'yang' quality (Tisserand, 1985). The species referred to was also misinterpreted (see above). René-Maurice Gattefossé (1937), the so-called pioneer of modern aromatherapy, actually used perfumes or at most deterpenated essential oils and not pure natural plant essential oils. Aromatherapy involves massage using a very diluted essential oil or mixture of essential oils (1–2 %) in a carrier oil such as almond oil or addition of essential oil to the bath or a basin of hot water, or using burners (Lis-Balchin, 2002d).

17.4 Functional properties and toxicity

Lavender has antimicrobial, pharmacological, physiological and miscellaneous functions.

17.4.1 Pharmacological effects

Plant (1920) applied 'waters of lavender' to the intestine of dogs *in vivo* and reported increased activity, which was sometimes followed by relaxation and decreased peristaltic activity. Linalool was reported to relax the small intestine of the mouse (Imaseki and Kitabatake, 1962) while Shipochliev (1968) observed a spasmolytic action on rabbit and guinea pig gut by the essential oil of lavender (*L. spica* L.). Reiter and Brandt (1985) report that linalool relaxes the longitudinal muscle of guinea pig ileum. A spasmolytic activity of *L. dentata* L. oil and its components 1,8-cineole and α- and β-pinene, has been observed on rat duodenum. Izzo *et al*. (1996) showed that the essential oil of *L. angustifolia* Mill. relaxed both longitudinal and circular muscle of the guinea pig ileum. There appears therefore to be good agreement that the oils of lavender are spasmolytic on intestinal muscle but Lis-Balchin *et al*. (1996a, 1996b) and Lis-Balchin and Hart (1999) reported that, with some commercial samples, the spasmolytic action is preceded by a contraction on guinea pig ileum.

Recent experiments using three different extracts of several *Lavandula* species, including a cold methanolic extract, a tea (made with boiling water) and a hydrosol (the water remaining after steam/water distillation) showed that the methanolic extracts of *L. angustifolia* dried flowers, *L. angustifolia* fresh flowers and fresh leaves, assessed separately, *L. stoechas* leaves and *L. viridis* leaves have a spasmolytic action on the guinea pig ileum. All the teas and hydrosols, except for *L. angustifolia* dried flowers and *L. angustifolia* fresh leaves, were also spasmolytic, while the water-soluble tea extract of *L. angustifolia* dried flowers and the leaves of *L. angustifolia* showed an initial spasmogenic action (Hart and Lis-Balchin, 2002). Brandt (1988) reported the spasmolytic actions of linalool on tracheal muscle.

Action on skeletal muscle of the essential oil of *L. angustifolia* Miller and also linalool and linalyl acetate produced a reduction in the size of the contraction in response to stimulation of the phrenic nerve and also when the muscle was stimulated directly (Lis-Balchin and Hart, 1997a). Thus the action would appear to be

myogenic; however, Ghelardini *et al*. (1999) interpret their similar results as showing a local anaesthetic action; similarly, Re *et al*. (2000) conclude from experiments on mouse neuromuscular junction that linalool has a local anaesthetic action. Linalyl acetate also caused an increase in baseline or resting tone (Lis-Balchin and Hart, 1997a), while limonene caused a rise in tone, with a decrease in the size of the contractions.

Lavender oil, linalool, linalyl acetate, α and β-pinene and 1,8-cineole reduce uterine activity at concentrations that are spasmolytic on intestinal muscle (Lis-Balchin and Hart, 1997b).

Mode of action

All essential oils of different lavenders showed a post-synaptic effect on the guinea pig ileum and none possesses atropine-like activity (Lis-Balchin and Hart, 1999) or appears to stimulate adrenoceptors. Lavender oil and linalool, appear to mediate a spasmolytic action on intestinal smooth muscle via a rise in cAMP (Lis-Balchin and Hart, 1999). There is no evidence of the use of calcium channels except at very high concentrations. This is in contrast to other essential oils (Vuorela *et al*., 1997). There is no evidence for potassium channel opening. The essential oil from *L. dentata* L., and its component 1,8-cineole, has been shown to inhibit calcium-induced contraction of rat duodenum. There is recent evidence to show that the methanolic extracts of *L. angustifolia* (dry flowers, fresh flowers and fresh leaves) are calcium channel blockers, as are the leaves of *L. viridis* and *L. stoechas* (Hart and Lis-Balchin, 2002).

The fact that some extracts of *L. angustifolia* have a strong spasmogenic action (dried flowers and fresh leaves) is somewhat disturbing as so many modern herbal and aromatherapy books state that the teas are sedative and are often prescribed for upset stomachs. The results support the findings (Castle and Lis-Balchin, 2002; Lis-Balchin, 2002a,d) that the information on lavender has been mistakenly transcribed from early herbals, such as those of Culpeper (1653), where *L. spica*, a more camphoric lavender, was used medicinally and not the very floral *L. angustifolia*. The spasmolytic results shown for the water-soluble extracts of the more camphoraceous *L. stoechas* again supports the well-quoted action of the camphoraceous spike lavender over the centuries and emphasizes the confusion.

17.4.2 Physiological effect

Evidence for the sedative properties of the EO of lavender after inhalation in animals is provided by Buchbauer *et al*. (1991, 1993) as it significantly decreased the motility of 'normal' test mice as well as that of animals rendered hyperactive or 'stressed' by an intraperitoneal caffeine. The main constituents of this oil, linalool and linalyl acetate, elicited a similar effect, which was dose related. The absorption of linalool from percutaneous application of lavender oil (Jager *et al*., 1992) provided some evidence for the aromatherapeutical use of lavender. Stress and travel sickness of pigs was reduced by lavender straw, measured by concentrations of cortisol in the pigs' saliva (Bradshaw *et al*., 1998). Linalool, which has a dose-dependent, sedative effect on the central nervous system of rats (Elisabetsky *et al*., 1995a), may be caused by its inhibitory activity on glutamate binding in the cortex (Elisabetsky *et al*., 1995b). Potentiation of GABAA receptors expressed in *Xenopus*

oocytes by perfumes and phytocides, including lavender oils and lavender perfumes, (shown by benzodiazepine, barbiturates, steroids and anaesthetics, which induce an anxiolytic, anticonvulsant and sedative effect) was investigated by Aoshima and Hamamoto (1999).

Swiss mice showed sedation after lavender oil (1/60 in olive oil) was orally administered (Guillemain *et al*., 1989). Lavender inhalation showed a similar effect (Komori *et al*., 1997). The positive effects of lavender oil as treatment for insomnia was indicated in a limited study of four elderly people (Hardy *et al*., 1995). A Japanese patent application for the usage of several monoterpenes (which can be incorporated into food such as chewing gums) as brain stimulants and/or enhancers of brain activity was filed by Nakamatsu (1995). Certain central neurotropic effects of lavender essential oil were shown by Atanassova-Shopova and Roussinov (1970). A more detailed account of physiological and other effects is given by Buchbauer (2002).

17.4.3 Psychological effects

Scientific research into the psychological (often referred to as psychopharmacological) effects of lavender is limited; however, there is a long history of it being regarded, and used, as a sedative or calming agent (Kirk-Smith, 2002). The effects on cells and brain tissues also suggest both reduction in electrical activity and an anti-convulsant effect. Both laboratory and clinically based studies reveal that responses to lavender may be determined not only by these pharmacological sedative effects, but by individual, situational and expectational factors independent of the lavender odour itself.

Many fragrances have been shown to have an effect on mood and, in general, pleasant odours generate happy memories, more positive feelings and a general sense of well-being (Warren and Warrenburg, 1993). Inhalation of lavender was found to have a sedative effect on people (judging by CNV studies) (Kubota *et al*., 1992; Torii *et al*., 1988; Manley, 1993). This was in agreement with the reduced motility in mice (Buchbauer, 1992; Jager *et al*., 1992; Kovar *et al*., 1987; Ammon, 1989).

Inhalation studies in people, of rosemary oil versus lavender oil using EEG and simple maths computations, showed that lavender increased α-power, suggesting drowsiness, while rosemary instigated decreased frontal alpha and beta power, suggesting increased alertness with faster and more accurate results in the maths (Diego *et al*., 1998). These results seem to show that odour has an effect on performance *per* se, but Knasko *et al*. (1990), who lied to their subjects that odour would be given, also showed an improvement in carrying out tasks, i.e. mind over matter! Karamat *et al*. (1992), however, found that lavender had a stimulant effect on decision times in human experiments. Subjects in a group given an ambient odour of dimethyl sulphide were less happy than those in the lavender group on both odour and non-odour days (Knasko, 1992). Ambient odours of lavender and cloves given to 72 volunteers (Ludvigson and Rottman, 1989) showed that lavender adversely influenced arithmetic reasoning. Lavender (at imperceptible levels) reduced the number of errors made in the arithmetical and concentration tasks compared to jasmine (Degel and Koster, 1999) and reduced stress in flight controllers (Leshchinskaia *et al*., 1983).

Most clinical studies initiated by aromatherapists used lavender oil, and showed little, if any, benefit (Vickers, 1996; Cooke and Ernst, 2000; Lis-Balchin, 2002d). There was no significant difference shown between the use of aromatherapy (with lavender), massage and periods of rest in an intensive care unit (Dunn *et al*., 1995). Aromatherapy massage on four patients with severe dementia and disturbed behaviour proved detrimental for most (Brooker *et al*., 1997).

The main action of essential oils is probably on the primitive, unconscious, limbic system of the brain (Lis-Balchin, 1997), which is not under the control of the cerebrum or higher centres and has a great subconscious effect on the person. Mood and behaviour could be influenced by odours, and memories of past odour associations could also be dominant, an area that needs to be fully explored before aromatherapy is used by psychologically unqualified persons in the treatment of Alzheimer's or other ageing diseases. Aromatherapy can, however, be effective in reducing stress and improving moods of terminally ill patients, but only in association with touch and the time to listen to the patient, as aromatherapy, like other alternative medicines, has a placebo effect owing to the greater time spent by the therapist with the patient, the belief imparted by the therapist and the willingness of the patient to believe in the therapy (Benson and Stark, 1996).

17.4.4 Antimicrobial effects

The antimicrobial activity of lavender oil against different bacterial species of lavender is moderate, in contrast to the considerable antimicrobial status awarded to lavender by aromatherapists (Deans, 2002). Lavender was found to be most effective against *Enterococcus faecalis* out of 25 bacteria, but *Klebsiella pneumoniae* enhanced growth! The genus *Bacillus* has been shown to be susceptible to lavender volatile oil by Jeanfils *et al*. (1991) and Lis-Balchin *et al*. (1998), the latter also showing differences in activity of different lavenders against 25 bacteria. Similarly, using 20 strains of *Listeria monocytogenes*, Lis-Balchin and Deans (1997) showed a wide variation in activity of different commercial lavenders. Vokou *et al*. (1993) suppressed potato sprout growth using crude herb material. Lavender also possesses antifungal properties, e.g. against *Aspergillus niger*, *A. ochraceus* and *Fusarium culmorum*, which all reacted differently to the oils (Lis-Balchin *et al*., 1998).

17.4.5 Other properties of lavender oil or its components

A study on mast cell-mediated immediate-type allergic reactions induced by an irritant in test animals showed a dose-dependent beneficial effect of lavender oil administered either topically or intradermally (Kim and Cho, 1999). Lavender flowers had a protective effect against enzyme-dependent lipid peroxidation (Hohmann *et al*., 1999). Lipid peroxidation and lipid metabolism studies in patients with chronic bronchitis showed normalization of the level of total lipids by lavender oil (Siurin, 1997). Inhalation of lavender oil had no effect on the content of cholesterol in the blood, but reduced its content in the aorta and atherosclerotic plaques (Nikolaevskii *et al*., 1990). Linalool showed only marginal effects on lipid peroxidation of polyunsaturated fatty acids (PUFAs) (Reddy and Lokesch, 1992). Yamada *et al*. (1994) showed anticonvulsive effects of inhaling lavender oil vapour and

Elizabetsky *et al*. (1999) showed similar effects for linalool in glutamate-related seizure models.

A hypoglycaemic effect of various species of lavender was shown by Gamez *et al*. (1987a,b). Linalool leads to a hepatic peroxysomal and microsomal enzyme induction in rats (Roffey *et al*., 1990; Chadba and Madyasthe, 1984) and choleretic and cholagogic activity of Bulgarian lavender oil and a mixture of linalool and α-terpinol was found by Peana *et al*. (1994) and Gruncharov (1973). Some periodontal diseases can be treated with a mixture of EOs, including lavender (Sysoev and Lanina, 1990). Lavender oil was said to be suitable for prevention and treatment of decubitus ulcers, insect bites, athletes' foot and skin rash and can also be used for the topical treatment of acne, prevention of facial scarring and blemishes of the face and body (Hartwig 1996). The EO of lavender was used in a mixture as a hair growth stimulant and for the treatment of *Alopecia areata* (Hay *et al*., 1998) and in a pilot study to determine possible novel, safe pediculicides in children. Skin penetration enhancers, especially for the transdermal absorption of various drugs and medicaments have included lavender oil with Nifedipine (Thacharodi and Rao, 1994). Research into cell cultures of *L. vera* for rosmarinic acid production was discussed by Ilieva-Stoilova *et al*. (2002).

There are numerous miscellaneous uses for lavender flowers, both fresh and dried (Lis-Balchin, 2002d) e.g. herbal pillows, lavender bags, household cleaning products and scented candles. Spike lavender is included in some veterinary shampoos and other products as an insect, especially flea repellent (Potter, 1988). Lavender oil is used as a component in topical formulations to relieve the pain associated with rheumatic and musculo-skeletal disorders, acting as a potent radical scavenger (Billany *et al*., 1995).

Perillyl alcohol, a minor component of lavender and the most important metabolite of D-limonene, is a chemo-preventative and chemo-therapeutic agent (Reddy *et al*., 1997; Bellanger, 1998), e.g. against rat liver cancer and rodent mammary and pancreatic tumours). Pancreatic tumours were inhibited completely by geraniol at 20 g/kg diet and 50 % by perillyl alcohol at 40 g/kg diet in hamsters. Patents have been taken out for various uses of perillyl alcohol including: antibiotic and antifungal action (US Patent 5,110,832) and carcinoma regression (US Patent 5,414,019).

Contemporary patents for lavender include: wound treatment (US Patent 4,318,906); treating skin and scalp conditions (US Patent 4,855,131); minor skin irritations, promoting healing, resisting insects (US Patent 5,620,695); fly and mosquito attractant (US Patent 5,635,174) and control of dermatomycoses and dermatophytoses of skin ailments with Tinea pedis (US Patent 5,641,481).

17.4.6 Toxicity of lavender essential oils

Culpeper (1653) said that lavender (*L. vera*) 'provokes menses of women, and expels both a stillborn child and afterbirth' (the only reference to lavender as an abortifacient).

The BIBRA Working Group (1994) showed little or no irritation to human and animal skin, but it has caused sensitization, photosensitization and pigmentation. Patch tests have shown a few allergies due to photosensitization and also pigmentation (Brandao, 1986; Nakayama *et al*., 1976). Its principal effect following

administration by oral, injection or inhalation routes to rodents was sedation. Linalool was irritant to the skin of various species of laboratory animals. There was the danger of causing dermatitis in sensitive people (Rudzki *et al*., 1976), e.g. an occupational allergy to a lavender shampoo used by a hairdresser (Brandao, 1986; Menard, 1961). Facial 'pillow' dermatitis due to lavender oil allergy was described by Coulson and Khan (1999). Facial psoriasis caused by contact allergy to linalool and hydroxycitronellal in an after-shave was described by De Groot and Liem (1983). Patch testing using lavender oil at 20 % in petrolatum on patients suspected of suffering from cosmetic contact dermatitis over a nine-year period in Japan showed a dramatic increase in 1997, which coincided with the importation of the aromatherapy trend for using lavender oil and dried flowers. There is also the danger of airborne contact allergic dermatitis through overuse of essential oils and their storage (Schaller and Korting, 1995), which produced a severe response in a man who had been active with essential oils.

17.4.7 D-Limonene toxicity

Although present in small quantities in most lavenders, except *L. stoechas*, the dangers of D-limonene sensitization have become more prominent as it is used in so many industrial processes, e.g. degreasing metal before industrial painting, cleaning assemblies and as a hand cleanser. It oxidizes to *R*-(–) carvone, *cis* and *trans*-isomers of limonene oxide and hydroperoxides, all potential contact allergens (Karlberg *et al*., 1994). Two per cent of dermatitis patients gave a positive patch test to D-limonene (Karlberg and Dooms-Goossens, 1997), especially when aged (Chang *et al*., 1997). Pulmonary exposure of human volunteers to D-limonene caused a decrease in the lung vital capacity (Falk-Filipsson *et al*., 1993). The major volatile component of lactating mothers' milk in the USA contained D-limonene (von Burg, 1995), thus making it possible that the baby could develop an allergic response soon after birth. Cats and dogs, too, are very susceptible to insecticides and baths containing D-limonene.

In contrast to all the toxicity, anticarcinogenic properties of D-limonene were shown *in vitro*, when applied subcutaneously to mice that were then injected with benzopentaphene, but although the lung tumours took longer to develop and therefore the animals lived longer, it did not prevent them forming (Homburger *et al*., 1971).

17.5 Quality issues and adulteration

17.5.1 Quality specifications of essential oils of lavender and solvent extracts

Boelens (1995) reviewed the chemical and sensory evaluation of *Lavandula* oils. The true oil is almost colourless and has a sweet, floral, herbaceous, refreshing odour with a pleasant, balsamic-wood undertone and a fruity-sweet top-note.

Definition of lavender and lavandula oils

The International Organization for Standardization (ISO) defines Oil of French Lavender, ISO 3515 as 'The oil obtained by steam distillation of recently picked

Table 17.1 ISO composition of *Lavandula angustifolia* P. Miller, ISO 3515 (a), its components (b), EC Regulations (c), and sensitization values (d)

(a)	Optical rotation	–11 to –7	
	Ester min.	38 %; max. 58 % as linalyl acetate	
(b)	**Components**	Min	Max
	trans-β-ocimene	2	6
	cis-β-ocimene	4	10
	Octanone-3	–	2
	1,8-cineole	–	1.5
	Limonene	–	0.5
	Camphor	–	0.5
	Linalool	25	38
	Linalyl acetate	25	45
	Terpinen-4-ol	2	6
	Lavandulol	0.3	
	Lavandulyl acetate	2	
	α-terpineol	–	1

(c) **EC regulations 2002 (CHIP)**

Lavender oil, CAS No. 8000-28-0; EEC No. 289-995-2; Hazard symbol: Xn; Risk phase: R65; H/C 15 %; Safety phase S62

Lavandin oil, CAS No. 8022-15-9; EEC No. 294-470-6; Hazard symbol: none; Risk phase: none; H/C: none; Safety phase: none

Lavender spike oil, CAS No. 8016-78-2; EEC No. 284-290-6; Hazard symbol: none; Risk phase: R10; H/C: none; Safety phase: none

D-Limonene, CAS No. 5989-27-5; EEC No. 227-813-5; Hazard symbol: Xn N; Risk phase: R10, 38, 43, 50/53; H/C 100 %; Safety phase S24, 37, 60, 61

L-Limonene, CAS No. 5989-54-8; EEC No. 228-813-5; Hazard symbol: Xn N; Risk phase: R10, 38, 43, 50/53; H/C 100 %; Safety phase S24, 37, 60, 61

Linalyl acetate, CAS No.115-95-7; EEC No. 204-727-6; Hazard symbol: none; Risk phase: none; H/C: none; Safety phase: none

Linalool, CAS No.78-70-6; EEC No.201-134-4; Hazard symbol: none; Risk phase: none; H/C: none; Safety phase: none

Maximum levels of fragrance allergens in aromatic natural raw materials:

European Parliament and Council Directive 76/768/EEC on Cosmetic Products, 7th Amendment 2002: The presence of the substances must be indicated in the list of ingredients when its concentration exceeds 0.001 % in leave-on products and 0.01 % in rinse-off products.

(d) **Sensitisers present in lavender oils (EFFA)**

Lavender: coumarin: below 0.1 %; geraniol, 1.1; limonene, 0.6; linalool, 38; Total: 39.7

Lavender and lavandin absolute: coumarin: 6; geraniol, 0.3; limonene, 0.7; linalool, 28; Total: 35

Lavandin oil: coumarin: below 0.1 %; geraniol, 0.4; limonene, 1; linalool, 37; Total: 38.4

Spike lavender: coumarin: below 0.1 %; geraniol, below 0.1 %; limonene, 1; linalool, 46; Total: 47

lavender flowers (*Lavandula angustifolia* P. Miller) either growing wild or cultivated in France'. The established chromatographic profile includes the main identifying components (Table 17.1).

Spike lavender (*Lavandula latifolia* (L.) Medikus) has a separate ISO (4719: 1992), as does Oil of Lavandin abrialis (*Lavandula angustifolia* P. Miller × *Lavandula latifolia* (L.) Medikus), France. The latter has a requirement for a minimum linalyl acetate content of 27 %/37 % maximum and linalool 28 %/38 % with camphor

at 7 %/11 % maximum. Oil of Lavandin *grosso* (*Lavandula angustifolia* P. Miller × *Lavandula latifolia* (L.) Medikus), France also has an ISO.

Terpeneless lavender oil is produced by careful vacuum distillation; a 'topping off' of about 10 % of the oil is sufficient to make it mellower, softer and more soluble in dilute alcohol. Of course, it has increased stability and is more useful in foods.

17.5.2 Lavandin oil

This was first produced in the late 1920s, but has since escalated well above that of true lavender. Many different hybrids, growing all over the world, give a higher yield than the shorter lavender. The oil is pale yellow to almost colourless and has a strongly herbaceous odour with a distinctive top-note which is fresh camphene cineole-like (Arctander, 1960). Lavandin oil is used in large quantities for a fresh note in perfumes and in detergents.

17.5.3 Lavender and lavandin absolute and concrete

Lavandula angustifolia P. Miller (or *L. officinalis*) absolute is produced from direct extraction of the herb with solvents and thence extraction with absolute alcohol after chilling and this is then evaporated continuously under reduced vacuum; it can also be produced from the distillation water by extraction with benzene or petroleum ether and thence reextracted with alcohol.

Lavandin absolute, like the lavender absolute, is a viscous, dark green liquid of herbaceous odour, resembling the flowering plant. Both are sweeter than the essential oil and are used in similar fragrances (Wells and Lis-Balchin, 2002).

17.5.4 Adulteration of lavender oil

Adulteration of lavender oils is primarily with lavandin oils and its fractions (as it is so much cheaper, being produced in at least a ten-fold excess), but other synthetic and natural fractions occur. Adulterants include: acetylated lavandin, synthetic linalool and linalyl acetate, fractions of ho leaf oil and rosewood oil, terpinyl propionate, isobornyl acetate, terpineol, fractions of rosemary, aspic oil, lavandin, etc. (Arctander, 1960; Lis-Balchin, 2002e).

Ordinary gas chromatography can be used to detect diluting solvents; however, GC, with or without mass spectrometry (MS) or other identification facilities, such as infra-red (IR), are not sophisticated enough to find most adulterations when fractions of other oils or synthetic components are used. Synthetic adulteration with linalool and/or linalyl acetate could often be detected by the presence of dehydrolinalool, dihydrolinalool, dehydrolinalyl acetate and dihydrolinalyl acetate, but detection was perfected by the use of enantiomeric (chiral) columns), mainly composed of an α-cyclodextrin phase (Ravid *et al.*, 1992; Lis-Balchin, 2002e). Pure lavender oil had either (3*R*)-(−)-linalyl acetate or *R*-(−)-linalyl acetate. Chiral columns can also be used by those involved in adulteration of essential oils to separate out the enantiomers, then add them in the correct proportion for a given essential oil!

17.6 References

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18

Lemongrass

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Abstract: Lemongrass (*Cymbopogon*) is a tropical perennial plant which yields aromatic essential oil. This chapter reviews the classification, morphology, origin and distribution of lemongrass, before describing the chemical composition and physicochemical properties of the essential oil and the oleoresin yield. Cultivation systems and harvesting are discussed and the different methods of essential oil distillation and oleoresin extraction. The many uses of lemongrass – culinary, medicinal, cosmetic – are described and the chapter finishes with an examination of quality issues and toxicity.

Key words: lemongrass, *Cymbopogon*, essential oil, citral, aromatic.

18.1 Introduction

Lemongrass is a tropical perennial plant which yields aromatic essential oil (see Fig. 18.1). The name lemongrass is derived from the typical lemon-like odour of the essential oil. The annual world production of lemongrass oil is around 1000 tonnes from an area of 16000 ha. The crop is extensively cultivated in marginal and waste lands and also along the borders as live mulch. The major share of lemongrass oil produced in the world is either from *Cymbopogon flexuosus* or from *C. citratus*. The taxonomic position of lemongrass is as follows:

- kingdom: Plantae
- sub-kingdom: Tracheobionta
- superdivision: Spermatophyta
- division: Magnoliophyta
- class: Liliopsida
- sub-class: Commelinidae
- order: Cyperales
- family: Poaceae
- genus: *Cymbopogon*

Fig. 18.1 Lemongrass field.

18.1.1 Classification

Three species are commonly cultivated

- ***Cymbopogon flexuosus* (Nees ex Steud) Wats.**: Known as East Indian, Cochin or Malabar grass, *C. flexuosus* (2n = 20, 40) is a tufted robust perennial grass of about 2 m in height. It flowers freely. Under this species, two types, *viz*. red grass and white grass, are identified based on stem colour. East Indian lemongrass oil of commerce is popularly known as Cochin oil in world trade, since 90 % of it was shipped from Cochin Port in ancient times.
  - *C. flexuosus* var. *flexuosus*:
    C. flexuosus var. *flexuosus* is known as red grass as the stem and leaf sheath are reddish or purple. It is recognized as the true lemongrass and is commercially cultivated. The oil content of the herb is 0.3–0.5 %. The essential oil contains 75–85 % citral, is superior in quality to oil from other types, exhibits good solubility in alcohol and is hence preferred for direct use in perfumery (Guenther, 1950).
  - *C. flexuosus* var. *albescens*:
    C. flexuosus var. *albescens* is known as white grass and is characterized by its white stem colour. The plant is normally seen wild. The oil content of the herb is 0.4–0.7 %. The essential oil contains 55–70 % citral, exhibits poor alcohol solubility and is inferior in quality.
- ***Cymbopogon citratus* (DC) Stapf.**: *C. citratus* (DC) Stapf. is known as West Indian or American lemongrass (2n = 40, 60). It is a stemless perennial grass with numerous stiff tillers arising from a short rhizomatous rootstock, making large tussocks. It seldom flowers under cultivation. The oil content of the herb is 0.3–0.7 %. The essential oil contains 74–76 % citral and exhibits poor alcohol solubility.

- ***Cymbopogon pendulus* (Nees ex Steud) Wats.**: *C. pendulus* (Nees ex Steud) Wats. is known as Jammu lemongrass. It is white stemmed, dwarf and frost resistant. The oil content of the herb is 0.3–0.7 %. The essential oil contains around 75–80 % citral and exhibits medium solubility in alcohol (Joy *et al*., 2001).

18.1.2 Morphological characteristics

C. flexuosus

C. flexuosus is a densely tufted robust perennial aromatic grass of about 2 m height. The leaves are linear and lanceolate, often very coarse, 50–120 cm long and 0.25–2.0 cm wide; glumes are 0.4–0.5 cm long. The inflorescences are very large and highly branched terminal drooping panicles bearing paired spikes on tertiary branches (see Fig. 18.2). The panicles are often greyish or greyish green (rarely with a touch of purple), decompound, 60 × 30 cm in size, spreading and slightly hairy. The raceme pairs are in dense masses. The spikes are borne on tertiary branches with slender, long, flexuous and comparatively inconspicuous spathes. Spikes bear spikelets in pairs of which one is sessile and the other pedicellate. The sessile spikelet is an awned bisexual floret whereas the pedicellate is an awnless staminate floret. The lower glumes of the sessile spikelets are 4–5 mm long and 1 mm wide with one to three definite or obscure intra-carinal nerves. They are shallowly concave with one or two depressions.

C. citratus

C. citratus is a perennial grass which is less robust in nature than *C. flexuosus*. Culms are 1–2 m long. Leaf blades taper towards the sheath and are 45–90 cm long, 1–2 cm wide, narrow, linear, glaucous and drooping with scabrous margins. Ligules are truncate. Inflorescences are rarely produced and the spathes are long and narrow.

Fig. 18.2 Inflorescence of *Cymbopogon flexuosus*.

The inflorescence is compound, paniculate, 30–60 cm long and open. The inflorescence is composed of racemes, terminal or axillary, subtended by a spatheole and enclosed. Spikelets, which are linear or lanceolate, dorsally compressed, 5–6 mm long and 0.7 mm wide, are found in pairs. Fertile spikelets are sessile and awnless, whereas sterile spikelets are pedicelled. The glumes are dissimilar, exceed the apex of the florets and are firmer than fertile lemma.

C. pendulus
C. pendulus is white stemmed and dwarf in nature. The leaves are linear and lanceolate, narrow, 100 cm long, 3 cm wide and upright when young, but trailing at maturity. The colour is usually a light to medium green, is affected by environment and is an unreliable guide to identification.

18.1.3 Origin and distribution

C. flexuosus is indigenous to India. It grows wild in India from sea level to an altitude of 4200 m. It is also distributed in Guatemala and China. *C. citratus* (DC) Stapf. is believed to have originated either in Malaysia or in Sri Lanka. It is widely distributed throughout the tropics and is grown in the West Indies, Sri Lanka, Java, Guatemala, Brazil, Mexico, Congo, Tanzania, India, Thailand, Bangladesh, Madagascar, Guinea, China and the Philippines. Jammu lemongrass is indigenous to sub-Himalayan areas of North India, mostly confined to North Indian states such as Jammu and Kashmir, Sikkim, Assam, Bengal and Madhya Pradesh (Handa and Kaul, 2001).

18.2 Chemical composition

18.2.1 Essential oil

C. flexuosus oil contains 75–85 % of aldehydes, made up largely of citral. The main constituents of the oil are geranial (citral a – 51.19 %), neral (citral b – 26.21 %), geraniol (5.00 %), citronellol (0.44 %), nerol (2.20 %), limonene (2.42 %), geranyl acetate (1.95 %), linalool (1.34 %), citronellal (0.37 %), α-pinene (0.24 %), caryophyllene (0.32 %), β-thujene (0.03 %), myrcene (0.46 %), *cis*-β-ocimene (0.06 %), *trans*-β-ocimene (0.07 %), terpenolene (0.05 %), methyl heptanone (1.50 %) and α-terpineol (0.24 %) (Weiss, 1997; Ranade, 2004). The essential oil of *C. citratus* contains citral a (41.82 %), citral b (0.18 %), myrcene (12.75 %), geranyl acetate (3.00 %), methyl heptanene (2.62 %), geraniol (1.85 %), β-elemene (1.33 %), elemol (1.2 %), citronellyl acetate (0.96 %), citronellal (0.73 %), β-caryophyllene oxide (0.61 %), dipentene (0.23 %), β-cymene (0.2 %), β-caryophyllene (0.18 %), β-pinene, δ-3-catrene (0.16 %), α-pinene (0.13 %) and β-phellandrene (0.07 %) (values are approximate) (Saleem *et al*., 2003a,b). The oil of *C. citratus* is less soluble in 70 % alcohol than that of *C. flexuosus*. The lower solubility of *C. citratus* oil is due to the presence of myrcene, an oleific terpene in the foreruns; this readily polymerizes on exposure to air and light.

C. pendulus oil contains citral a (43.29 %), citral b (32.27 %), geraniol acetate (3.58 %), linalool (3.07 %), geraniol (2.6 %), elemol (2.29 %), β-caryophyllene

(2.15 %), β-caryophyllene oxide (1.56 %), methyl heptanone (1.05 %), citronellyl acetate (0.72 %), β-elemene (0.7 %), citronellal (0.49 %), *p*-cymene (0.36 %), dipentene (0.35 %), phellandrene (0.3 %), pinene (0.19 %), β-pinene (0.16 %), car-3-ene (0.04 %), myrcene (0.04 %) and camphene (0.01 %) (Shahi *et al*., 1997; Sharma *et al*., 2002). Figures 18.3 and 18.4 show the chemical structures of important constituents of lemongrass essential oil and the gas chromatogram of the oil, respectively.

The two isomers of citral, *viz*. citral a (geranial) and citral b (neral), constitute the bulk of lemongrass oil. Citral has a citrus flavour. Geraniol, linalool and citronellol are the most important acyclic terpene alcohols that can be separated from

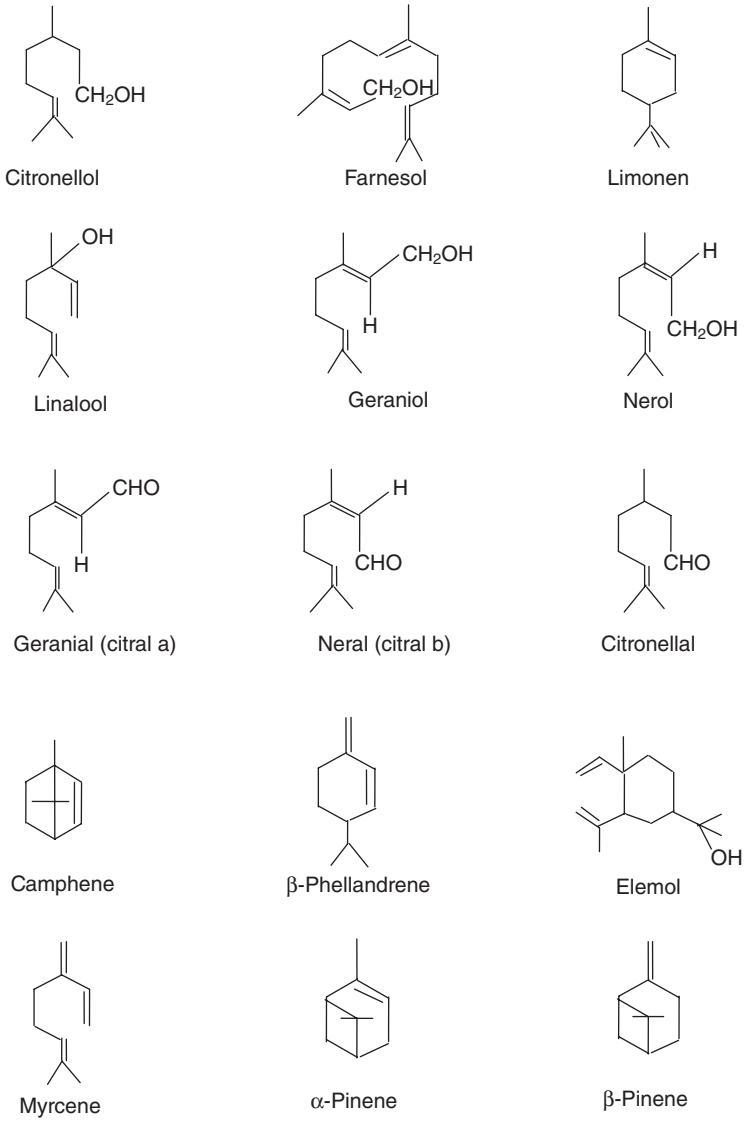

Fig. 18.3 Chemical structures of important constituents of lemongrass essential oil (Skaria *et al*., 2006).

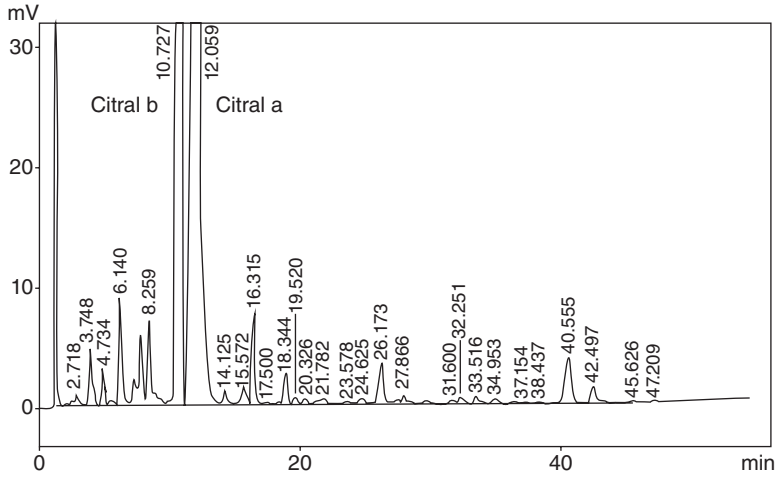

Fig. 18.4 Gas chromatogram of lemongrass oil (*Cymbopogon flexuosus*). GC conditions – column: 3.2 mm diameter, 3 m long stainless steel column filled with 5 % OV-17 on 80–100 mesh Chromosorb W (HP), oven temperature programmed to rise from 110 °C to 230 °C at 2 °C min^{-1}, injector and detector temperature: 250 °C, nitrogen flow rate: 30 ml min^{-1}, injection volume: 2 µl (Skaria *et al*. 2006).

Table 18.1 Physicochemical properties of lemongrass oils

Property	*C. flexuosus*		*C. pendulus*	*C. citratus*
	Red grass	white grass		
Specific gravity at 30 °C	0.881	0.931	0.915	0.898
Refractive index at 30 °C	1.482	1.498	1.489	1.491
Optical rotation at 30 °C	–3 to 1	–	–0.36	–0.62
Total aldehydes	80–89 %	76.4 %	75–80 %	74.96 %
Solubility in 70 % alcohol	2.8 vol.	Insoluble	–	–

Source: Joy *et al*. (2001).

lemongrass oil and used as flavour and fragrance substances. In flavour compositions, geraniol is used in small quantities to accentuate citrus notes. Nerol and citronellol are used for bouquetting citrus flavours. Pinene is an important starting material in the fragrance and flavour industry.

Physicochemical properties of oil

The physicochemical properties of *C. flexuosus* red and white types, *C. pendulus* and *C. citratus* are given in Table 18.1. Oil of lemongrass is a viscous liquid, yellow to dark yellow or dark amber in colour turning red on prolonged storage. Presence of water imparts a turbid appearance. Differentiation of lemongrass oils into West Indian and East Indian in trade has no geographical significance as oils from both the species are produced in both the areas. However, the West Indian oil has less citral and more myrcene than the East Indian oil. Although both oils have a

pronounced fresh lemony fragrance, the odour of East Indian is stronger (Kamath *et al*., 2001). East Indian is considered sweeter and more refreshing.

18.2.2 Oleoresin

Oleoresin, comprising the volatile and non-volatile components responsible for the characteristic flavour and aroma, can be separated by subjecting the herb to extraction with a suitable solvent or a mixture of solvents. The solvent residue in the product should be minimal; typically less than 25–30 ppm. The oleoresin is a concentrated wholesome product with better storage characteristics than oil.

East Indian lemongrass contains 17.3 % oleoresin in the leaves and 15.6 % in the inflorescence (Joy *et al*., 2009a). Another study showed that lemongrass leaf lamina (17.29 %) and leaf sheath (17.28 %) contain more oleoresin compared to the stem (11.33 %) and inflorescence (15.64 %). Air-dried fine powder of leaf yields maximum oleoresin (12.66 %) among four different particle sizes tested (Joy *et al*., 2009b).

Out of 406 lemongrass accessions evaluated at Aromatic and Medicinal Plants Research Station, Odakkali, Kerala, India, OD-410 was found to be the best for oleoresin yield. It contains 18.57 % oleoresin and annual yields is 2683 kg/ha. Methanol was the best solvent for oleoresin extraction among eight solvents tested (Joy *et al*., 2009b).

18.3 Production

18.3.1 Climate and soil

C. flexuosus and *C. citratus* flourish in sunny, warm, humid conditions of the tropics. Lemongrass grows well between 900 and 1250 m above mean sea level. Both species produce highest oil yield per tonne of herbage where the rainfall averages 2500–3000 mm annually (Weiss, 1997). Rain-fed areas with annual precipitation of more than 500 mm and with limited availability of irrigation water, salt-affected areas, wind and erosion prone areas, slopping lands, river/canal banks, areas prone to damage of agricultural crop by wild animals, orchards and plantations with about 30 % shade, etc. can be utilized for lemongrass cultivation. Lemongrass can perform satisfactorily even in semi-arid regions receiving low to moderate rainfall. In areas where rainfall is poor, it can be grown with supplemental irrigation. A day temperature of 25–30 °C is considered optimum for maximum oil production, with no extremely low night temperatures. Short periods above 30 °C have little effect on the plants, but severely reduce oil content.

Both species can be grown on a range of soils; it appears that good drainage is the most important factor. Sandy loam to loam soils with assured drainage and average soil fertility are considered ideal for cultivation, but lemongrass flourishes in soils ranging from rich loam to poor laterite. In sandy loam and red soils, the plant requires good manuring. Calcareous and water-logged soils are unsuitable (Farooqi and Sreeramu, 2001). Plants growing in sandy soils have higher leaf oil yield and citral content. Soils of pH 5.5–7.5 are suitable. *C. citratus* is more commonly grown on soils with higher acidity than *C. flexuosus*. In India, the highest herb and oil yields of *C. flexuosus* are obtained in soils of pH 7.5. Lemongrass will grow and

produce average herbage and oil yields on highly saline soils. In pot trials *C. flexuosus* grown in soils with electrical conductivity of 11.5,10 and 5.5 mmhos/cm showed no significant reduction in herb and oil yield and the citral content was unaffected by increasing salinity levels up to 15 mmhos/cm (Weiss, 1997).

18.3.2 Planting

For the cultivated varieties of lemongrass see Table 18.2.

C. flexuosus

C. flexuosus is generally propagated through seeds. It can also be propagated through division of clumps. Small plantations of lemongrass can be established by planting of slips. Hussain *et al*. (1988) reported that propagation through vegetative means from selected clones is better as seed propagation tends to cause considerable genetic heterogeneity resulting in deterioration of yield and oil quality. Lemongrass seeds have a dormancy of a few weeks and they lose viability in a few months. The seeds collected during the months of January–February are usually sown in the nursery during April–May. Germination is very poor if sown after October. The soil is made to fine tilth by repeated ploughing. Beds of 1–1.5 m width and convenient length are prepared. Seed is mixed with dry river sand in the ratio of 1:3 and sown in the field at the rate of 20–25 kg/ha. Alternatively, seedlings can be raised in a nursery in one-tenth of the area of the main field and transplanted after 45 days. This method requires only 3–4 kg seeds/ha and is ideal for uniform stand and better growth of the plants. The seeds are uniformly broadcasted on the beds and covered with a thin layer of soil. The seed bed is irrigated frequently. Seeds germinate in 5–7 days. The seedlings raised in nursery beds are transplanted in the field at the six to seven leaf stage. Seedlings 50–70 days old are planted during the monsoon season. A spacing of 30 cm × 30 cm with a plant density of 111 000/ha is recommended. A wider spacing of 60 cm × 45 cm for seedlings and 90 cm × 60 cm for slips has been recommended for fertile, irrigated land under North Indian conditions (Farooqi *et al*., 1999).

C. citratus

C. citratus seldom flowers under cultivation and hence is propagated through division of clumps (Anon., 1983). With the onset of rains, slips separated from existing healthy clumps are used for planting. Clumps are uprooted, top portion cut at a height of 20–25 cm and roots pruned before separation of slips. Selected slips are planted in the field at a spacing of 90 cm × 60 cm.

18.3.3 Manuring

Spent lemongrass compost at 10 t/ha and wood ash at 2 t/ha, by-products of grass distillation, are applied at the time of bed formation (Hussain *et al*., 1988). Lemongrass requires 275 kg N, 50 kg P_2O_5 and 175 kg K_2O/ha/year. Under rainfed conditions of Kerala, application of 100 kg N in three to four split doses was found to be optimum, although a response up to 200 kg was recorded (Thomas, 1989). The application of 50 kg/ha each of P_2O_5 and K_2O as a basal dose gave encouraging

Table 18.2 Released varieties of East Indian lemongrass (*Cymbopogon flexuosus*)

Name of variety	Character	Release centre	Adaptability
Sugandhi (OD-19)	Red stemmed variety with plant height 1 to 1.75 m and profuse tillering. Adapted to a wide range of soil and climatic condition. Oil yield – 100–125 kg/ha Citral – 84–86%	AMPRS, Odakkali, Kerala, India	Major lemongrass growing areas
Pragati	Dwarf with broad, erect and dark green leaves Average oil content – 0.63%t Citral – 75–82%	CIMAP, Lucknow, U.P., India	High fertile / irrigated soils. Suitable for north Indian Plains and tarai belt of subtropical and tropical climate.
Nima	Tall, high yield of bio mass (25–28 Mt/ha) with high oil yield (230–250 kg/ha.)	CIMAP, Lucknow, U.P., India	Indian Plains
Cauvery	Tall, white stemmed selection from OD-19 with an improvement in yield in terms of oil and citral content.	CIMAP, Lucknow, U.P., India	Requires high soil moisture and is evolved for river valley tracts of Indian Plains
Krishna	Medium tall, high tillering High yield of bio mass (25–28 Mt/ha) with high oil yield (230–250 kg/ha)	CIMAP, Bangalore centre, India	Indian Plains
NLG – 84	100–110 cm tall with broad leaves and dark purple sheath. Citral content – 84%	AINRP on M & AP, NDUAT, Faizabad, U.P., India	Uttar Pradesh, India

Source: NMPB (National Medicinal Plants Board) (2009), High–Yielding Varieties of some medicinal and aromatic plants with general guidelines for seed production and certification. Published by Alakananda Advertising Pvt. Ltd., New Delhi. p. 19–21.

results in West Bengal. It is recommended to apply 60:45:35 kg/ha N, P_2O_5 and K_2O basally and 60 kg N in three to four splits/annum as top dressing during the growing season as an optimum dose. Lemongrass also responds well to the application of copper, iron, calcium and sulphur. It was reported from CIMAP, Lucknow, that boron (2.5 ppm) in combination with chloride salts (chloride salinity) can be beneficial for the crop (Farooqi and Sreeramu, 2001).

In chromate overburdened soil, application of lime at 6 t/ha and fertilizer at 100 kg N, 50 kg P_2O_5 and 50 kg K_2O/ha produced higher plant height, tiller number and herb yield of *C. pendulus* (Behura *et al*., 1998). Optimum application of fertilizers increased citral content of oil (Ghosh and Chatterjee, 1991). Excess fertilizer application is undesirable as it promotes vegetative growth and oil with less citral content (Joy *et al*., 2001). Among the five manurial practices tested, NPK (100:50:50 kg/ha) was superior with an oleoresin yield of 2400 kg/ha/year (Joy *et al*., 2009a).

18.3.4 Irrigation

In case of drought, the crop should be irrigated every alternate day for about a month after planting. It is recommended that four to six irrigations are given during the period from February to June under North Indian conditions for optimum yield. Soil moisture regimes maintained at 0.80 IW: CPE (cumulative pan evaporation) ratio significantly increased crop growth, herbage and essential oil yields. Quality of the essential oil is not affected by soil moisture regimes (Singh *et al*., 1997).

18.3.5 Weed control

The first 25–30 days after planting (or harvest) is the crop–weed competition period. For a good establishment of the crop, the field should be kept weed free for the initial period of 3–4 months after planting. Generally, two to three weedings are necessary in a year. Among herbicides, diuron at 1.5 kg ai/ha and oxyfluorfen at 1.5 kg ai/ha are effective for weed control (Hussain *et al*., 1988). Duhan and Gulati (1973) and Khosla (1979) observed a significant control of dicot weeds with the application of 2-4-D (sodium salt). Spraying paraquat at 2–2.5 l/ha in 500 l of water immediately after cutting the grass is also an effective method of weed control. Application of distillation waste of aromatic crops as organic mulch at 3–5 t/ha can check weed growth.

18.3.6 Intercropping

The plant does not tolerate shade and oil yield is drastically reduced when the crop is grown under diffused light (Pareek and Gupta, 1985). Studies at the Aromatic and Medicinal Plants Research Station, Odakkali, South India, indicated poor tillering, lean and lanky growth as well as decreased oil yield when *C. flexuosus* was grown as intercrop in coconut gardens; the oil content was also found to be reduced by 20 %. In contrast, intercropping in cinnamon plantation which is regularly pruned for extraction of bark and leaf oil was found to be profitable. In new plantations of cashew, mango and coconut, lemongrass is cultivated during the initial 4–5 years of

Table 18.3 Pests of lemongrass

Pest	Stage of pest/symptom of damage	Management measures
Spittle bug: *Clovia bipunctata* Walk. (Hemiptera: Cercopidae)	Adults and nymphs – stunted growth	Not serious
Stem borer: *Chilotrea* sp. (Lepidoptera: Pyralidae)	Caterpillar – death of tillers	Spray mercaptothion 50EC @0.2 %
Nematodes: *Tylenchorhynchus vulgaris* *Rotylenchus reniformis* *Helicotylenchus* spp. *Pratylenchus* spp.	Adults and nymphs – root damage causing stunted growth	Not serious

plantation establishment. *C. citratus* is seldom intercropped or under-planted. An interesting method of integrating *C. flexuosus* into plantations of other crops was proposed for Bangladesh, but not widely implemented (Khan, 1979). *C. citratus* has been under-planted in young rubber plantations in Malaysia and elsewhere to help defray cost of plantation establishment. Pratibha and Korwar (2003) suggested lemongrass for crop diversification in semi-arid regions.

18.3.7 Plant protection

Pests and diseases of lemongrass are listed in Tables 18.3 and 18.4. Leaf spot caused by *Helminthosporium cymbopogi* is a serious disease of lemongrass. Brown tip disease causes browning and curling of affected leaves. This is a physiological disease resulting from the low water content of the grass at the end of the dry season. Symptoms of rust disease (*Puccinia nakanishikii*) of lemongrass causing elongated, stripe-like, dark brown lesions on both sides of leaf surfaces have been described by Koike and Molinar (1999).

Under rain-fed conditions, the field gives a dried appearance during the months of December–May. The dry grass and stubble of the crop is set on fire in May, prior to the onset of monsoon. This practice kills termites attacking crop stubble and also helps to rejuvenate the old clumps and ward off pests and diseases (see Figs 18.5 and 18.6).

18.3.8 Factors affecting growth, yield and quality

Oil is concentrated in the leaf blade and it increases from base to tip. Morphological characteristics like plant height, number of tillers/plant and number of leaves/plant are significantly correlated with essential oil yield. Only young and rapidly expanding leaves synthesize and accumulate essential oil, and the various biosynthetic pathways have been partially determined (Sangwain and Farooqi, 1993). A study of the pattern of formation of citral in *C. flexuosus* oil revealed that levels of the

Table 18.4 Diseases of lemongrass

Disease	Causal organism	*Management measures*
A. Blights		
Curvularia leaf blight (CLB)	*Curvularia andropogonia*	Spray Bordeaux mixture (BM) 1 % or zineb 0.3 % or mancozeb 0.2–0.3 % thrice at 15-day intervals
Leaf blight	*Rhizoctonia solani*	
Grey blight	*Pestalotiopsis magniferae*	
B. Leaf spots		
Leaf spot (eye spot)	*Helminthosporium saccharii* *H. leucostylum* *Dreschlera Victoria* *D. helmi*	Spray BM 1 % or copper oxychloride (COC) 0.2 % or zineb 0.3 % thrice at 15-day intervals
Curvularia leaf spot (CLS)	*C. andropogonia*	
Leaf spot	*C. veruciformis* *C. trifolii* *Colletotrichum graminicola* *H. cymbopogi*	
Leaf spot and clump rot	*Fusarium equisetti* *F. verticillium*	
C. Wilts and rots		
Root rot	*Botrydiplodia theobromae*	Spray BM 1 % or COC 0.2 % or zineb 0.3 % thrice at 15-day intervals
D. Rust, smut, etc.		
Smut	*Tolyposporium christensenni* *Ustilago andropogonis*	Spray BM 1 % or COC 1 % or zineb 0.3 % thrice at 15-day intervals
Leaf rust	*Puccinia nakanishikii*	Spray any contact fungicide such as mancozeb 0.3 % or zineb 0.3 %
E. Virus diseases		
Little leaf	Virus	Destruction of affected plants and control of vectors
F. Other malformations		
Little leaf (malformation of inflorescence)	*Balencia sclerotica*	Spray BM 1 % or COC 0.2 % or zineb 0.3 % thrice at 15-day intervals
Brown tip disease	Physiological disease	Proper management

constituents increased up to the reproductive phase and then declined, then increased again after the post-reproductive phase of the plant (Ghosh and Chatterjee, 1991). Maximum elimicin content as a major chemical constituent of oil has been observed at flowering stage. A significant correlation was observed between essential oil content and crop growth rate ($r = 0.6018$) as well as net assimilation rate ($r = 0.9474$). In commercial oil production, only selected plants are allowed to flower, since profuse flowering prior to cutting substantially reduces oil yield (Anon., 1983). A direct relationship between chlorophyll and odour-bearing constituents was noted (Sharma *et al*., 1988). Repeated applications of 10–100 ppm of indole acetic acid (IAA), indole butyric acid (IBA), naphthalene acetic acid (NAA) or gibberellic acid (GA3) increased oil content significantly, although herbage yield and citral content were not affected.

Fig. 18.5 Stubble burning of lemongrass.

Fig. 18.6 Lemongrass field after stubble burning.

18.4 Harvesting and processing

18.4.1 Harvest

Stage of harvest is a major factor determining oil yield and quality. Grass is harvested when individual tiller has four to five fully opened leaves. The first harvest is obtained 4–5 months after transplanting. The plants are cut about 10–20 cm above ground level. Subsequent harvests are taken at an interval of 60–90 days, depending upon the fertility of soil and seasonal factors. Citral content in lemongrass oil increases with time and reaches a maximum in 2–5 months, depending on the season. Therefore, taking early harvests of lemongrass should be discouraged (Rao *et al*., 2005). Young and tender leaves, harvested in the early season, give oil low in aldehyde content (60–70 %) and of poor solubility. Later, the aldehyde content of oil increases to 75 % and more. The yield of oil also increases. In general, the recovery of oil is lower in rainy seasons (June–August) than in the summer. Under normal conditions, two to three harvests are possible during the first year and three to four

in subsequent years, depending on the management practices followed. However, Subramanyam and Gajanana (2001) reported that three cuttings are obtained during the first year of planting and five to six cuttings per year in the subsequent years. Sunny days are preferable, since cloudy and misty conditions tend to depress leaf oil content. However, during the rainy season, harvest can begin as soon as the leaves get dry, as wet grass quickly ferments. The crop should not be allowed to produce inflorescence as this adversely affects growth and development of plants on subsequent harvests. Herbage yield of 10–15 t/ha/harvest is attainable. The average oil yield is 125–175 kg/ha/year. In second and subsequent years, 200–250 kg oil/ha may be produced with good management and use of recommended variety.

18.4.2 Seed collection

Lemongrass for seed purpose is not cut as yield of seeds from plants subjected to regular harvest is very low. Generally, the plant flowers during November–December in the plains and mature seeds are collected during January–February (see Fig. 18.7). A healthy plant produces 10–20 g of seeds. The whole inflorescence is cut and dried in the sun and seeds are collected by thrashing against the floor or beating with sticks. Seed germination is very poor in fresh seeds due to dormancy; the seeds can be sown after two months of storage. Seeds lose viability beyond six months of storage (Thomas, 1995).

18.4.3 Distillation of essential oil

Lemongrass oil is collected by steam distillation of the herbage. On cooling, the distillate separates out into a layer of oil, floating over bulk of water. To obtain good quality oil, steam distillation in stainless steel units is preferred at a steam pressure of 18–32 kg/cm^2 in the boiler. The grass is distilled either fresh or after wilting. Wilting herbage prior to distilling reduces moisture content and increases

Fig. 18.7 Lemongrass field for seed collection.

oil recovery. Drying in the sun reduces oil recovery but has little effect on oil composition.There are three types of distillation.

1. **Hydro-distillation**: In this method, the herb is packed in a vessel and partly filled with water. The vessel is heated by direct fire, steam jacket or immersed steam coil. These units work without an external boiler and the required steam is generated within the distillation still itself.
2. **Hydro-steam distillation**: The plant material is packed on a grid fitted at a height above the base of the still. The lower part of the still is filled with water to a level below the grid and heated from below. In this method, the steam is always fully saturated, wet and never superheated. The plant material is in contact with only steam and not with boiling water.
3. **Steam distillation** (see Figs 18.8 and 18.9): In this method, no water is added to the still. Instead, saturated or superheated steam generated in an external boiler is introduced into the chamber through steam coils. For the large-scale production of oil, this method is more suitable.

Lemongrass leaves can be dried and then extracted using a non-polar solvent such as hexane. An oil of softer note is obtained by this method. Distillation, being a high-temperature process, yields oil with a burnt note. Also it is devoid of fine volatile fractions. However, the solvent extraction process is more expensive than steam distillation and hence not practised commercially.

Purification

Freshly distilled oil is cloudy due to the presence of suspended dirt and moisture. Insoluble particles can be removed by filtration. For the removal of moisture, anhydrous sodium sulphate at the rate of 2 g/kg oil is added, kept overnight and then

Fig. 18.8 Boiler and distillation chamber of steam distillation unit.

Fig. 18.9 Condenser and oil separator of steam distillation unit.

filtered. If the colour of the oil changes due to ageing, contamination, etc., it can be refined by a steam rectification process.

Storage

Small quantities of oil can easily be stored in glass bottles. For large quantities, stainless steel or aluminium containers are preferred. Oil being corrosive in nature, plastic containers are unsuitable. During storage, containers should be filled up to the brim and kept in shaded or cool areas away from direct heat and sunlight.

The oil of lemongrass is chemically reactive. The terpene mixture undergoes a series of complex reactions when exposed to air and sunlight and slowly gets converted into a dark viscous resinous substance. However, if stored in aluminium or stainless steel vessels without contact with air, water and light, the oil is stable for long time.

18.4.4 Extraction of oleoresin

For extraction of oleoresin, 75 minutes boiling with methanol, 10 minutes rinsing and one washing operation is the best protocol. However, cold extraction is better for preserving fresh aroma and for flavouring purpose. A harvest interval of 30 days is better for higher oleoresin recovery. Among five sources of organic and inorganic fertilizers, NPK (100:50:50 kg/ha) has more favourable effect on oleoresin yield

producing 2400 kg/ha/year. Storage studies for six months showed that density and viscosity increased, solubility in methanol decreased and refractive index and optical rotation remained unchanged on storage (Joy *et al.*, 2009a).

18.4.5 Production of citral

Citral, the major constituent of lemon grass oil, is responsible for its characteristic lemon odour and flavour. It is separated by fractional distillation and is used for flavouring purposes. Citral is used as the starting material in the synthesis of vitamin A and a number of other industrially important products.

18.5 Main uses of lemongrass

18.5.1 Flavouring

Leaves

Lemongrass is commonly used in Asian cooking. It is used extensively in Thai cuisine in both fresh and dried forms in dishes including soup, grilled chicken and curries. It adds a citrus taste to the food. When Thai food was embraced in the USA, lemongrass became a household name. A blend of lemongrass, garlic, ginger and oil is stable in the freezer during winter. This paste can be fried until fragrant and then cooked down with a can of coconut milk for a delicious sauce for noodle, vegetable or seafood dishes. Dried lemongrass leaves are widely used as a lemon flavour ingredient in herbal teas, prepared either by decoction or infusion of two to three leaves in 250 or 500 ml of water (Wannmacher *et al.*, 1990) and other formulations. Lemongrass tea is a diuretic and imparts no biochemical changes to the body in comparison to ordinary tea. Lemongrass iced tea is prepared by steeping several stalks in a few quarts of boiling water. This can also be combined with green or black teas. A simple syrup made by steeping lemongrass in a mix of equal parts hot water and sugar can be used to enhance fruit salads or to make homemade soda.

Essential oil

Lemongrass oil is used in culinary flavouring. It is used in most of the major categories of food, including alcoholic and non-alcoholic beverages, frozen dairy desserts, candy, baked foods, gelatins and puddings, meat and meat products and fats and oils. Lemongrass oil is also used in instant beverages. Lemongrass oil has an edge over lemon since it is not acidic in nature. It also promotes digestion of fat.

Oleoresin

Lemongrass oleoresin is mainly used in flavouring foods, drinks and bakery preparations. It is ideal for flavouring tea (Joy *et al.*, 2009a).

18.5.2 Herbal medicine

Lemongrass oil is used in traditional herbal medicine as an analgesic, antidepressant, antimicrobial, antipyretic, anti-inflammatory, antioxidant, antiseptic, astringent,

carminative, deodorant, diuretic, febrifuge, fungicide, galactogogue, nervine tonic and sedative. Some of these properties have been investigated in recent studies.

- **Analgesic**: Lemongrass oil helps relieve pain in muscles and joints and is effective in toothache and headache resulting from viral infections like cough and cold, influenza, fever, pox, etc. It also helps to relieve fatigue (Lorenzetti *et al*., 1991).
- **Antidepressant**: Lemongrass is considered to boost self-esteem, confidence, hope and mental strength, uplift the spirit and fight depression (Costa *et al*., 2011).
- **Antibacterial**: Lemongrass is effective in inhibiting bacterial infections in the colon, stomach, urinary tract and respiratory system and in wounds. It helps to suppress skin diseases and body odour resulting from bacterial infections (Mickiene *et al*., 2011).
- **Antifungal**: Lemongrass oil has good fungicidal properties and may be used to cure fungal infections, both external and internal (Handique and Singh, 1990; Alam *et al*., 1994; Mehmood *et al*., 1997). Its oil is highly effective in vapour phase against *Candida albicans* (Tyagi and Malik, 2010a,b). Essential oil completely inhibited the growth of *Aspergillus flavus* at 750 ppm and also exhibited a broad fungitoxic spectrum (Singh *et al*., 2010).
- **Antipyretic**: The oil when given with tea can bring down fever. It acts by controlling infections as well as by increasing perspiration.
- **Anti-inflammatory**: Francisco *et al*. (2011) supported the use of *C. citratus* leaves extract in traditional medicine as a safe anti-inflammatory drug due to its polyphenolic compounds. According to Figueirinha (2010), a *C. citratus* infusion has anti-inflammatory properties due to its flavonoid fraction, particularly luteolin glycosides. These properties could be explored for the treatment of inflammatory diseases, in particular of the gastrointestinal tract.
- **Antioxidant**: Studies by Tiwari *et al*. (2010) revealed cytoprotective, antioxidant and anti-inflammatory properties of *C. citratus* when used as a dietary component and also in formulations against lung inflammatory diseases where oxidative stress plays an important role.
- **Astringent**: Lemongrass is thought to promote the contraction of gums, hair follicles, muscles, skin and blood vessels, thereby preventing loosening and fall of teeth and hair.
- **Carminative**: Lemongrass oil can relieve gas trouble.
- **Deodorant**: In diluted form, lemongrass oil serves as an efficient deodorant.
- **Diuretic**: Lemongrass increases urination, both in frequency and in quantity (Carbajal *et al*., 1989).
- **Nervine**: Lemongrass helps cure many nervous disorders such as shivering of hands and limbs, nervousness, vertigo, convulsions, sluggishness, lack of reflexes, etc. (Blanco *et al*., 2009). It may be of use to those suffering from Alzheimer's disease and Parkinson's disease.
- **Sedative**: This is perhaps one of the most important and most appreciated medicinal properties of lemongrass oil. It has a great soothing, sedating and calming effects on the mind, cures inflammation and itching of skin and relieves tension and anxiety. This feature can also help in cases of insomnia (Blanco *et al*., 2009).
- **Tonic**: Lemongrass tones all the systems functioning in the body, such as the respiratory system, digestive system, nervous system and excretory system and

facilitates absorption of nutrients in the body, thus providing physical strength and improving immunity.

18.5.3 Perfumes and cosmetics

Lemongrass essential oil is appreciated for its fresh, earthy and lemony fragrance. It is used as an ingredient in the manufacture of citrusy soaps and perfumes. The extract is often used in floor cleaning preparations and personal care products, like deodorant.

Lemongrass also finds use in aromatherapy. Fresh herb or oil of lemongrass can be used for a foot bath. The patient additionally benefits by inhaling the scent. It also ameliorates pain arising from rheumatism and nerve conditions. Its refreshing fragrance reduces headache and prevents drowsiness and rejuvenates mind and soul. It is also used to make aromatherapy candles. Lemongrass oil is caustic and should be mixed with a carrier oil for application on the body. It may cause irritations on the body, hence may be avoided during pregnancy. The oil can be blended with essential oils of basil, cedar wood, geranium, jasmine, lavender and tea tree.

18.5.4 Insect repellent

Lemongrass is quite popular as an insect repellent and widely used in mosquito repelling formulations. It helps to keep pets clean of fleas, ticks and lice. It is used in pet shampoos as a bug repellant.

18.5.5 By-product utilization

After extraction of oil, substantial quantities of ligno-cellulosic residues are available which often pose disposal problems. About 12–15 tonnes of residues are available from 1 ha of the crop every year (Atal and Kapur, 1982). It can be utilized as organic manure or mulch in agriculture. The residue can also be used as fuel in oil distillation and ash can be applied to crops. Spent material has been found useful in mushroom production and as raw material in the paper industry.

Atal and Kapur (1982) described the use of ligno-cellulosic waste from distillation as a raw material for mini paper mills. Pulping of such materials is easier than wood, since it requires less drastic pulping conditions. Left over ligno-cellulosic marcs (residue) responds well to pulping by chemical and semi-chemical methods and pulp recoveries are as good as any other pulpable raw material. Lemongrass marc can also be converted into strawboard and fibreboard. A small-scale strawboard unit can be set up alongside an essential oil distillation plant, thereby, utilizing ligno-cellulosic marc available after distillation.

18.6 Quality issues

18.6.1 Essential oil

The Statutory Indian Specifications for East Indian lemongrass oil are given in Table 18.5.

Table 18.5 Statutory Indian specifications of East Indian lemongrass oil

i.	Colour and appearance	Dark yellow to light brown red mobile liquid
ii.	Odour	Lemon-like
iii.	Specific gravity at 30 °C/ 30 °C	0.888–0.898
iv.	Optical rotation	+1°–3°
v.	Refractive index at 30 °C	1.4786–1.4846
vi.	Solubility in 70 % alcohol	2 to 3 vols
vii.	Citral percentage	Special grade: 80 and above A grade: 76 and above, but below 80

Indian Standard Specification for Oil of lemongrass (East Indian Oil of lemongrass) UDC 668-524-26(083-75) 540

Citral estimation

The most important quality characteristic of lemongrass oil is the citral content. This is determined by the bisulphite or the sulphite method (Guenther, 1948). In this method, in addition to citral, other aldehydes and a part of methyl heptenone are estimated. Consequently, higher results are obtained. Since error is insignificant, it is commonly used in quality evaluation of oil in trade. Citral can be precisely estimated by gas chromatography (see Section 18.2.1 and Fig. 18.3).

Organic lemon grass oil

Many countries have set up organic production standards for growing, processing, packaging and shipping of lemongrass oil. Since lemongrass production mainly uses no chemical pesticides and fertilizers, the oil is generally considered to be 'organic'. For any product to be 'organic', though, a set of procedures and regulations have to be observed which vary from country to country. Certification should be sought before labelling the product as organic. Federal Organic Legislation in the USA has defined three types of organic products based on the ingredients used in the product. They are:

(1) **100 % organic** if entire ingredients including lemongrass oil are 'certified organic'.
(2) **Organic** if 95 % ingredients including lemongrass oil are 'certified organic'.
(3) **Made with organic ingredients** if a minimum of 70 % organic ingredients are used.

18.6.2 Oleoresin

The quality of oleoresin is generally evaluated based on the 'bite' imparted by the resin portion containing combinations of alkaloids, gums, pigments, etc. and 'aroma' imparted by the volatile or essential oil component. Consistency of flavour, colour, viscosity, pourability, dispersibility, blending, etc. are the important parameters for its use as a flavour ingredient.

18.6.3 Common adulterants

Adulteration is the deliberate addition of cheaper alternative oils, oil fractions, by-products, isolates, synthetics, etc. to pure lemongrass oil. Lemongrass oils should

be 100 % pure and wholly extracted from the specified *Cymbopogon* species. Several contaminants are observed in marketed oil. These rarely occur due to deliberate addition, but are probably due to defective production and processing practices. Adulteration with synthetic citral and other synthetic odourants may change the beneficial properties of the oil and may also impart toxicity and/or irritancy to the products. The presence of adulterants can be easily detected by poor or uncharacteristic odour profile and variation in physicochemical characteristics. Advanced analytical techniques such as GC–MS, GC–FTIR, GC–13C NMR, HPLC–MS, etc. can also be used. Techniques requiring less capital investment such as thin-layer chromatography (TLC) will be more practical and appropriate.

Lemongrass oil is reported to be occasionally adulterated with litsea. Jammu lemongrass oil also is used as an adulterant. Adulteration with petroleum and coconut oil can be recognized by the incomplete solubility of the oil. Presence of moisture is also observed if the oil does not separate properly after distillation. Lemongrass oil adulterated with citronella oil will have remarkably low citral content. Adulteration with acetone can be easily detected by a low specific gravity although the citral content of the oil is normal since, on estimation of the citral, acetone combines with bisulphite and increases the citral value. Traces of residual organic solvents are found as a result of extraction practices. The presence of pesticides and heavy metals can also occur as a result of poor agricultural and improper storage practices.

18.6.4 Toxicity

Lemongrass oil has no adverse effects on the blood, liver function, kidney function, protein, carbohydrate and lipid metabolism of rats. Studies have failed to detect mutagenic or toxicological reactions in humans (Leung and Foster, 1996).

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19

Lovage

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Abstract: Lovage (*Levisticum officinale*) is a perennial spice crop which belongs to the Apiaceae (Umbelliferae) family and the plant order Apiales. This chapter deals with the origin, botanical characteristics and chemical composition of lovage, as well as cultivation and production of the plant. Biotechnological approaches for the production of the plant essential oil compounds have been reviewed, such as falcarinol, (*Z*)-ligustilide, (*Z*)-3-butylidenephthalide, *trans*-β-farnesene, β-phellandrene, *n*-octanal, γ-elemene and palmitic acid. The application of the plant in food is also discussed, including the description of some European recipes for dishes where lovage appears as an important ingredient. Finally, health benefits of the plant have also been discussed.

Key words: lovage, *Levisticum officinale*, Apiaceae, ligustilide, phthalide, essential oil, food.

19.1 Introduction

Lovage (*Levisticum officinale* W.D.J. Koch) has been grown for its aromatic fragrances, ornamental aspects and medicinal properties for a long time and its use can be traced back to ancient Rome. The plant was called by Diosecorides, 'libysticon' or 'lygisticon'. Many authors considered its name to be derived from the Latin word 'levare' (lighten) (Hornok, 1992). According to Stuart (1989), the plant name is derived from lovage's reputation in many European countries as a love charm or aphrodisiac.

Lovage is known as *Celeri perpetuel* in French; *Badekraut* in German; *Levistico* in Italian; *Ligustico* in Spanish; *Levistiko* in Greek; *Goritsvet* in Russian; *Selam out* in Turkish; *Robejji* in Japan; and *Anjedan e roomi* in Iran.

In a twelfth-century manuscript attributed to Roger of Salerno, there is an early description of the use of a soporific mixture used to induce relief of pain in a patient about to undergo surgery. This medication was composed of the bark of mandragora, hyoscyamus and lovage seed, which were mixed together, ground and then applied wet to the forehead of the patient (Corner, 1937). This herb was plentiful in monastery gardens during the Middle Ages. Hildegard used it for soothing coughs and against lung and chest complaints. It was also thought that lovage increased the

urine flow and expelled gas and so was used for kidney and intestinal complaints (Holtom and Hylton, 1979).

19.1.1 Origin

Lovage is originally native to Southwest Asia (Hazaran Mountain; Kerman province; Iran at an altitude of 2500–3400 m) and southern Europe, but it is naturalized in many temperate regions and has for a long time been cultivated elsewhere (Tutin, 1968; Rechinger, 1987; Mozaffarian, 1996). It thrives on sunny mountain slopes (Chevallier, 1996).

19.1.2 Botanical characteristics

Lovage (*Levisticum officinale* W.D.J. Koch) is a hardy perennial dicotyledon plant belonging to the family Apiaceae (Umbelliferae) and the order Apiales. The plant has been alternatively classified as *Ligusticum levisticum* L., *Levisticum persicum* Freyn & Bornm., *Hipposelinum levisticum* Britt. and *Angelica levisticum* Baillon (Simon *et al*., 1984; Rechinger, 1987). The name of the genus *Ligusticum* is said to be derived from Liguria in Italy, where it once grew in abundance. The plant is diploid, 2n = 22, robust, glabrous, perennial with a clump-forming habit and reaching 1 m spread. The stems are stout, furrowed, striate and tubular, branching and reaches a height of 2–2.5 m. The leaves are alternate, 0.5–0.6 m long, dark green, shining, toothed, petiolate with stipules, radical, hairless, 2–3 pinnate, roughly triangular in outline and rhombic.

The petiole is hollow and inflated near the base. The grey–brown rhizome is vertical, penetrates the soil up to 0.4–0.5 m in depth and terminates in a taproot, which is ringed cross-wise. The roots have a thick yellowish white bark separated from a brownish yellow radiate wood by a dark line. Essential oil-bearing structures are visible in the outer regions of the transverse section. The inflorescence is flat, compound umble with 5–15 axes and 5.0–7.5 cm wide. The bracts are numerous, linear lanceolate, long acute and deflexed with a scarious margin. The greenish yellow flowers are small, hermaphrodite and produced in large numbers. The fruit is flat, 5–7 mm, broadly elliptical and yellowish brown winged twin achene. The seeds are fertile with an average germination capacity of 68 %. The weight of 1000 seeds is 3.7 g (Tutin, 1968; Rechinger, 1987; Jia *et al.*, 1989; Hornok, 1992; Evans, 2002).

19.1.3 Trade and commerce

Lovage is known as a small spice crop, and it is difficult to obtain accurate or reliable figures for it. Information about the commercial production of essential oil from lovage was not available in the surveyed literature, but the leaf of lovage as a condiment is sometimes produced in large commercial quantities. According to Lawrence (1985), the world production of lovage root and seed oil in 1984 was 500 kg and 300 kg, respectively. In 1993, the estimated annual world value of lovage essential oil was approximately £800 000 (Hogg, 2001). Lawrence (1993) noted lovage herb as being one of the main essential oils to be in short supply in the world market. The worldwide production of root, herb and seed essential oils of the plant is 2000, 1500 and 900 kg, respectively. In 2005, 15 ml, 100 ml and 1 kg of lovage oil

was priced at $30, $140 and $900, respectively (www.rangeproducts.com.au). The most important producers of lovage are Germany, Hungary, the Netherlands, Poland, Belgium, Finland and the USA.

19.2 Chemical composition

All parts of the plant contain essential oil. The herb oil (*Levistici herba*) is colourless or very pale yellow and extremely diffusive. Lovage root oil (*Levistici radix*) is an amber to olive-brown coloured liquid with root-like odour, suggestive of celery, angelica, liquorice extract, oleoresin and oak moss. The yield and its chemical composition differ significantly depending on the individual genetic and geographical variability, plant age, different plant parts and developmental stages, as well as any post-harvest treatments (Mirjalili *et al*., 2010). The presence and concentration of certain chemical constituents also fluctuates according to the season, climatic condition and the origin of the plant. Extraction methods (i.e. hydro- and steam distillation, supercritical CO_2, solvent extraction, etc.), solvent composition and sample preparation affect the chemical profile of extracts (Cu *et al*., 1990; Daukšas *et al*., 1999; Bylaite *et al*., 2000; Daukšas *et al*., 2002; Menaker *et al*., 2004).

The essential oil content (w/w%) in different plant parts is 0.05–1.0 % in the rhizome and roots, 0.1–0.4 % in the leafy stem bearing green seed, 0.08–0.2 % in the leaves and 0.8–2.7 % in the ripe seeds. Essential oil composition of lovage has been studied extensively, and more than 190 compounds were reported in its root, seed and leaf oil (Naves, 1943; De Pooter *et al*., 1985; Toulemonde and Noleau, 1988; Cu *et al*., 1990; Szebeni-Galambosi *et al*., 1992; Venskutonis, 1995; Bylaite *et al*., 1998; Daukšas *et al*., 1998; Mirjalili *et al*., 2010). The chemical compositions of essential oils distilled from separate botanical parts of this plant are rather different (Bylaite *et al*., 1998; Novak and Nemeth, 2002; Dyduch *et al*., 2003). Volatile oil is composed of phthalides (butylidene-, dihydrobutyliden-, butyl- and propylidenephthalide; sedanonic anhydride; *cis*- and *trans*-ligustilide; senkyunolide; isosenkyunolide, validene-4,5-dihydrophthalide) with lesser amounts of terpenoids (α- and β-pinenes, α- and β-phellandrenes, γ-terpinene, carvacrol, eugenol and l-α-terpineol) and volatile acids (butyric acid, iso-valeric acid, maleic acid and angelic acid) (Gijbels *et al*., 1981, 1982; Toulemonde *et al*., 1987; Cu *et al*., 1990; Ibrahim, 1999; Hogg, 2001; Hogg *et al*., 2001).

The most important compounds of essential oils from lovage are phthalides, which constitute more than 70 % of the total volatile oil from roots, 25 % from the leaves, 14.5 % from the stems and about 6 % from the seeds (Daukšas *et al*., 1998). The chemical structures of major phthalides are shown in Fig. 19.1. It was found that in the flowers and seeds β-phellandrene (40.8 % and 61.5 %, respectively) were main constituents, while α-terpinyl acetate (≈ 70 %) was reported as the principal constituent of the leaves and stems oils (Bylaite *et al*., 1998). The oil of lovage fruits was reported to contain β-phellandrene (69.3 %), α-terpinyl acetate (4.2 %) and α-terpineol (2.1 %) as the major components (Dyduch *et al*., 2003). Samiee *et al*. (2006) reported α-terpinyl acetate (40.5 %) and β-phellandrene (16.7 %) as the main constituents in essential oil and β-phellandrene (23.0 %), naphthalene (20.6 %) and γ-terpinene (12.1 %) as the major components in methanol extract of the plant aerial parts. The major volatile oil components of lovage parts are shown in Table 19.1.

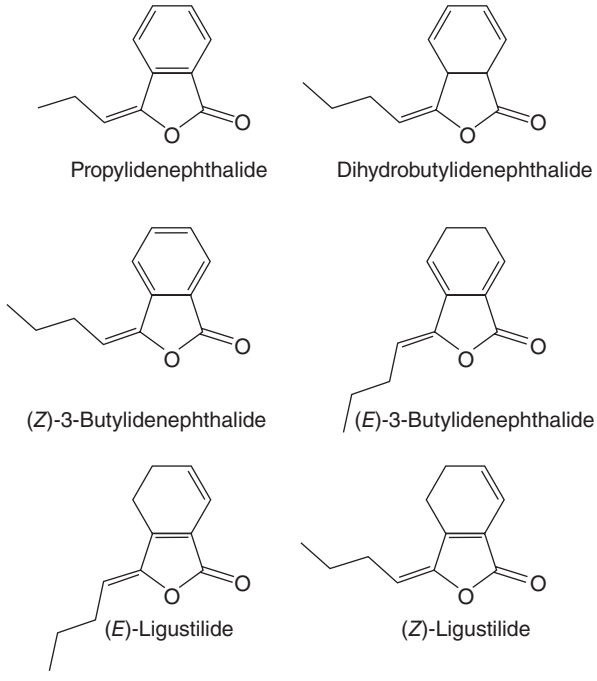

Fig. 19.1 Chemical structures of major phthalides in the essential oil of lovage.

Table 19.1 The major volatile oil constituents of lovage parts

Constituent	Retention indices	Leaves (%)	Stems (%)	Flowers/seeds (%)	Roots (%)
α-Pinene	928	0.4–0.8	1.0–1.2	2.9–5.3	2.0–12.7
β-Pinene	967	1.0–1.7	0.2–0.8	2.9–17.7	2.5–6.6
Myrcene	981	1.6–4.4	1.2–3.4	2.2–7.1	0.3–0.8
α-Phellandrene	994	0.4–1.2	0.1–1.2	1.0–2.9	0.2–0.5
β-Phellandrene	1019	13.4–26.5	10.8–28.5	11.7–63.1	1.7–15.5
Pentylcyclohexadiene	1125	0.3–0.9	0.2–0.5	0.2–0.4	7.4–29.3
α-Terpinyl acetate	1338	49.7–70.0	48.2–68.9	4.5–16.2	0.1–0.2
(*Z*)-Ligustilide	1697	4.4–11.7	4.8–13.8	5.6–16.0	37.0–67.5

Source: Hogg (2001).

In the study by Stahl-Biskup and Wichtmann (1991), the essential oil composition of lovage root between seedlings and adult plants was compared. In essential oil from adult plants, *Z*-ligustilide and the biosynthetically related pentylcyclohexa-1,3-diene form more than 50 % of the oil, while germacrene-B and β-phellandrene are the minor components. These findings revealed that the production of pentylcyclohexadiene and phthalides begins at about 11 and 18 weeks after germination, respectively, and after 20 weeks of germination, the amount of *Z*-ligustilide reaches about 30 % of essential oil.

Seasoning-like flavour substances of the commercial lovage extract were studied by Blank and Schieberle (1993). Aroma extract dilution analysis resulted in six

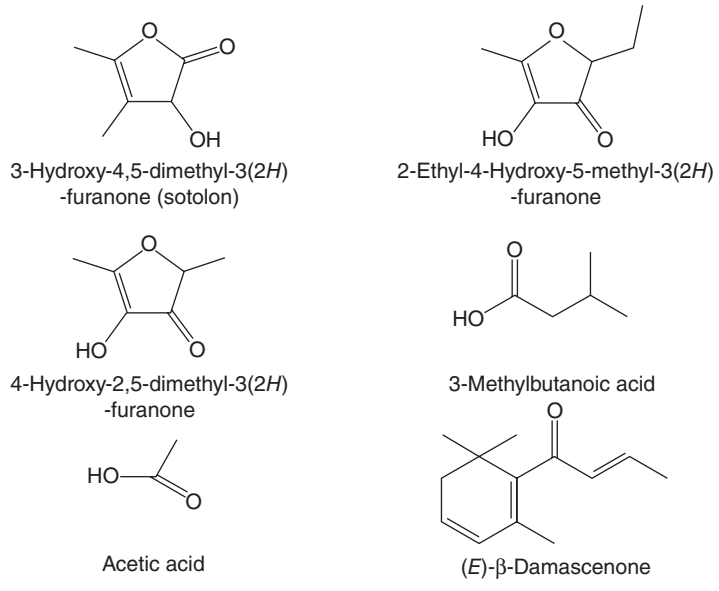

Fig. 19.2 Chemical structures of seasoning-like substances of lovage.

odorants having high sensory relevance. They were identified as 3-hydroxy-4,5-dimethyl-3(2*H*)-furanon (sotolon) with seasoning-like odor, (*E*)-β-damascenone with honey-like odor, 2-ethyl-4-hydroxy-5-methyl-3-(2*H*)-furanone (homofuraneol) and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanon with caramel-like odor, 3-methylbutanoic acid with rancid odor and acetic acid with pungent odor (Fig. 19.2). Sotolon was reported as the key aroma compound of the acidic fraction of lovage extract due to its characteristic seasoning-like flavor and high flavor dilution factor.

Lovage, like the other plants of the Apiaceae family, contains furocoumarins (Fig. 19.3) (Nielsen, 1970; Murray *et al*., 1982). Some furocoumarins, such as psoralen, 5-methoxypsoralen (5-MOP) and 8-methoxypsoralen (8-MOP), are potent photosensitizers when activated by near-UV light (300–380 nm). They intercalate readily into DNA and form light-induced mono- or di-adducts with pyrimidine bases. Thus, they are phototoxic, mutagenic and photocarcinogenic. Severe dermatitis can result after contact with furocoumarin-containing plants in the presence of sunlight (Pathak, 1974). The fruits of lovage contain imperatorin as a major compound and small amounts of 5-MOP, 8-MOP and psoralen (Naves, 1943; Dauksha and Denisova, 1969; Ceska *et al*., 1987). Psoralen was identified with 5-MOP by Karlsen *et al*. (1968) as being present in the lovage root. Other cumarines such as umbelliferone and apterin were also isolated and characterized from the lovage (Karlsen *et al*., 1968; Fischer and Svendsen, 1976) (Fig. 19.3). Quercetin as a flavonol was reported by Wojdylo *et al*. (2007) in lovage (923 mg/100 g). Recently, some polyacetylenes such as 3*R*,8*S*-falcarindiol, 3*R*-falcarinol and 3*R*,8*R*-dehydrofalcarindiol have also been identified from the dichloromethane extract of the roots of lovage (Schinkovitz *et al*., 2008) (Fig. 19.4). Najda *et al*. (2003) determined the phenolic acids and tannins content of different anatomical parts of the plant (Table 19.2). Total phenolic acid content of different parts of the plant has been reported as: roots

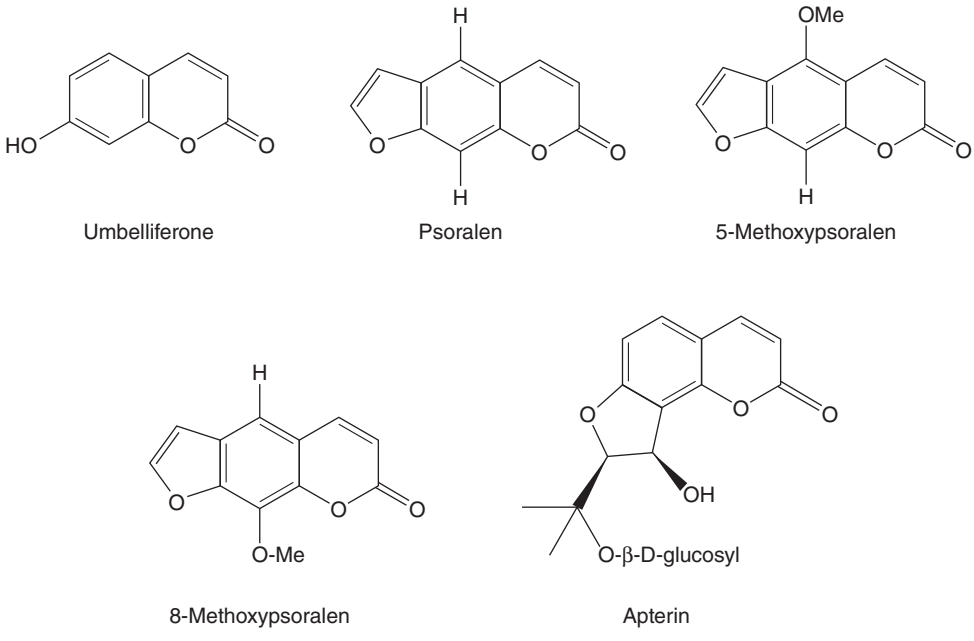

Fig. 19.3 Chemical structures of some coumarins isolated from lovage.

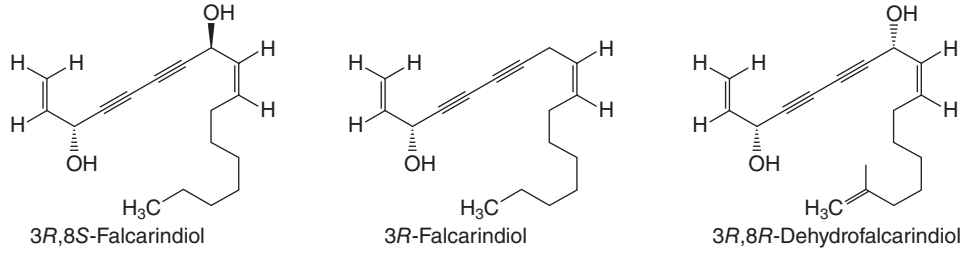

Fig. 19.4 Chemical structure of polyacetylenes from lovage.

Table 19.2 Water content, tannins and total phenolic acids in different anatomical parts of lovage

Anatomical part	Water content (%)	Tannins (%)	Total free phenolic acids (mg/100 g dry mass)			
			Chlorogenic	Caffeic	*p*-Coumaric	*m*-Coumaric
Roots	7.0	6.6	0.123	0.264	0.044	0.052
Herbs	8.6	5.3	1.362	2.121	0.063	0.098
Stalks	9.3	7.4	0.645	0.148	0.032	0.048
Blades	6.0	2.7	2.012	2.657	0.110	0.123
Fruits	9.4	1.8	2.123	3.067	0.758	0.214

Source: Najda *et al.* (2003).

(0.12–0.16 %), herb (0.88–1.03 %), stems (0.30–0.39 %), leaf (1.11–1.23 %) and fruits (1.32–1.41 %) and for tannins as: roots (6.6 %), herb (5.3 %), stems (7.4 %) leaves (2.7 %) and fruits (1.8 %). The total phenolic content of ethanol extracts of lovage leaves and their stability throughout the storage period have been recently evaluated by Materska (2010). Chlorogenic acid content of the plant (5.3 mg g^{-1} fresh weight) showed the highest stability throughout the storage period. Lovage also contains β-sitosterol (Nielsen and Kofod, 1963).

19.3 Cultivation and production

19.3.1 Ecological requirements

Lovage as a hardy perennial plant can be cultivated in any temperate climate since it is able to survive harsh winters. It has been reported that the plant could survive a temperature of –35 °C during the winter with no damage (Szebeni-Galambosi *et al*., 1992). The preferred temperature range is between 6 and 18 °C with annual precipitation of 500–1500 mm. Although lovage is not sensitive to low temperatures, high quality in roots yield and oil can be obtained in warm regions. In very hot locations, some shade is necessary. The root system is in a relatively thin soil layer (0.4–0.5 m), and water-absorbing roots do not penetrate the soil deeply. Water demand in lovage is high because of the large surface area of foliage which leads to high evaporation and transpiration; therefore supplemental irrigation is necessary in arid regions (Omidbaigi, 2000). Recently, lovage has been adapted to semi-arid conditions for commercial production (Evin, Tehran, Iran, 35° 48′ N, 51° 23′ E and 1785 m altitude with an averages temperature of 15 °C and 244.6 mm annual precipitation).

19.3.2 Soil and fertilization

Lovage grows well in many types of soils except heavy clay. Deep and well-drained soils with full sun are ideal conditions for this plant; however, it can grow in partial shade. Lovage prefers a well-drained deep sandy loam soil, rich in nutrients and humus with a pH range of 5.0–7.8. Soils originating from swamp are especially suitable for cultivation and harvesting, and rooting is easy in these types of soils. For cultivation, the field is prepared for autumn sowing with 30–50 cm deep ploughing in August. For sowing, it is necessary to prepare the soil so as obtain a fine structure and a well-compacted seedbed. Direct application of organic manure is not recommended for lovage. However, if lovage is used as part of an intercopping system or as a follow-on crop then manuring of the alternative crop is beneficial. In the autumn, prior to planting, 60–70 kg/ha of N, 100–120 kg/ha of P_2O_5 and 140–150 kg/ha of K_2O active material should be introduced into the soil (Hornok, 1992). Lovage is the same as other Apiaceae family plants such as angelica and fennel in that it extracts a large amount of nutrients from the soil; therefore, a sufficient supply of nutrients is also necessary during later years.

The response of lovage to N-fertilization is quite strong. According to Galambosi and Szebeni-Galambosi (1992), increasing the N level significantly affects the vegetative growth and root yield of lovage plants. Fresh and dry yield of both aerials

and roots was doubled by the application of 120 kg/ha of N fertilization. Heavy mulching with hay or straw is recommended to conserve moisture. It also encourages earthworms to digest the mulch and increases calcium availability.

19.3.3 Propagation

Lovage can be propagated by direct seeding, dividing roots or transplanting the seedlings. Seeds retain their viability for 2 years. The best sowing date in the case of direct seeding is late autumn (November). It is mentioned that seed germination capacity increases during winter (Omidbaigi, 2000). As a frequent result of late sowing, the rosettes would not develop during the winter and consequently the plants would not develop their generative organs even in the second year. The initial development of germinated plants is slow and only rosettes are formed in the first year. Lovage is generally sown in a row spacing of 0.5–0.7 m by application of 10–12 kg/ha of seeds (70–80 seeds/m). The sowing depth should not be over 20 mm, because of uneven sprouting which usually happens in deeper sowings.

For transplant production, 1.0–1.5 kg/ha of seeds is required to produce 42 000–55 000 transplants at a distance of 20–25 cm between rows (Hornok, 1992). The best seed sowing time for this purpose is in mid-March and transplants will be ready in early autumn. Transplants are so susceptible to freeze injury that they should be transplanted to the field before early autumn freezing. Root division is another method of propagation which is rarely used. Each divided root should have at least one healthy vegetative bud to be planted. Root division is preferably made in September, as is usually the case with other spreading rooted plants. According to Kolodziej and Najda (2007), method of plantation establishment (seedlings and direct sowing) significantly affects plant height, number of leaves or lateral shoots per plant, yields of herbs and roots (per plant and per area unit), essential oil content and yield. Transplants production had positive effect on plant size and raw material yield from the area unit. However, an increase of essential oil content in raw material obtained with direct sowing was noted. In contrast, essential oil yield was higher on plots with seedlings transplanting.

19.3.4 Pests and diseases

The leaf miner (*Liriomyza* sp.) is the first threat to the wellbeing of a lovage plant. These pests are tiny black flies, 0.1 in long, with yellow stripes. Their larvae develop from eggs laid on the underside of the leaves. In the spring, the larvae tunnel inside the leaves and stems, damaging tissues and spreading rot diseases. The meandering white or translucent trails they blaze through foliage are symptoms of their presence in the leaves. The larvae eventually drop to the ground and pupate in their cocoons, emerging later as adults (Ganter, 1997; Stuart and Trumble, 2002). Cleanliness is the best defence against this pest. Remove and destroy infested leaves. Shallow cultivation of the earth in autumn helps by exposing the pupae to cold. Agricultural fleece (row covers) may protect small plants from egg-laying flies, but this is not a permanent solution. Handpicking of the chalky white, dry eggs is effective if it is done systematically, once a week for a month, followed up by a spray of light horticultural oil, which will suffocate any menacing remnant. Sometimes lovage seed

heads attract aphids, but this problem is succinctly solved by gently bending the heads into a basin of soapy water and swishing them around to dislodge the insects. Naturally, this should be done before the seeds are fully ripe (Ganter, 1997).

Against the plant louse, some pesticides such as Pirimor® (pirimicarb), Wofatox (methyl parathion) and Phosdrin® (mevinphos) may be used. Lovage is frequently damaged by a fungus disease such as peronospora (*Plasmopora nivea*), powdery mildew (*Erysiphe polygoni*) and septoria (*Septoria apiicola*). According to Hornok (1992), the best protection is provided with a 0.1–0.2 % benomil solution by spraying every 10–12 days until mid-September. Powdery mildew can also be effectively controlled by spraying the plant with wettable sulphur at the initial stage of infection. Recently, *Puccinia bornmuelleri* has been reported as causing rust disease of lovage from Poland (Wolczanska and Wojciak, 2010).

19.3.5 Weed control

Weed control is important in successful lovage production. Early weed control is especially critical. Cultivation is an effective control method for weeds in lovage, especially young plants. Weed control is usually performed by cultivating between the rows. Mechanical cultivation can be replaced by the application of herbicides. Chemical weed control in the autumnal sowing can be accomplished sufficiently by the application of Maloran (chlorbromurion) before sowing (2.5–3.0 kg/ha). In the spring, Merkanzin (prometrin) can be used before sowing in amounts of 4–5 kg/ha. Maloran is also used at 8–10 kg/ha on lovage plantations in their second or later years, before sprouting in the early spring (Hornok, 1992).

19.3.6 Harvesting and handling

Lovage can survive for 6–8 years; however, in practice it is only maintained in production for 3–4 years because later than that the stem and leaf development diminishes and roots become hollowed and rotten (Hornok, 1992). The plant has a rosette form in the first year. The stem emerges in the second and later years. Cutting leaves from the base of 1 year-old plants in the autumn, and just before the frosts, strengthens the roots.

According to Szebeni-Galambosi *et al*. (1992), the fresh leaf yield depends on the dryness of the summer and pest damage, but it could be 0.5 and 3.9 kg/m^2 for the first and second year, respectively. The aerial parts of lovage (leaves and stems) can be harvested a few times per season, especially in the second and later years. It is also reported that the highest yield of total fresh leaf is obtained during flower stalk emergence. The plant height and fresh leaf yield can be varied with an increased number of harvests. According to Galambosi and Szebeni-Galambosi (1992), the plants that were harvested once or twice during the vegetative growth period produced a higher fresh leaf yield than plants harvested only at the end of the growing season, but this was due to the higher moisture content (about 90 %) of aerial parts harvested during the growth cycle. The average yield of aerial parts of lovage is 4–6 t/ha, from which 2–4 kg of essential oil can be isolated (Hornok, 1992).

Harvesting time can also affect essential oil yield and composition of aerial parts of lovage. In the study by Bylaite *et al*. (1998), the highest amount of essential oil

(2.7 %) based on dry weight was in the middle of July, when seeds were formed. The essential oil yield of 1.53 % was determined in the flowers, which were harvested at the end of flowering in July, whereas the highest concentrations of essential oil in leaves and stems were 1.35 and 1.16 %, which were harvested on June 9 (growing phase) and June 16 (formation of buds), respectively.

One of the major components of essential oils in lovage is α-terpinyl acetate with fresh bergamot-lavender odour (Bauer *et al*., 1990). The highest content of α-terpinyl acetate (70 %) has been detected from the essential oil of leaves collected during a first harvesting on 15 May. The percentage of this compound in the leaves and stems was decreased during the flowering period of the plants. In the flowers, it constituted only 16.27 % (end of flowering), but the lowest amount of α-terpinyl acetate (4.56 %) was determined in the seeds (19 July) (Bylaite *et al*., 1998).

Harvesting of lovage seeds depends on the market demand and intended usage. The average lovage seed yield is 0.4–0.6 t/ha, which gives 3–6 kg of seed essential oil (Hornok, 1992). The essential oil content and composition of seeds can also change during maturation. Immature seeds contain the highest essential oil content (1.5 %); however, it decreases in subsequent harvestings, i.e., green mature seed (1.0 %) and ripened seed (0.6 %), respectively. β-phellandrene, as one of the principal compounds of lovage oil, increased significantly after seed formation and constituted 62.4 %, 60.5 % and 56.4 % of green mature, immature and ripened seed oils, respectively.

The roots of lovage can be harvested in the autumn. The roots are ploughed out after cutting the foliage. On a large scale, the roots can be harvested with rotating forked potato-harvesting machines (Omidbaigi, 2000). Related reports revealed that the root yield was significantly affected by plant age. In a study by Szebeni-Galambosi *et al*. (1992), the highest fresh root yield was obtained from 3–4 year-old plants. According to Hornok (1992), the fresh root yield of 3–4 year-old plants is 6–8 t/ha, from which 5–6 kg essential oil can be extracted. The average yield of lovage roots in Lithuania was reported as 9–10.5 t/ha (Daukšas *et al*., 1999).

The essential oil content and composition of lovage root also can be influenced by harvesting time and plant age. In the study by Penka and Kocabova (1962), the oil content of lovage root increased as the plant grew older. In another report from Finland, the root oil content varied from 0.12 to 1.36 % depending on the transplantation and harvesting times. Also, in the 1 year-old roots the relative amount of phthalides as major compounds of roots oil was significantly higher than in older roots (Szebeni-Galambosi *et al*., 1992). After harvesting, the roots must be cleaned and dried. The soils is shaken off the roots and then, before processing, the roots are washed, before being split into pieces 0.1–0.15 m in length and dried under shade conditions or by artificial driers at 40–50 °C.

19.3.7 Biotechnological approaches for production of essential oil compounds

Biotechnology offers an opportunity to exploit cells, tissues and organs by growing them *in vitro* for large-scale propagation and genetically manipulating them to obtain secondary metabolites. Since the world population is increasing rapidly, there is extreme pressure on cultivable land to produce food. Therefore, for other uses such as production of pharmaceuticals and chemicals from plants, the available land

should be used effectively. Hence, it is appropriate to develop modern technologies leading to plant improvement for better utilization of the land (Ramachandra Rao and Ravishankar, 2002).

In the past years, considerable interest has also been shown in transformed organ cultures, mainly hairy root cultures, which grow rapidly in media without any exogenous growth regulators. Being genetically and biochemically more stable than cell suspension cultures, hairy root cultures are very useful tools in the study of *in vitro* secondary metabolite production (Wysokinska and Chmiel, 1997). Moreover, the need for safer drugs without side-effects has led to the use of natural ingredients with proven safety. These factors have laid emphasis on the use of biotechnological methods to enhance the production of pharmaceuticals and food additives, both in quality and quantity. Transformed root cultures of lovage have been established by inoculation of aseptically grown seedlings with *Agrobacterium rhizogenes* strain A4 carrying plasmid pRiA4::70GUS (Santos *et al*., 2005). Hairy roots growth in four different types of liquid culture media, i.e. SH (Schenk and Hildebrandt, 1972), B5 (Gamborg *et al*., 1968), half-strength B5 (B5/2) and half-strength MS (MS/2), was determined by the dissimilation method and by measuring the fresh and dry weight of the roots. The hairy roots grown in SH medium showed a higher biomass increase, expressed either in dissimilation, fresh weight or dry weight, when compared with those grown in B5/2 and MS/2 media. Dissimilation and fresh weight growth curves of the hairy roots grown in B5 medium were similar to those of the hairy roots grown in SH medium. The hairy roots essential oil yields ranged from 0.006–0.018 % (v/fr. wt), while the yield of the oil from the lovage plant roots was 0.16 % (v/fr. wt). The main components of the oil samples from the hairy roots culture were falcarinol, (*Z*)-ligustilide, (*Z*)-3-butylidenephthalide, *trans*-β-farnesene, β-phellandrene, *n*-octanal, γ-elemene and *n*-heptanal, in varying amounts depending on the culture media tested. The main components of the oil from the lovage plant roots were (*Z*)-ligustilide, β-pinene, pentylcyclohexadiene and α-pinene. (Santos *et al*., 2005). The effect of different NH_4^+:NO_3^- ratios on growth and volatile components of lovage hairy roots in darkness and under photoperiod conditions has also been reported (Costa *et al*., 2008). Most of the mixtures of volatiles isolated from the hairy roots were dominated by *n*-octanal, (*Z*)-falcarinol or both components in about the same relative amounts.

Recently, the biotransformation capacity of the plant hairy root cultures has been also studied by evaluating the effect of the addition of 25 mg/L menthol or geraniol on morphology, growth and volatiles production (Nunes *et al*., 2009). Lovage hairy root cultures were maintained for 7 weeks in SH medium, in darkness at 24 °C and velocity of 80 r.p.m., and the substrates were added 15 days after inoculation. Growth was evaluated by measuring fresh and dry weight and by using the dissimilation method. Hairy roots morphology and growth were not influenced by substrate addition. No new volatiles were detected after menthol addition and, as was also the case with the control cultures, volatiles of these hairy roots were dominated by (*Z*)-falcarinol (1–45 %), *n*-octanal (3–8 %), palmitic acid (3–10 %), and (*Z*)-ligustilide (2–9 %). The addition of geraniol induced the production of six new volatiles: nerol/citronellol/neral (traces-15 %), α-terpineol (0.2–3 %), linalool (0.1–1.2 %), and geranyl acetate (traces-2 %). The relative amounts of the substrates and some of their biotransformation products decreased during the course of the experiment.

Following the addition of β-glycosidase to the remaining distillation water, analysis of the extracted volatiles showed that lovage hairy roots were able to convert both substrates and their biotransformation products into glycosidic forms.

19.4 Main uses in food

All parts of the plant are edible and used for culinary purposes. The leaves and stems are used as a celery substitute in soups, salads, pizzas, stews, sauces and with meat and poultry. The stems can also be blanched and served as a culinary herb. Seed could be used for seasoning meat, bread, potatoes, cheese spreads, pickles, rice and chicken dishes, confectionery and liqueurs (Launert, 1981). The essential oils from leaves (*Levistici folium*), fruits (*Levistici fructus*) and roots (*Levistici radix*) are used in the food, beverage, perfume and tobacco industries (Chiej, 1984; Bown, 1995). Lovage is widely used as a flavouring ingredient, too, in various liqueurs, herb bitters and sauces (Grieve, 1984; Chevallier, 1996). The powdered root was once applied as a substitute for pepper. The essential oils and extracts are used as flavour components in major food products, such as beverages, frozen dairy desserts, candy, gelatins and puddings, meat and its products. Average dosage levels used are generally below 0.005 %, with the exception of 0.017 % and about 0.013 % reported for lovage extract in sweet sauces and in frozen dairy desserts, respectively. Lovage (crude) is also mentioned in alcoholic beverages, baked foods, savory and sweet sauces. In this case, the largest level used is 0.015 % in beverages (Leung and Foster, 1996). According to Opdyke (1978), root oil has an acute oral toxicity with LD_{50} of 3.4 g/kg and an acute dermal toxicity with LD50 of >5 g/kg. In the industry, lovage usage is restricted almost wholly to confectionery and tobacco products (Cu *et al*., 1990). Following the literature, some European recipes for dishes where lovage appears as an important ingredient are given below (Weishan, 2010):

Lobster and potato salad with lovage

Ingredients	*Amount*
Cooked lobster meat	1 kg
Red bliss potatoes (cooked and cut into 1.5 cm dice)	0.5 kg
Mayonnaise	½ cup
Sour cream	½ cup
Freshly squeezed lemon juice	1 tablespoon
Chopped shallots	3 tablespoons
Chopped fresh flat leaf parsley	½ cup
Chopped lovage leaves	½ cup
Salt and freshly ground black pepper	to taste
Red leaf lettuce and fresh chives	for garnish

Method of preparation

1. Combine the mayonnaise, sour cream, lemon juice, shallots, parsley and lovage leaves in a small bowl.
2. Add the mayonnaise mixture to the lobster and potatoes.
3. Toss gently until the mixture is combined.

4. Season to taste with salt and ground black pepper.
5. Garnish with lettuce and chives.

Corn chowder with lovage

Ingredients	*Amount*
Diced bacon	1/2 cup
Butter	2 tablespoons
Chopped onion	1 cup
Chicken broth	6 cup
Red potatoes (scrubbed cut into 1.5 cm dice)	0.5 kg
Fresh com kernels	3 cup
Milk or light cream	2 cup
Chopped lovage leaves	1/2 cup
Salt and freshly ground black pepper	to taste

Method of preparation

1. Cook the bacon in a large soup pot over medium heat until crisp.
2. Add butter and melt.
3. Add onions to the pot and sauté until wilted, about 7 minutes.
4. Add broth and potatoes.
5. Bring the broth to a boil, and then lower the heat and simmer for about 20 minutes or until the potatoes are tender.
6. Add the corn, the lovage, and the milk or cream and continue to cook for an additional ten minutes, but do not allow boiling after adding the milk or cream.
7. Season to taste with salt and ground black pepper.

Marinated cherry tomatoes with lovage

Ingredients	*Amount*
Red cherry tomatoes	1 pint
Yellow pear cherry tomatoes	1 pint
Finely chopped lovage leaves	1/4 cup
Extra-virgin olive oil	1/4 cup
Balsamic vinegar	3 tablespoons
Salt and freshly ground black pepper	to taste

Method of preparation

1. Combine the tomatoes, lovage leaves, olive oil, vinegar, salt and pepper in the small bowl.
2. Cover and let marinate at room temperature for at least an hour.

Bloody marys

Ingredients	*Amount*
Tomato juice	1 quart
Lime juice	1/2 cup
Prepared horseradish	2 tablespoons
Tabasco sauce	1 tablespoon
Vodka	1 1/2 cup

Freshly ground black pepper	1 teaspoon
Lovage stalks	15–25 cm

Method of preparation

1. Combine all ingredients except lovage in a pitcher and stir well.
2. Pour over ice in six tall glasses.
3. Garnish with the lovage stalks, which should be used as straws.

Cream of lovage soup

Ingredients	*Amount*
Butter	2 tablespoons
Onion (chopped)	2 medium
Potato (peeled and diced)	3–4 medium
Carrot (peeled and diced)	2–3 medium
Fresh lovage leaves (chopped)	½ cup
Chicken or vegetable stock	3 cups
Milk or light cream	1 cup
Grated nutmeg	to taste
Salt and pepper	to taste

Method of preparation

1. Melt the butter and gently sauté the onions, potatoes and carrots for 5 minutes.
2. Add the lovage and cook for 1 minute longer.
3. Add the stock, bring to a boil, cover, and simmer gently until the potatoes and carrots are soft, about 15 minutes.
4. Purée in a blender or push through a sieve and return to the pot.
5. Add a grating of nutmeg and salt and pepper to taste and reheat.
6. Stir in milk or cream but do not allow the soup to boil. It can be served hot or cold with chopped lovage as garnish.

19.5 Functional properties

Lovage has long been used in traditional medicine, particularly as carminative, digestive, diuretic, expectorant, antispasmodic and diaphoretic (Holtom and Hylton, 1979). In Iranian folk medicine, lovage is used for the treatment of several gastrointestinal, nervous and rheumatic disorders (Zargari, 1990). Its properties are similar to those of angelica, but lovage is less known as a herb. The leaves and seeds are often used in seasoning, and the rhizomes and roots are used medicinally. Today, lovage is still the principal ingredient in many diuretic tea mixtures, and it is used to treat kidney stones, jaundice, malaria, sore throat, pleurisy, rheumatism, gout and boils (Bown, 1995). Lovage promotes menstruation and relieves menstrual pains. It also improves circulation. An infusion of lovage leaves used to be accounted a good emmenagogue (Grieve, 1984).

The roots, leaves and seeds are used internally in the treatment of stomach disorders, especially cases of colic and flatulence in children, feverish attacks, kidney stones, tonsillitis and cystitis (Bown, 1995). The roots are externally used in the

treatment of sore throats, haemorrhoids and skin ulcers. Lovage is helpful in treating jaundice, chronic constipation and skin diseases. It can also relieve inflammation of the eyes (Chevallier, 1996).

In aromatherapy, it is used to alleviate conditions of the muscles, joints and circulation, and also the digestive and genito-urinary systems. Today, lovage root is occasionally used in digestive formulations in capsules and tablets and as a tea ingredient; however, the use of lovage as a herb has caveats (Leung and Foster, 1996). It is not recommended for pregnant women, as it is known to promote the onset of menstruation. People suffering from kidney disease should not use this herb either, due to its irritant effect, which in excessive doses can cause kidney damage. Herbalists usually prescribe it in admixture with other drugs (Evans, 2002).

In recent years, the medicinal properties of some chemical constituents of lovage were investigated. Two constituents of lovage, butylphthalide and ligustilide, have been shown to have antispasmodic and antiasthmatic actions (Bisset, 1994). The phthalides have been reported to be sedative in mice, and some coumarins have been associated with a phototoxic reaction in humans as well as being useful in treating psoriasis (Bruneton, 1999). Phototoxic reactions are fairly common, ranging from a simple erythema to blisters. Lovage extracts and essential oil have been shown to have strong diuretic effects on mice and rabbits (List and Hörhammer, 1976; Leung and Foster, 1996). Lovage has been indicated for pedal oedema in humans and to dissolve phlegm in the respiratory tract (Bisset, 1994).

Bioactivity of lovage oil has been investigated by Hogg (2001) and dosages of oil of 40 ppm have a value for potential use in antitumour research; 1 ppm as a pesticide has been reported. In the study by Zheng and Wang (2001), the antioxidant capacity (oxygen radical absorbance capacity, ORAC) and total phenolic contents in extract of lovage was determined. The ORAC value and total phenolic content were 21.54 μmol of Trolox® equivalent (TE)/g of fresh weight and 2.63 mg of gallic acid equivalents (GAE)/g of fresh weight, respectively. The extract of the plant has shown insecticidal effect against the confused flour beetle, *Tribolium confusum*, one of the most serious stored product insect pests worldwide (Hrudova *et al*., 2006). Recently, the repellency of lovage root oil alone or in combination with *Calophyllum inophyllum* L. (Clusiaceae) nut oil (tamanu oil) against female *Stomoxys calcitrans* (L.) (Diptera: Muscidae) has been evaluated by the exposed human hand bioassay (Hieu *et al*., 2010). Based on the protection time (PT), lovage root essential oil (0.5 mg/cm^2) showed potent repellency (3.36 hours). Tamanu oil was found to synergize the repellency of lovage root essential oil in that the binary mixtures of the essential oil with tamanu oil (PT, 2.68 hours) resulted in significantly greater repellency than either lovage root essential oil (1.13 hours), tamanu oil (0.56 hours), or DEET (N,N-diethyl-3-methylbenzamide) alone (2.20 hours) based on the protection time (Hieu *et al*., 2010).

The essential oil of lovage seeds has been shown to have antibacterial effects against gram-positive and gram-negative bacteria, i.e., *Bacillus subtilis* ATCC 9372, *Enterococcus faecalis* ATCC 15753, *Staphylococcus aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27852 and *Klebsiella pneumoniae* ATCC 3583 (Mirjalili *et al*., 2010) (Table 19.3). The oils indicated high activity against tested gram-positive bacteria especially,

Table 19.3 Antibacterial activity of the essential oil of lovage fruits

Microorganisms	IF		MF		RF		Ampicillin*
	DD	MIC	DD	MI	DD	MI	DD
Bacillus subtilis	25	3.75	36	0.93	35	0.93	14
Enterococcus faecalis	19	15	17	7.5	13	7.5	11
Staphylococcus aureus	17	3.75	21	3.75	16	3.75	13
Staphylococcus epidermidis	23	1.87	26	0.93	25	1.87	19
Escherichia coli	19	15	18	7.5	15	7.5	12
Klebsiella pneumoniae	10	>15	10	>15	9	>15	–
Pseudomonas aeruginosa	11	>15	8	>15	9	>15	9.7

IF = immature fruit (mm); MF = mature fruit (mm); RF = ripened fruit (mm); DD = diameter of inhibition zone (mm) including disk diameter of 6 mm; MIC = minimum inhibitory concentration, values as mg/ml oil. Inactive (–), moderately active (7–14), highly active (>14).
* Tested at a concentration of 10 µg/disk.
Source: Mirjalili *et al*. (2010).

B. subtilis that was more sensitive than others and a gram-negative bacterium, *E. coli.*

The antibacterial activity of the oils has also been determined by measuring the minimal inhibitory concentrations (MICs) against tested bacteria (Table 19.3). The essential oils of mature and ripened seeds exhibited the highest activity against *B. subtilis* with MIC value of 0.93 mg/ml. Also high sensitivity of *S. epidermidis* to the mature seed oil was observed with a MIC value of 0.93 mg/ml. The oils showed lowest activity against *K. pneumoniae* and *P. aeruginosa*, with MIC values more than 15 mg/ml (Mirjalili *et al*., 2010). In the study by Schinkovitz *et al*. (2008), the antimycobacterial activity of the polyacetylenes against *Mycobacterium fortuitum* and *M. aurum* in a microtitre plate dilution assay from dichloromethane extract of lovage roots has been reported. The MIC of 3(*R*)-falcarinol against *M. fortuitum* and *M. aurum* was 16.4 µM while that of 3(*R*)-8(*S*)-falcarindiol was 30.7 µM against *M. fortuitum* and 61.4 µM against *M. aurum*, respectively. Previously, 3(*R*),8(*R*)-dehydrofalcarindiol was isolated from *Artemisia monosperma* and, surprisingly, this polyacetylene exhibited no antimycobacterial activity at 128 µg/mL. Reference antibiotics ethambutol and isoniazid exhibited an activity of 115.5 µM and 14.6 µM against *M. fortuitum* and 3.4 µM and 29.2 µM against *M. aurum*, respectively (Schinkovitz *et al*., 2008).

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20

Nigella

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Abstract: Nigella (*Nigella sativa* L.) is grown for its seeds which are used dried in foods, pickles, baked goods and confectionery and in the perfumery and medicinal industries. This chapter begins with a description of nigella and details of production methods. Post-harvest handling, main products and uses in food are then reviewed. Several processed products from nigella whole seed, such as the essential oil, fatty oil and mucilage, are in demand in the food and medicinal products industries. The bioactive compounds present in essential oil from the seeds are detailed, key constituents being thymoquinone and various other monoterpenes. The major functional properties of nigella are outlined. It has antifungal, antibacterial, antiparasitic, anti-inflammatory, antioxidant and several other immunological activities. Toxicity information and quality specifications for different nigella products are also given.

Key words: nigella, cultivation, chemical composition, main products, functional properties, medicinal uses, toxicity, quality specifications, adulteration.

20.1 Introduction and description

Nigella sativa L., a common species of the genus Nigella, is native to an area stretching from the Mediterranean through West Asia to northern India. Categorized as a minor seed spice, nigella has long been domesticated and is known to be cultivated from Morocco to Northern India, in sub-Saharan Africa (particularly Niger), Eastern Africa (especially Ethiopia) (Jansen, 1981), and in Russia, Europe and North America; it was reportedly introduced to Britain in 1548. In South East Asia, nigella seeds are mainly used for medicinal purposes while in India, where nigella can be found in the wild, it has been used as a spice and condiment since ancient times.

Nigella is mentioned in ancient Greek, Roman and Hebrew texts, under the name 'black cumin', as both a condiment and a component of herbal medicine. The earliest reference to nigella is found in the Old Testament book of Isaiah. In a treatise on the early origins of Indian and Chinese drugs, Al-Biruni (973–1048) refers to the black seeds of nigella as a kind of grain known as *alwanak* in the *Sigzi* dialect of Kazakhstan; this was confirmed by Suhar Bakhat, who cites its use during the tenth and eleventh centuries. Moreover Ibn Sina (980–1037), a contemporary of Hippocrates, listed the medicinal benefits of nigella in the book *The Canon of Medicine*. Nigella has been used since antiquity by Asian herbalists and pharmacists and was

used for culinary purposes by the Romans. It is best known thanks to the saying of the prophet Muhammad, that black cumin is a remedy for every illness except death. Nigella seeds were found in the tomb of pharaoh Tutankhamun in ancient Egypt, suggesting that they play a critical role in ancient embalming practices. Dioscorides, a Greek physician of the first century AD, reported that black cumin seeds taken orally can treat headaches, nasal catarrh, toothache and intestinal worms, as well as acting as a diuretic and increasing production of breast milk.

The name nigella derives from the Latin *nigellus* or *niger*, meaning black. Common names applied to the genus Nigella are devil-in-a-bush or love-in-a-mist. As well as being commonly called black cumin, it is also known by a variety of popular names in different countries. It is called small fennel (or black cumin) in English; *cheveux de venus, nigelle, cumin noir* or *poivrette* in French; *nigella* in Italian; *Schwarz kümmel* in German; *neguilla* or *pasinara* in Spanish; *kolongi* in Turkish; *habba tu sawda* in Arabic; *shonaiz* in Persian; *jinten hitan* in Indonesian and Malay; and *kala zira*, *kalongi*, *krishanjirka*, *mangrail* and many other vernacular names in India. Some of the popular names of nigella are very similar to those given to other spices of the Apiaceae family, *viz*. *Siah Zira* (Black Cumin–*Carum carvi* L.), *Kala Zira* (Black Cumin – *Bunicum persicum* Bioss. Fedtsch syn. *Carum bulbocastanum* Koch.). Botanically and structurally, the nigella seed is entirely different from the seed spices mentioned above and belongs to a different family. In order to avoid confusion, it is therefore advisable to refer to this spice as nigella.

20.1.1 Classification

The genus Nigella contains more than 116 species, the most popular of which is *Nigella sativa* L. As per the conventional classification of spices, it is classified as a mild spice; in addition, in terms of the plant organs used, nigella is classified as a seed spice because it is mostly the dried seeds that are used as a spice. With regard to pollination behaviour, *N. sativa* L. is a cross-pollinated crop and has a somatic chromosome number of 2n = 12 (Jha and Roy, 1979). The flowers are self-fertile, but cross-pollination occurs through insects. It belongs to the buttercup family (Ranunculaceae) and to the order Ranunculales. The other closely related species, *N. damascena* L. and *N. arvensis* L., are mostly used as ornamental plants and in medicines.

20.1.2 Chemical structure

A qualitative examination of *N. sativa* L. seeds revealed the presence of sterols, triterpenes, tannins, flavonoids, cardiac glycosides, alkaloids, saponins, volatile oils, coumarins, volatile bases, glucosinolates and anthraquinones (Al-Yahya, 1986). It has been shown that *N. sativa* seeds contain >30 % of a fixed oil and 0.40–0.45 % w/w of volatile oil. Qualitative analysis of *N. sativa* seed oil using the capillary gas chromatography–mass spectrometry (GC–MS) technique has allowed the 67 different compounds to be identified; when these compounds were classified into various functional groups, the following results were obtained: monoterpenes (46 %); carbonyl compounds (25 %); phenols (1.7 %); alcohols (0.9 %) and esters (16 %) (Aboutable *et al.*, 1986). In the volatile oil of *N. sativa*, Adamu *et al.* (2010) analysed

2-methyl-5 (1-methyl ethyl)-bicyclo[3.1.0]hex-2-ene as the major constituent (62.28 %) of the volatile oil of *N. sativa,* while α-pinene was a minor constituent (2.28 %). El-Tahir *et al*. (1993) showed that the volatile oil contained 18.4–24 % thymoquinone and a total of 46 % of various monoterpenes such as *p*-cymene and pinene.

The presence of thymoquinone, dithymoquinone, thymohydroquinone, thymol, carvacrol, oxy-coumarin, 6-methoxy-coumarin and 7-hydroxy-coumarin, α-hedrin and steryl-glucoside as well as large amounts of flavinoids, tannins, essential fatty acids, essential amino acids, ascorbic acid, iron and calcium has been reported (Omar *et al*., 1999). According to Weiss (2002), the seeds contain 0.5 % volatile oil, of which the seven main constituents and their approximate proportions are *p*-cymene (31 %), thymoquinone (25 %), ethyl linoleate (9 %), α-pinene (9 %), ethyl hexadecanoate (3 %), ethyl oleate (3 %) and β-pinene (2 %). Sharma *et al*. (2009) found that in the essential oil (mean 0.5 %, max. 1.5 %), thymoquinone was identified as the main component (up to 50 %) alongside *p*-cymene (40 %), pinene (up to 15 %), dithymoquinone and thymohydroquinone. Other terpene derivatives were found only in trace amounts: carvacrol, carvone, limonene, 4-terpineol, citronellol. Furthermore, the essential oil contains significant (10 %) amounts of fatty acid ethyl esters. On storage, thymoquinone yields dithymoquinonene and higher oligo condensation products, which provide the spice with its aromatic flavour. Nickavara *et al*. (2003) investigated the chemical composition of the volatile oil of *N. sativa* seeds grown in Iran using GC and GC–MS methods and identified 32 compounds (86.7 %) in the volatile oils. The major compounds were *trans*-anethole (38.3 %), *p*-cymene (14.8 %), limonene (4.3 %), and carvone (4.0 %). The results are given in Table 20.1 (Nickavara *et al*., 2003).

Variations in the volatile composition have been observed in the two species, *N. damascene* and *N. sativa*: the former contained sesquiterpenes, including a large proportion of β-elemene (27.7 % extract, 54.7 % oil) and methyl 3-methoxy-*N*-methyl anthranilate (30.7 % extract, 12.7 % oil), which account for the characteristic aroma of this species; in the latter, however, only monoterpenes, including *p*-cymene (49 % extract, 47.4 % oil) and thymoquinone (20.6 % extract, 20.8 % oil), were detected (Rchid *et al*., 2004). Moretti *et al*. (2004) reported that the main components of *N. sativa* were *p*-cymene (33.8 %) and thymol (26.8 %), with only a small amount of thymoquinone (3.8 %) whereas *N. damascene* oil contained almost 100 % sesquiterpenes, 73.2 % of which was made up by β-elemene.

Traces of two different types of alkaloids have been found in nigella seeds: isochinoline alkaloids, represented by nigellimin and nigellimin-N-oxide, and pyrazol alkaloids, including nigellidin and nigellicin. Rajkapoor *et al*. (2002) reported the alkaloids present in the seeds to be nigellicin, nigellidin, quanazoline, tannin, steroid α-spinasterol, campsterol, cholesterol, stigmas 7-en-3-β-ol, stigmasterol and flavonoids of trigillin quercetin-3-glucoside. Four dolabellane-type diterpene alkaloids have been isolated from the seeds of *Nigella sativa* (Morikawa *et al*., 2004 a, b). The active principles, nigellone and nigellidine, are reported to contain an indazol nucleus (Rahman *et al*., 1995). The chemical structure of the active principle compound nigellone is given in Fig. 20.1 (Harborne *et al*., 1999). Three flavonoid glycosides and triterpene saponins were also identified from *N. sativa,* together with four phospholipid classes: phosphatidylcholine, phosphatidylethanolamine,

Table 20.1 Chemical composition of the volatile constituents

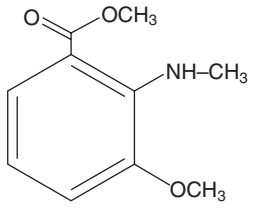
Compound	Percentage
n-Nonane	1.7
3-Methyl nonane	0.3
1,3,5-Trimethyl benzene	0.5
n-Decane	0.4
1-Methyl-3-propyl benzene	0.5
1-Ethyl-2,3-dimethyl benzene	0.2
n-Tetradecane	0.2
n-Hexadecane	0.2
Total non-terpenoid hydrocarbons	*4.0*
α-Thujene	2.4
α-Pinene	1.2
Sabinene	1.4
β-Pinene	1.3
Myrcene	0.4
α-Phellandrene	0.6
p-Cymene	14.8
Limonene	4.3
γ-Terpinene	0.5
Total monoterpenoid hydrocarbons	*26.9*
Fenchone	1.1
Dihydrocarvone	0.3
Carvone	4.0
Thymoquinone	0.6
Total monoterpenoid ketones	*6.0*
Terpinen-4-ol	0.7
p-Cymene-8-ol	0.4
Carvacrol	1.6
Total monoterpenoid alcohols	*2.7*
α-Longipinene	0.3
Longifolene	0.7
Total sesquiterpenoid hydrocarbons	*1.0*
Estragole	1.9
Anisaldehyde	1.7
trans-Anethole	38.3
Myristicin	1.4
Dill apiole	1.8
Apiole	1.0
Total phenyl propanoid compounds	*46.1*
Total compounds	*86.7*

Source: Nickavara *et al.* (2003).

phosphatidylserine, and phosphatitdylinositol (Merfort *et al.*, 1997; Ramadan and Mörsel, 2002).

Fatty acid content of the oil

The seeds also contain a fatty oil rich in unsaturated fatty acids. The fixed oil is mainly composed of unsaturated and essential fatty acids (principally linoleic acid, followed by oleic acid) (Al-Jassir, 1992) whereas the composition of the volatile oil has been described above. The fatty acid composition of *N. sativa* differs according to the



Nigellone

Fig 20.1 Chemical structure of nigellone and thimoquinone – chemical formula: $C_{10}H_{13}NO_3$; Molecular weight: 195.22.

origin of the seed. Several varieties have been investigated in this regard, including those of Turkish (Aitzetmuller *et al.,*1997), Saudi (A1-Yahya, 1986), Iranian (Nickavara *et al*., 2003) and Indian (Bhakare *et al*., 1992) origin, with each found to contain a specific pattern of certain fatty acids. The seeds of Turkish origin contained dihomolinoleic acid (20: 2n-6, or 11, 14-*cis*, *cis*-eicosadienoic acid, 20:23 llc, 14c) (Sener *et al.,* 1985; Aitzetmuller *et al.,*1997) which differs from the edible oils. Ansari *et al.* (1975) determined the seed oil content of *N. sativa* of Indian origin using thin-layer chromatography (TLC) and gas–liquid chromatography (GLC) chromatographic techniques. *N. sativa* seed oil from Egyptian varieties has been shown to have the following fatty acid composition in the triglyceride portion: linoleic (59.6 %), oleic (23.8 %), palmitic (12.4 %) and stearic (< 1 %). The major triacylglycerols found were trilinoleoyl (24.6 %), oleoyldilinoleoyl (19.6 %), palmitoyldilinoleoyl (17.5 %), palmitoyloleoyllinoleoyl (12.9 %) and dioleoyllinoleoyl (9.6 %) (El-Sayed *et al.,* 1997; Abdel-Ghany *et al.,* 1998). Nickavara *et al*. (2003) investigated the total fatty acid composition of the extracted fixed oil of *N. sativa* seeds grown in Iran using GC and GC/MS methods. Eight fatty acids (99.5 %) were identified in the fixed oils. The main fatty acids of the fixed oil were linoleic acid (55.6 %), oleic acid (23.4 %) and palmitic acid (12.5 %). The results are given in Table 20.2 (Nickavara *et al*., 2003). The autoxidative stability and peroxide value (76 mg/kg after 5 days) of crude *N. sativa* oil of Egyptian origin was determined by Zeitoun and Neff (1995). A study examining the link between the degree of unsaturation and the tocopherol content of the oil was carried out by Kamal-Eldin and Anderson (1997).

Total lipid content and lipase activity of the oil

The lipid content has been reported to be 31.8 %, the majority of which is represented by neutral lipids, while the minor lipids include glycolipids (monogalactosyl diglyceride, digalactosyldiglyceride, acylated-sterylgalactoside and sterylgalactoside) and phospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, candiolipin and phosphatidylglycerol) (Bhakare *et al.,* 1992; Abdel-Ghany *et al.,* 1998). The enzyme lipase has been isolated from the seeds and its hydrolytic action with commercial detergents has been investigated for potential use as a biodegradation agent (Saad, 1995). The native lipase of *N. sativa* has been used in oleochemical reactions to esterify the fatty acid obtained from oleic acid, sunflower oil and coconut oil with glycerol and methanol (Mert *et al.,* 1995; Dandik and Aksoy, 1996). This method provided a very high yield (81.7 %) of synthesized triglyceride (triolein) (Dandik and Aksoy, 1996). The enzymatic hydrolysis of used

Table 20.2 Fatty acid composition of the fixed oil of *Nigella sativa* L

Fatty acid	Percentage
Lauric acid	0.6
Myristic acid	0.5
Palmitic acid	12.5
Stearic acid	3.4
Oleic acid	23.4
Linoleic acid	55.6
Linolenic acid	0.4
Eicosadienoic acid	3.1
Total fatty acids	*99.5*

Source: Nickavara *et al.* (2003).

frying oil catalysed by the native lipase of *N. sativa* seed has also been investigated along with the kinetics of the lipolysis process (Dandik *et al.,* 1993; Dandik and Aksoy, 1996).

Composition of nigella mucilage
The mucilage separated from *N. sativa* is an amorphous, brown-coloured solid that is soluble in water. The average soaking factors have values from 8 (root) to 11 (flowers and seedless fruits). The mucilage separated from *N. sativa* is of the ozuronic type and has been found to contain six components after mucilage hydrolysis: galacturonic acid (26.08 %), glucuronic acid (11.40 %), galactose (12.39 %), glucose (18.21 %), arabinose (20.03 %) and rhamnose (11.89 %) (Toma *et al.*, 2004).

20.2 Production and international trade

N. sativa is native to the Mediterranean regions and is now cultivated in other parts of the world including the Middle East, North Africa and Asia (Durani *et al.*, 2007). Nigella has been observed growing in the wild in India and Egypt, and in Iran, where eight species have been recorded (Mozoffarin, 1998). *N. damascene* also grows wild in grassland areas of temperate Europe (Tutin *et al.*, 1964).

India is known to be the largest producer and exporter of nigella in the world. The other producing countries are Sri Lanka, Bangladesh, Nepal, Egypt, Iraq and Pakistan. In India, it is commercially cultivated in Punjab, Himachal Pradesh, Madhya Pradesh, Bihar, Jharkhand, Assam, West Bengal and Andhra Pradesh. Precise information on the areas and extent of production is not available; it is estimated that around 9000 ha are devoted to the production of nigella in India, with an output of about 7000–8000 tonnes of which about 2800 tonnes are exported.

20.2.1 Cultivation

Nigella is a cool season crop requiring a frost-free growing season. Cold weather is desirable for the early growth period, but warm sunny weather is required during

seed formation. Plants are frost-sensitive at any growth stage and this limits its range in Europe and highland areas of the tropics. In the northern hemisphere, nigella is sown in late spring–early summer, but in regions with wet and dry seasons, it is sown just after the first rains. Regional cultivars can be grown from sea level up to 2500 m, while cultivars able to withstand considerable moisture stress have been developed in North Africa and West Asia. Nigella is often intercropped with barley or wheat in Ethiopia, and is strip cropped in North Africa and elsewhere (Weiss, 2002). The farmers grow both local cultivars and others that have been developed through selection from land races. Regional cultivars are more popular in areas where production is on a smaller scale, such as Sri Lanka, Bangladesh, Nepal, Egypt, Iraq and Pakistan. Recently the NRCSS AN-1 variety has been developed by the National Research Centre on Seed Spices in India, and has been recommended for release (Malhotra and Vashishtha, 2008). Nigella is propagated by seed, and sown at row spacing of 30 cm, plant spacing of 15–20 cm and seed rate of 8 kg/ha. The nigella crop takes 140–160 days to reach maturity. An average yield of 600–800 kg can be obtained from 1 ha of land (Malhotra, 2006).

20.2.2 Post-harvest handling and processing

The freshly-harvested nigella seed crop is allowed to dry in order to facilitate threshing to separate the seeds. The fresh seeds are taken to an oil extraction unit to recover the essential oils. Seed dried in the shade has a higher oil content than sun-dried seeds. In India, nigella seed is dried in zero-energy solar drier tunnels to avoid entry of dust and foreign materials. The seed is cleaned easily with a screening mill followed by a gravity separator. The seeds are well dried (to a water content of 8–9 %), cleaned, graded through sieving and stored in polyethylene lined gunny bags in a cool dry place (Malhotra, 2010). Nigella essential oil is obtained by steam distillation or hydrodistillation of fruits.

The volatile oil content of *N. sativa* has been determined to be 0.4–0.5 % w/w (El-Alfy *et al*., 1975). However, an improved method for isolating the volatile oil involves Soxlet extraction of the seeds with petroleum ether to give an oil (35 %) which on steam distillation provided a much higher yield of volatile oil (1.5 %) (Rathee *et al*., 1982). The volatile oil is a pale yellow liquid with a characteristic aromatic odour and taste which is readily soluble in organic solvents such as ether, chloroform and ethanol but only sparingly soluble in water (E1-Alfy *et al*., 1975). A large proportion of essential oil content can also be recovered through the supercritical fluid extraction method. Isolation of the fixed oil by extraction of the crushed seeds of *N. sativa* with hot petroleum ether is a more efficient procedure that produces a higher yield 35 % (Rathee *et al.*, 1982) or 42.01 % (Ebrahiem, 1998). The oil obtained by pressing the seeds has been found to be quite rich in fatty acids (Turkay *et al.*, 1996). The acidity in the oil arises as a result of the enzyme lipase which is present in the raw seeds, hydrolysing the fatty acid esters *in situ* (Dandik and Aksoy, 1996). A deacidification process has been devised for *N. sativa* oils, involving extraction with supercritical carbon dioxide. Using this method, the amount of neutral oil that is co-extracted with the fatty acids has been increased to 94 % (Turkay *et al.*, 1996). A variety of stability tests on a number of Egyptian oils, including black seed oil, have shown variable results that were dependent on the type of method

employed (E1-Sayed *et al.*, 1977). Nigella oleoresins can also be prepared but, due to their lower commercial value, they are not very popular.

20.2.3 Main products

Whole seed

The dried seeds of nigella are a major commercial product, used in foods, pickles, baked goods and confectionery and in the pharmaceutical and perfumery industries. Nigella seeds are generally dry-roasted or fried before being used for a variety of culinary purposes. The flavour is like that of oregano, with a bitterness similar to that of mustard seeds. In India, nigella seeds have been used as spices and preservative agents since ancient times for the preparation of pickles, and they are widely used in Indian cuisine as a spice or pepper substitute to flavour vegetable and *dhal* dishes and curries, particularly mildly braised lamb dishes such as *korma*. They can also be sprinkled onto naan bread before baking, and are one of the ingredients in some garam masalas. Along with cumin, fennel, mustard and fenugreek, nigella seed is one of the five spices used without roasting or grinding in *panch phoran*, a famous spice mix of Bengali origin. Nigella is also used in Middle Eastern cuisine, where it is added to bread dough and is an essential constituent of *choereg* rolls and some Turkish breads. The seeds of *N. sativa* can also be ground to a paste and mixed with melted honey to make 'henlava', a Middle Eastern confection that usually uses toasted sesame seeds instead of nigella. In Europe, the seeds may occasionally be used as a pepper substitute, and, under the name *charnushka,* are often found in Jewish and Russian rye bread, flat breads and savoury pastries, and even in Polish coffee cake. The whole seed can also be processed to give essential oils, which are not used for culinary purposes, and fatty oils, which are discussed below.

Essential oil and other extracts

The nigella seed contains 0.4–0.5 % volatile oil which is yellowish brown in colour. It has an unpleasant odour but has been recognized for its nutritional and health-promoting properties. The essential oil is not used in food but, owing to its many functional properties, has number of medicinal values for treatment of diseases (detailed in Section 20.3). The essential oil contains thymoquinone by which it acquires an aromatic flavour. Adamu *et al*. (2010) advocated that essential oil from *N. sativa* be utilized for the manufacture of perfumery products, antimicrobial and antiseptic agents. *N. sativa* crude extracts possess a number of medicinal properties. The alcoholic extracts are mostly prepared and are used in various formulations. Supercritical extraction with carbon dioxide is a feasible technique for isolation of active substances from *N. sativa*. The supercritical extract is characterized by higher concentration of the main extracted aromatic compound thymoquinone (Alhaj *et al*., 2008). The supercritical extracts, being stronger and more effective, are increasingly replacing organic solvents, e.g. *n*-hexane, dichloromethane chloroform and so on, that are conventionally used in industrial extraction.

Fatty oils

The fatty oil obtained by the expression of seeds can be used for edible purposes. Since *N. sativa* has positive effects on human nutrition and health, fatty oils from

nigella seeds are now being considered as a new source of edible oils (Gharib Zahedi *et al*., 2010). Extraction with benzene and subsequent steam distillation of the extract to remove the volatile components yields the fixed oil. The fixed oil from black seeds has the same faint peppery/spicy odour of the seed, whereas the essential oil has an extremely intense odour. The latter is also very expensive, due to the low volatile oil content in the seeds (0.5–1.5 %, compared to 35–40 % for fixed oil), and is thus very rarely used in its pure form. Nigella seed, powder and oil are used as adjuncts for flavouring foods, as preservative in confectioneries and in the pharmaceutical industry. Nigella fixed oils are used as stabilizing agents for edible fats (Pruthi, 2001).

20.3 Functional properties

The nutritional constituents of nigella seed from Europe and Ethiopia are given in Table 20.3. Proximate analysis of *N. sativa* seeds showed that the moisture content ranged from 5.52–8.50 %, crude protein from 20–26.7 %, ash from 3.77–4.86 %, total carbohydrates from 23.5–33.2 % and ether extractable lipids from 34.49–38.72 % (Takruri and Dameh, 1993; Salma *et al*., 2007). Chemical analysis showed that nigella seed is a significant source of essential fatty acids, proteins, carbohydrates and other vitamins and minerals (Takruri and Dameh, 1993; Durani *et al*., 2007; Salma *et al*., 2007). *N. sativa* seeds include nutritional components such as carbohydrates (glucose, xylose, rhamnose and arabinose), vitamins (thiamine, riboflavin, pyridoxine, niacin and folic acid) (Khan, 1999), mineral elements and proteins. *N. sativa* seeds are also a source of calcium, iron, potassium and alkaloids (nigellidine, nigellimine and nigellicine) (Khan *et al*., 2003). The amino acids present

Table 20.3 Nutritional constituents of nigella seed (per 100 g)

Constituents	European seed	Ethiopian seed
Moisture (g)	4	6.6
Protein (g)	22	13.8
Fat (g)	41	32.2
Carbohydrate (g)	17	–
Fibre (g)	8	16.4
Ash (g)	4.5	7.5
N (g)	–	2.2
Na (g)	0.5	–
K (g)	0.5	–
Ca (g)	0.2	0.5
P (g)	0.5	0.6
Fe (mg)	10	17
Thiamine (mg)	1.5	0.62
Niacin (mg)	6	9.5
Pyridoxine (mg)	0.7	–
Tocopherol (mg)	34	–

Sources: Takruri and Damah, (1993); Nergiz and Ottles, (1993).

in dormant seeds are cystine, lysine, aspartic acid, glutamic acid, alanine and tryptophan (Al-Jassir, 1992).

Nigella seed, its extracts and oils are known to have several pharmacological properties: some traditional medicinal preparations that contain *N. sativa* are listed in Table 20.4. The seeds are employed as a corrective of purgatives and other medicines and are believed to possess diuretic, anthelmintic and emmenagogue properties, useful in indigestion, loss of appetite, fever, diarrhoea, dropsy, puerperal diseases, etc. They act as a galactagogue, and are therefore given in combination

Table 20.4 Some of the medicinal preparations using *Nigella sativa*

S.No.	Remedy	Use	Reference
1.	Two drachm of lightly roasted nigella seeds, with the addition of an equal quantity of treacle	For the relief of intermittent fever	Nadkarni (2001)
2.	10–20 grains of nigella seed	For the treatment of dysmenorrhoea; in large doses it may induce abortion	
3.	A confection made of nigella seeds, cumin seeds, black pepper, raisins, tamarind pulp, pomegranate juice and sonchal salt with treacle and honey	As a cure for loss of appetite and distaste for food	
4.	Nigella seeds with the addition of long-pepper, sonchal salt and wine	For the relief of pain experienced after childbirth	
5.	10 g of each of the following: nigella seeds, cumin seeds, anise seeds, ajowain seeds, carum seeds, *Anethum sowa*, fenugreek, coriander, ginger, long pepper, long pepper root, plumbago root, habusha (an aromatic substance), dried pulp of *Ziziphus jujuba*, root of *Aplotaxis auriculate* and Kamala powder, combined with 1000 g treacle, one seer (about 1 l) of milk and 40 g butter, boiled together. The resulting confection is known as *pancha jiraka paka*, and should be used in doses of about one drachm every morning	For the relief of illness related to childbirth, including fever, loss of appetite and disordered secretions after delivery	
6.	Crushed seeds in vinegar	To ease symptoms of skin disorders such as ring worm, eczema and baldness	Weiss (2002)
7.	Tea made from powdered nigella seeds, fenugreek, garden cress, *Commiphora* spp. and dried leaves of *Cleome* spp., *Abrosia maritina* L. and *Centaurium pulchellum* (SW) Druce (used mainly in Egypt)	As a cure for diabetes	
8.	500 mg Egyptian nigella oil capsules	As an antihistamine	

with other medication to women who have recently given birth. The seeds also have antibilious properties and may be administered internally to arrest vomiting. The seeds are fried, bruised and tied in a muslin bag and the smell inhaled to relieve the symptoms of colds and nasal congestion.

Important functional properties reported for *N. sativa* are:

- antifungal;
- antibacterial;
- antiparasitic;
- anti-inflammatory;
- antioxidant;
- immunological;
- anticarcinogenic;
- antidiabetic;
- hepatoprotective;
- antispasmodic, antihypertensive, analgesic, growth-regulating, bronchodilatory and gastroprotective.

These properties are considered in the sections below.

20.3.1 Antifungal activity

The essential oil of *N. sativa* has shown excellent activity against a number of fungi (Agarwal *et al.*, 1979; Bourrel *et al.*, 1995; Aboul Ela *et al.*, 1996). The inhibition of aflatoxin formation by a number of medicinal plants including *N. sativa* at different concentrations has been studied. In one of these studies, the powdered seed and essential oil effectively inhibited the growth and aflatoxin production of a toxigenic strain of *Aspergillus flavus* (E1-Shayeb and Mabrouk, 1984; EI-Sayed *et al.*, 1997; Ozcan, 1998). The essential oil has also been reported to be effective against *Colletotrichum capsici*, *Pythium vexans* and *Sclerotinia trifolium*; while the seeds are ineffective against *A. flavus*, *A. niger*, *Geotrichum candidum* and *Penicillium roquefortii* (Rathee *et al*., 1982; Akgul and Kivanc, 1989). Moreover, *N. sativa* oil could be valuable in the protection of plants against the fungus *Candida olivacum*, a known parasite affecting the growth of economic crops such as rice, wheat and cotton (Aboul Ela *et al.*, 1996).

20.3.2 Antibacterial activity

Thymoquinone, primarily present in seeds and oils, exhibits strong antimicrobial properties and is believed to be the active principle responsible for the antimicrobial profile of *N. sativa* oil (E1-Alfy *et al.*, 1975; Morsi, 2000; O'Mahony *et al*., 2005). However, another study has indicated the complementary involvement of other fatty acid compounds in the antimicrobial properties of the oil (Bourrel *et al.*, 1993). Several studies have shown that nigella seed, extracts and oils have an antibacterial action that inhibits the growth of both gram-positive and gram-negative microorganisms except certain strains of *Pseudomonas pyocyanea [P. aeruginosa]* (Toppozada *et al.*, 1965; Hanafy and Hatem, 1991). Essential oil from the seeds was found to be active against *Vibrio cholera*, *Shigella shiga, S. dysenteriae*, *S. flexneri* and *Escherichia coli* (Rathee *et al*., 1982; Ferdous *et al*., 1992). Water extract and

hexane extract, however, have only weak activity against *Streptococcus* (Naqvi *et al*., 1991). The antibacterial activity of the essential oil of *N. sativa* has also been further examined in a number of studies (Agarwal *et al.*, 1979; Rathee *et al.*, 1982; Saxena and Vyas, 1986; Hasan *et al.*, 1989; Karawya *et al.*, 1994; E1-Kamali *et al.*, 1998). During testing against 21 pathogenic bacteria, it was found that the antibacterial activity of the seeds was predominantly related to the volatile oil fraction and the activity was much higher for all of the gram-positive strains tested. It has also been suggested that the volatile oil would be a good substitute for common antibiotics (Rathee *et al.*, 1982; Hasan *et al.*, 1989).

The crude extracts of *N. sativa* were reported to have a promising effect on multidrug resistant organisms, including gram-positive and gram-negative bacteria; nigella oil was more effective against the former (*Staphylococcus aureus, S. epidermis*) than against the latter (*Staphylococcus, Streptococcus pyogenes*) (Alhaj *et al*., 2008; Salman *et al*., 2008). It has been reported to be effective against clinical isolates of methicillin-resistant *S. aureus* (Hannan *et al*., 2008) and *Helicobacter pylori* (O'Mahony *et al*., 2005; Syed *et al*., 2009; Salem *et al*., 2010). The biological activity of the oil, after fractionation, was ascribed to its phenolic content. A solution of the phenolic portion of the oil in propylene glycol had an enhanced antibacterial activity and was found to be non-toxic to humans with no adverse effects on blood pressure, heart or respiration (Toppozada *et al.*, 1965).

Nigella extract, in combination with the commercial diagnostic antibiotics streptomycin and gentamycin, has been shown to exert a synergic antibacterial action. In an *in vitro* study, the volatile oil has shown antibacterial action against 37 enteric organisms; promising results have been observed on strains *S. dysenteriae 1, S. flexneri, S. sonnei* and *S. boydii* (Ferdous *et al.*, 1992). Moreover, the aqueous methanolic extract of *N. sativa* has been screened against *S. mutans* and reported to show good antibacterial activity by preventing the adhesion of viable cells of *S. mutans* to smooth surfaces. The plant extract thus proved valuable in the prevention of dental caries and plaques (Namba *et al.*, 1985). In a collective study, some 32 plant species used in Saudi folk medicine including *N. sativa* exhibited antidiarrheal activity (Shah *et al.*, 1988). *N. sativa* oil (0.1 % w/w) has also proven very useful in food preservation, as a potent inhibitor of food spoilage and hazardous bacteria (Akgul and Kivanc, 1989; E1-Sayed *et al.*, 1997).

20.3.3 Antiparasitic activity

The volatile oil of *N. sativa* has been reported to exhibit fairly good antiparasitic activity, and particularly anthelmintic activity against earthworms (*Pheritima posthuma*), tapeworms (*Taenia solium*), hookworms (*Bunostomum trigonocephalum*) and nodular worms (*Oesophagostomum colombionum*), which was found to be comparable with that of the chemical agent piperizine phosphate (Agarwal *et al.*, 1979). Akhtar and Riffat (1991) reported the use of *N. sativa* seeds as a treatment against worms for children. The glycosides of *N. sativa* have considerable anticestodal potential in animals (Akhtar and Riffat, 1991; Mahmoud *et al*., 2002), with doses of 150 and 200 mg/kg showing activity comparable to that of levamisole hydrochloride and oxychozanide Nilzan™ after 10 and 15 days. A lower dose of 100 mg/kg of the glycosides after 15 days was almost as effective as Nilzan (Akhtar

and Aslam, 1997). Oil from *N. sativa* could therefore prove extremely useful as a natural antiparasitic treatment for children.

20.3.4 Anti-inflammatory activity

N. sativa seeds and oil are effective anti-inflammatory substances. The anti-inflammatory activities of the thymoquinone present in *N. sativa* are attributed to its antioxidant effects. It has been claimed that nigellone, the non-toxic carbonyl polymer of thymoquinone (Mahfouz and E1-Dakhakhny, 1966), thymoquinone (E1-Dakhakhny, 2000; El Gazzar *et al*., 2006) and thymohydroquinone (E1-Alfy *et al*., 1975) are the active principles responsible for the anti-inflammatory properties of *N. sativa.* Nigellone in low concentrations proved to be an effective inhibitor of histamine release induced by antigens and calcium ionophores. The mechanism of action is thought to involve the inhibition of the protein kinase C and a decrease in the intracellular calcium concentration.

The fixed oil prepared from *N. sativa* has been tested as a possible inhibitor of eicosanoid generation and membrane lipid peroxidation using thymoquinone, a potent anti-inflammatory agent, as a reference parameter. The crude fixed oil acted as an inhibitor of the cyclooxygenase and 5-1ipoxygenase pathways of arachidonic acid metabolism in rat peritoneal leucocytes stimulated with a calcium ionophore, but the activity was greater than would be anticipated from the thymoquinone content of the oil. This has led to the conclusion that the anti-inflammatory actions of the expressed fixed oil are not solely related to the thymoquinone content; the unusual C 20:2 fatty acids that are known to be present in the seed oil may also contribute (Houghton *et al.*, 1995). The anti-inflammatory effects of the volatile oil and thymoquinone were observed in the significant reduction of both carrageenan-induced oedema formation and cotton seed pellet granuloma weight. On the basis of these results, it has been concluded that the volatile oil has an anti-inflammatory action that involves the inhibition of eicosonoids and lipid peroxidation (Mutabagani and El-Mahdy, 1997). The soluble fraction of *N. sativa* seeds also had a stimulatory effect on the lymphocyte response to pooled allogenic cells, and also contributed to an increase in the production of interleukins (Haq *et al.*, 1995).

20.3.5 Antioxidant activities

N. sativa seeds and extracts have been reported to exhibit antioxidant properties, as they offer protection against damage caused by oxidation. Lado *et al*. (2004), Nagwa *et al*. (2006) and Adamu *et al*. (2010) have all found that nigella oils may be used as an antioxidant, while Musa *et al*. (2004) found that the ethanol extract can also generate antioxidants and was able to prolong the lifespan of mice. Recently Ibraheem *et al*. (2010) reported that *N. sativa* has calcium antagonist and antioxidant properties, both of which play a major role in the management of diseases. The highest percentage of hydrogen peroxide inhibition in hepatic microsomes of mice treated with lindane was shown by *N. sativa* seed extract (Awney *et al.*, 1997). Nour and Mourad (2010) also found that nigella oil had an antioxidant effect on monosodium glutamate-induced oxidative stress in the brain of rats, and nigella seed extract has also prevented oxidative deterioration.

The chemotherapeutic toxicity caused by cisplatin has been shown to be greatly reduced by using cisplatin in combination with plant extracts of *Crocus sativus* stigmas (50 mg/kg) and *N. sativa* seed (50 mg/kg) (El-Daly, 1998). Similarly, the oil has been shown to be useful as a protective agent against the side-effects of methotrexate chemotherapy (Labib *et al.*, 2009). On the basis of these results, it has been suggested that a nutritional supplement of nigella seed extract may offer better protection to the human body against oxidative damage than supplementation with synthetic antioxidants. In fact, the antioxidant activity of *N. sativa* extract was found to be comparable with that of t-butylhydroquinone (Atta and Imaizumi, 1998).

20.3.6 Immunological activities

N. sativa seed extract had an inhibitory effect on the human immunodeficiency virus protease; however, the active principle(s) responsible for the activity were not identified. A number of traditional medicines including the seeds of *N. sativa* have been examined *in vitro* for their HIV protease-inhibiting properties (Ma *et al.*, 1994). Studies have also been carried out on the effect of the volatile oil on T-cells (Nair *et al.*, 1991; Huschart *et al.*, 1994; Hailat *et al.*, 1995). From the effect of the volatile oil on Jurkat T-cell leukemia polypeptides, a possible post-translational modification of P24 protein has been suggested as a biological action (Hailat *et al.*, 1995). In mice, cisplatin-induced falls in haemoglobin levels and leucocyte count have been reduced by treatment with an extract of *N. sativa* seed. It has also been found that the plant extract modulates the immune system by increasing the number and activity of immune competent cells in humans (Medenica *et al.*, 1993; Kandil *et al.*, 1994). Nigella oil has played a significant role in altering the liver damage induced by *Schistosoma mansoni* infection in mice, and can help to improve the immunological system of the host (Mahmoud *et al.*, 2002). The immunomodulatory properties of *N. sativa* seed and thymoquinone support its traditional use for treatment in rheumatism and related inflammatory disorders. It may also be used as an immunopotentiating agent, and has strong immunomodulatory properties and interferon-like activity (Medenica *et al.*, 2000; Swamy and Tan, 2000). *N. sativa* seed extract modulates the neurotransmitter amino acid release in cultured neurons *in vitro* (El-Naggar *et al.*, 2010).

20.3.7 Anticarcinogenic activity

An organic fraction obtained from *N. sativa* seed was tested on cancer cells such as EAC, DLA, S-180 and was found to have anti-tumour properties (Salomi *et al.*, 1992). An alcoholic extract of *N. sativa* was screened for anticancer properties and was found to be active against Ehrlich ascites carcinoma in mice. The extract (160 mg/kg body wt) produced a significant increase of lifespan (Abdel-Salam *et al.*, 1998). *N. sativa* extract (100 mg/kg body wt) inhibited skin carcinogenesis in mice by delaying the onset of papilloma formation. The extract (100 mg/kg body wt) also restricted the incidence of tumour to 33.3 % in chemically-induced soft tissue sarcomas in albino mice (Salomi *et al.*, 1992). Nigella extract inhibits cancer and endothelial cell progression and decreases the production of the angiogenic protein

fibroblastic growth factor made by tumour cells. A monodesmosidic triterpene saponin, α-hederin, has also been isolated from the extract of nigella seeds and has been shown to exert antitumoural activity (Muthu Kumara and Huat, 2001).

20.3.8 Antidiabetic activities

Several studies have reported that *N. sativa* oil and extracts have an antidiabetic effect (Uddin *et al*., 2002; Kaleem *et al*., 2006; Bouchra *et al*., 2009). *N. sativa* and thymoquinone proved clinically useful for the treatment of diabetes and for protecting beta cells against oxidative stress (Abdel-Meguid *et al*., 2010). The oil has been reported to have significant effects in diabetic and dyslipidemic patients (Najmi *et al*., 2008). The blood sugar lowering effect of the plant extract has been investigated in animal experiments, and the results have indicated that the plant extract could act as a useful therapeutic agent in the treatment of non-insulin dependent diabetes mellitus (A1-Awadi *et al.*, 1985). Crude nigella appeared as effective as *n*-hexane extract for alleviating streptozotocin-induced diabetes mellitus (Khanam and Dewan, 2008). In a comparative study, the hypoglycaemic effect of the volatile oils of *N. sativa* and *Allium sativum* showed significant elevation of the serum insulin levels relative to glipizide (A1-Zuhair *et al.*, 1996). This synergic effect was higher for *A. sativum* than for *N. sativa. N. sativa* and four other medicinal plants of Egyptian origin have also been examined for their hypoglycaemic effect and a herbal formulation of the five plants was more effective in reducing blood glucose levels (Eskander *et al.*, 1995).

20.3.9 Hepatoprotective activities

Thymoquinone, found in *N. sativa* as a major constituent of its volatile oil, has been found to be an efficient cytoprotective agent against chemically-induced hepatic toxicity in animal experiments (Bashandy, 1996; Al-Gharably *et al*., 1997; El-Dakhakhny *et al.*, 2000; Kanter *et al*., 2005; Begum *et al*., 2008). Using the well-known hepatoprotective agent silybin as a reference, the hepatoprotective properties of thymoquinone have been further examined in isolated rat hepatocytes. Preincubation of the hepatocytes with thymoquinone or silybin (1 mM) yielded isolated hepatocytes that offered protection against tertbutyl hydroperoxide (TBHP)-induced injury (Daba and Abdel-Rahaman, 1998). *N. sativa* seeds are effective in treating patients suffering from liver cirrhosis and hepatocellular damage (Meral *et al*., 2001).

N. sativa oil (0.27 g/100 g body wt/day) was administered to adult and senile rats in order to determine its possible effect on the ageing process by measuring parameters thought be associated with ageing, such as functional and structural changes in the liver and kidney of the animals. The treated senile animals showed a decrease in serum cholesterol, total lipids, γ-glutamyl transferase, urea, uric acid and nuclear DNA content. The results have suggested that nigella seed oil may be able to slow down the ageing process in senile rats (Bashandy, 1996). *N. sativa* treatment has also been shown to protect the rat liver against hepatic ischemia-reperfusion injury (Yildiz *et al*., 2008).

20.3.10 Miscellaneous activities

Antispasmodic: In the early experimental work on *N. sativa,* which began in the 1980s, the alcoholic seed extract was fractionated by column chromatography on alumina to yield two organic fractions that showed hypotensive activities when tested on dogs. Fractionation of the alcohol–water extracts of the seeds afforded an organic fraction that displayed antispasmodic activity when tested on isolated rabbit intestine (Zawahry, 1963). The volatile oil was found to have some anti-oxytoxic potential based on *in vitro* experiments carried out on the uterine smooth muscle of rats and guinea pigs using isolated uterine horns (Aqel and Shaheen, 1996). However, the effects were concentration dependent and reversible by tissue washing. The volatile oil of *N. sativa* has been tested *in vitro* on the vascular smooth muscle, and it inhibited the norepinephrine-induced contractions of rabbit aortic rings in a solution containing Ca e+ ions; the activity was dose dependent and reversible (Aqel, 1992). In other studies the volatile oil functioned as a direct tracheal smooth muscle relaxant and a Ca 2+ antagonist (Aqel, 1992; Tanira *et al.,* 1996), in which contractions were induced by histamine and acetylcholine, respectively.

Anti-hypertensive: In animal experiments, the volatile oil showed potent centrally acting antihypertensive properties that were partly attributed to the presence of thymoquinone in the oil (Mahfouz *et al.*, 1962; E1-Tahir *et al.*, 1993). Experiments evaluating the effects of nigella volatile oil (30–120 zl/kg) on the arterial blood pressure and heart rate of urethane-anesthetized guinea pigs have indicated that the oil has cardiovascular-depressant effects which were speculated to be thymoquinone-related (El-Tahir and Ageel, 1994).

Analgesic: *N. sativa* oil has been shown to display CNS–depressant and potent analgesic properties in laboratory animal experiments. The analgesic activity of the oil has been attributed to the speculative presence of an opioid principle in the oil (Khanna *et al.*, 1993).

Growth-regulating: The effect of the seed oil on growth regulation in *Dysdercus similis* (F) has been examined. Petroleum ether fractions of the seeds at concentrations of 10.3–62.5 ppm were tested for growth–regulating [juvenile hormone (JH)] activity against larvae of *D. similis* (F). The *Nigella sativa* fraction showed high JH activity and this was considered to be due the fatty acid content in the seed extract (Kumar and Thakur, 1989).

Bronchodilatory: The bronchodilatory effects of the plant extract have long been known and studied (Mahfouz and El-Dakhakhny, 1966). Animal experiments have indicated that the volatile oil may act as a centrally acting stimulant of the respiratory system, provided the thymoquinone content in the oil can be removed (El-Tahir *et al.*, 1993). Nigellone is thought to be a promising substance for the prevention and control of bronchial asthma and other allergic conditions (Chakravarty, 1993). The role *N. sativa* oil in the management of wheeze associated lower respiratory tract illness in children has also been investigated (Ahmad *et al*., 2009).

Gastroprotective: *N. sativa* seeds in the diet have a favourable effect on the lipid profile by lowering the triglyceride, total cholesterol and LDL cholesterol and

increasing the HDL cholesterol in albino rats (Buriro and Tayyab, 2007). The gastroprotective effects of *N. sativa* oil on the formulation of stress gastritis have been reported in hypothyroidal rats (Abdel-Sater, 2009). Gastric antiulcer effects have also been reported by Rajkapoor *et al*. (2002).

Other health effects:

- *N. sativa* and gluthione have an antiperoxidative effect and are also beneficial in protecting against ionizing radiation-related tissue injury (Cemek *et al*., 2006). The radioprotective properties of the seed oil of *N. sativa* were discussed by Abdel Salarn *et al*. (1998); its use showed significant improvement in DNA, RNA, super oxide dismatase (SOD) and glutathurane (GSH) profiles and thereby enhanced longevity in animals. The expressed oil of *N. sativa* was the subject of earlier studies on radioprotection and has been shown to normalize enzymatic changes in the liver tissue that occur as a result of exposure to ionizing radiation in rabbits (Karawya *et al.*, 1994; E1-Bahy, 1997).
- Ethanolic nigella extract helped in reducing the number of calcium oxalate deposites on ethylene glycol-induced kidney calculi (Hadjzadeh *et al*., 2007).
- The use of *N. sativa* oil to treat and heal chemically-induced wounds in rabbit skin was found to be effective (Zinadah-Abu, 2009).
- The alcoholic extract of *N. sativa*, administered orally on a daily basis, clearly improved the reproductive performance of male rats (Noor, 2008; Al-Sa'aidi *et al*., 2009) and brought about increased spermatogenesis in male albino rats (Mohammed *et al*., 2009).
- Thymoquinone, the principal active component of nigella seeds (Hosseinzadeh and Parvardeh, 2004), has been shown to suppress epileptic seizures in rats (Hosseinzadeh *et al*., 2005).
- Nigella powder can be used as vinegar and applied on spots caused by vitiligo, followed by exposure to sunlight. A decoction of seeds mixed with sesame oil is used externally in various skin eruptions. Nigella has also proved useful in the treatment of dermatitis (Zedlitz *et al*., 2002).

20.4 Toxicity

Nigella seed yields a volatile oil containing melanthin, nigelline, damascene and tannin. Melanthin is toxic in large doses and nigelline causes paralysis, so this spice must be used in moderation. The traditional use of nigella seeds is supported by Zaoui *et al*. (2002b) for the treatment of dyslipidaemia, hyperglycaemia and related abnormities; however, the same study indicates that the plant can be relatively toxic. In another report, Zaoui *et al*. (2002a) reported acute and chronic toxicity caused by *N. sativa* fixed oil. The methanol extract from the related species *N. damascene* seeds showed a high oestrogenic activity. Among the purified phenolic compounds tested, the phenolic ester 1-0-(2,4-dihydroxy) benzolglycelrol showed the strongest oestrogenic activity due to the presence of flavonoid compounds (Agardi *et al.*, 2000). As a result, *N. damascene* should not be used as a substitute for *N. sativa* (Chevallier, 2001). The seed powder did not produce any toxic effect at very high doses (28 mg/kg orally) and oral thymoquinone was also found to be safe at LD50

of 2.4 g/kg (Tissera *et al*., 1997). Nigella is a safe and effective herb that can be used by almost anyone under the advice of a herbal practitioner. No health hazards or side-effects are associated with the proper administration of designated therapeutic dosages, although dermatitis has been reported in some cases (Sharma *et al*., 2009).

20.5 Quality issues

20.5.1 Specification for whole seed

The quality of nigella seed mainly depends on its appearance. The seeds should be matt-black, triangular, 1.5–3 mm long, uniform in size, shape and texture and have an oily, white interior. The odour of nigella seeds when crushed should resemble strawberry. Some authors have mentioned that its smell is similar to that of oregano or carrots.

The Indian Agmark grade specifications for nigella seeds as laid down in the Prevention of Food Adulteration Indian Act (PFA standards) are given below:

- Seed moisture: not more than 11 % by weight
- Total ash: not more than 6 % by weight
- Ash insoluble in acid: not more than 1 % by weight
- Organic extraneous matters: not more than 3 % by weight
- Inorganic extraneous matters: not more than 2 % by weight
- Volatile oil: not less than 1 % (v/w)
- Ether extract (crude oil): not less than 35 % (v/w)
- Alcoholic acidity as: not more than 7 % (v/w) oleic acid

ASTA, ESA and ISO have not laid down specifications for nigella seed. This may be because there is less demand for the spice in European and American countries. India exports nigella to neighbouring countries, the Middle East and the Gulf states, though, to satisfy a demand from the many expatriate Asian workers.

Nigella powder is produced by grinding dried, cleaned and sterilized seed. After sieving through the required mesh size, the powder should be packed in airtight containers. The freeze-grinding technique can be used to avoid loss of flavour during heat grinding. The powder is creamy white in colour with an aroma like strawberry. The specification for whole nigella seed should be strictly followed in powder production in addition to any seed powder quality specifications. The quality standards as laid down under the Prevention of Food Adulteration (PFA) Act and Rules for nigella powder are given below (Anon., 1998):

- Powder means the ground product of dried seeds of *N. sativa* L.
- Moisture: not more than 12 % by weight
- Total ash: not more than 7 % by weight
- Ash soluble in dilute HCL: not more than 1.5 % by weight
- Volatile oil: not less than 0.5 % (v/w)
- It should be free from added colouring matter.

20.5.2 Volatile oil and fixed oils

The volatile oil content of nigella seed averages 0.5–1.4 % and it contains primarily the glucosides melanthin and melathingenin, bitter substances, and a crystalline

active principle, nigellone. The aroma of nigella oil is warm, spicy and fatty and the flavour is strawberry-like with a burning sensation. The volatile oil of nigella is yellowish brown with an unpleasant flavour. The physiological properties of nigella oil are given below (Pruthi, 2001):

- Specific gravity at 15°C: 0.875–0.886
- Refractive index at 20°C: 1.4836–1.4844
- Optical rotation at 20°C: +1.43° to + 2.86°
- Acid value: up to 1.9
- Ester value: 1.0–21.6
- Ester value (after acetylation): 15–73
- Solubility: 2-4.5 or more vols alcohol

Fixed oils are also extracted from nigella seeds. The fatty oil obtained from seeds is used for edible purposes. Extraction with benzene and subsequent steam distillation of the extract to remove volatile oil gave about 31 % of a reddish brown, semi-drying oil with the following characteristics:

- Specific gravity at 25°C: 0.9152
- Refractive index at 21°C: 1.4662
- Acid value: 42.83
- Saponification value: 199.6
- Iodine value: 117.6
- RM value: 3.9 %
- Unsaponifiable matter: 0.03 %

Nigella oleoresins can be extracted from the seeds but have little commercial value. Therefore, there are no ISO, ASTA or ESA specifications for nigella oleoresins.

20.5.3 Adulteration

Nigella seed is available both whole and in ground form. The whole seed is subject to adulteration by onion seeds, because of their similarity. Onion seeds lose viability after one year and such unused seeds are used to adulterate batches of nigella seed. Another form of adulteration is when the exhausted seed or spent seed after oil extraction is mixed in whole seed or ground form with unprocessed nigella seeds. Essential oil extracted from seeds has also been found to be adulterated with chaff oil. The range of essential oil is 0.5–1.4 %. It should contain melangin as the major component, and levels of this compound should not go below 30 %. A high ratio of eicosadienoic acid to eicosamonoenoic acid combined with a high level of CO_2 fatty acids, is characteristic of nigella seed oils and could be used to identify genuine oil (Weiss, 2002). The adulterants can be detected through chromatographical techniques.

20.6 References

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21

Oregano

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Abstract: The present review focuses on the various *Oregano* species, oregano being one of the most famous – and economically important – culinary herbs in the world. The botany and chemotaxonomy of the species is thoroughly reported, along with cultivation methods and breeding techniques (including biotechnology), details of the chemical constituents of its essential oil, its traditional uses and medicinal uses (including its applications as an antimicrobial and antioxidant). Quality specifications and commercial issues are also covered.

Key words: oregano, biotechnology, botany, breeding, chemotaxonomy, cultivation, ethnobotany, medical uses, *Origanum* sp.

21.1 Introduction and description

Oregano is the common name for a general aroma and flavour primarily derived from a plethora of plant genera and species used all over the world as a spice, but usually refers to the genus *Origanum*, the European oregano, the name of which is derived from the Greek words *oros*, mountain and hill, and *ganos*, ornament. At least 61 species of 17 genera belonging to six families are mentioned under the name oregano. The family Lamiaceae (Labiatae) is considered to be the most important group containing the genus *Origanum* that provides the source of well-known oregano spices – Turkish and Greek types. Two genera of the Verbenaceae family (*Lanata* and *Lippia*) are used for production of oregano herbs. The other families (Rubiaceae, Scrophulariaceae, Apiaceae and Asteraceae) have a limited importance. However, we frequently encounter the herbs of the above-mentioned families under the name of oregano in the market (Bernath, 1997).

21.1.1 Botanical characteristics

Oregano is generally considered as a perennial herb, with creeping roots, branched woody stems and opposite, petiolate and hairy leaves (Grieve, 1994). The flowers are in corymbs with reddish bracts, a two-lipped pale purple corolla and a five-toothed calyx. In moderate climates, the flowering period extends from late June to

August. Each flower produces, when mature, four small seed-like structures. The foliage is dotted with small glands containing the volatile or essential oil that gives the plant its aroma and flavour (Simon *et al*., 1984).

21.1.2 Taxonomy and geographical distribution

During the past 150 years, more than 300 scientific names have been given to fewer than 70 presently recognized *Origanum* species, sub-species, varieties and hybrids. Within the genus *Origanum*, and based on a diverse palette of morphological characters, such as length of stems, indumentum of stems and leaves, number of sessile glands on leaves, arrangement of verticillasters, arrangements, number and length of branches, Ietswaart (1980) recognized three groups, 10 sections, 38 species, six sub-species and 17 hybrids. Since then, five more species (Duman *et al*., 1995; Danin and Künne, 1996; Skoula and Harborne, 2002) and one more hybrid (Duman *et al*., 1998) have been recognized, raising the number of species to 43 and the number of hybrids to 18.

Ietwaart's three groups are classified as follows:

- Group A has two- or one-lipped, rather large, calyces 4–12 mm long. Bracts are rather large 4–25 mm long, membranous, usually purple, sometimes yellowish green, more or less glabrous.
- Group B has two- or one-lipped, rather small, calyces 1.3–3.5 mm long. Bracts are rather small 1–5 mm, leaf-like in texture and colour, more or less hairy.
- Group C has calyces with five (sub)equal teeth.

The members of the genus are mainly distributed around the Mediterranean region: 35 out of 43 occur in the East Mediterranean, exclusively (Greuter *et al*., 1986); four species are found restricted in the West Mediterranean, while three are endemic to Libya. In addition, hybrids have been found when *Origanum* species co-occur, either in natural or in artificial conditions. Often hybrids have been considered initially as species, as in the case of *Majorana leptoclados* (*Origanum* × *minoanum*), *Origanum paniculatum* Koch. (*Origanum* × *aplii* Boros), *Amaracus lirius* Hayek (*Origanum* × *lirium* Heldreich ex Halacsy) and others (Skoula and Harborne, 2002). Taxonomic investigations on the genus are currently conducted with the aid of molecular markers (Katsiotis *et al*., 2009).

21.1.3 Chemical structure

Chemical composition of Origanum *species and their volatile oils*

Although abundant chemical compounds have been isolated from oregano, the most important group, from a commercial and application point of view, refers to its volatile oils, basically composed of terpenoids. A comprehensive review of the composition of a 'standard' essential oil is given in Table 21.1. However, composition may vary significantly among different genotypes. Oregano species are rich in phenolic monoterpenoids such as carvacrol (Fig. 21.1) and, secondarily, thymol (Fig. 21.2), while species rich in bicyclic monoterpenoids *cis*- and *trans*-sabinene hydrate (Fig. 21.3) are commercially designated as marjoram. It is quite easy to distinguish the difference between the pungent smell of oregano and the sweet smell of marjoram. In the first group are a number of chemically related compounds such

Table 21.1 Comprehensive composition of oregano essential oil

Cymyl- compounds	**Sesquiterpenoids**
p-Cymene	*allo*-aromadendrene
p-Cymenene	β-Bisabolene
p-Cymen-8-ol	β-Bourbonene
Carvacrol	γ-Cadinene
Carvacrol acetate	α-Cadinol
Carvacrol methylether	β-Caryophyllene
γ-Terpinene	Caryophyllene oxide
Thymol	α-Copaene
Thymol acetate	β-Cubonene
Thymohydroquinone	Germacrene-D
Thymoquinone	Germacrene-D-ol
	Bicyclogermacrene
Sabinyl- compounds	α-Humulene
Sabinene	α-Muurolene
Sabinene hydrate	γ-Muurolene
cis-Sabinene hydrate	
trans-Sabinene hydrate	**Diterpenoids**
cis-Sabinene hydrate acetate	Akhdarenol
trans-Sabinene hydrate acetate	Akhdardiol
cis-Sabinol	Akhdartriol
trans-Sabinol	Isoakhdartriol
Sabina ketone	
Sabinyl acetate	**Triterpenoids**
Thujene	β-Amyrin
	Betulic acid
Acyclic compounds	Betulin
Geraniol	Methyl-3β-21α-dihydroxyurs-12-en-28-olic acid
Geranyl acetate	
Linalool	Oleanolic acid
Linalyl acetate	Ursolic acid
β-Myrcene	Uvaol
Bornyl- compounds	
Borneol	
Bornylacetate	
Camphene	
Camphor	
Isoborneol	
Isobornyl aceate	

as γ-terpinene (Fig. 21.4), *p*-cymene, thymol and carvacrol methyl ethers, thymol and carvacrol acetates; compounds such as *p*-cymenene, *p*-cymen-8-ol, *p*-cymen-7-ol, thymoquinone and thymohydroquinone are also present. In the second group, α-thujene, sabinene, *cis*- and *trans*-sabinene hydrate acetates, *cis*- and *trans*-sabinol and sabina ketone can also be found (Skoula and Harborne, 2002).

Other chemical groups that are commonly detected in *Origanum* species are acyclic monoterpenoids such as geraniol, geranyl acetate, linalool, linalyl acetate and β-myrcene; bornane-type compounds such as camphene, camphor, borneol, and bornyl and isobornyl acetate; and sesquiterpenoids, such as β-caryophyllene, β-bisabolene, β-bourbonene, germacrene-D, bicyclogermacrene, α-humulene,

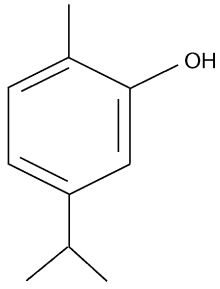

Fig. 21.1 Carvacrol.

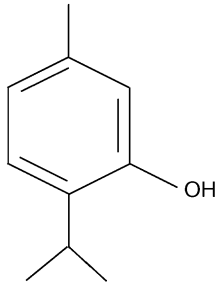

Fig. 21.2 Thymol.

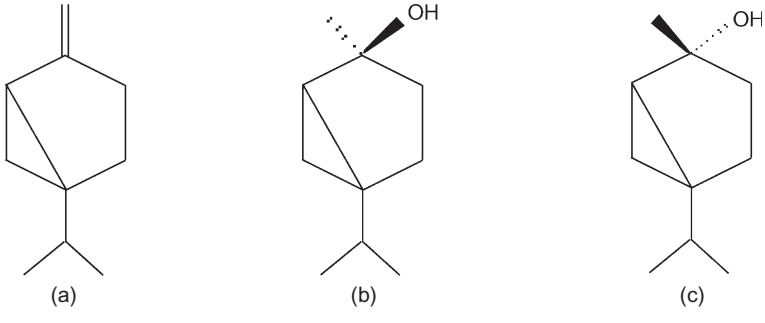

Fig. 21.3 (a) Sabiene, (b) *cis*- and (c) *trans*-sabiene hydrates.

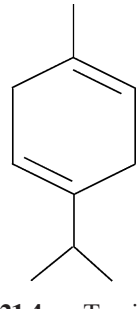

Fig. 21.4 γ-Terpinene.

α-muurolene, γ-muurolene, γ-cadinene, allo-aromadendrene, α-cubebene, α-copaene, α-cadinol, coryophyllene oxide and germacrene-D-4-ol.

Chemotaxonomy

From a chemotaxonomical point of view, the qualitative variation of the volatile compounds at the infrageneric level is quite considerable. At the infraspecific level, it has been reported that *O. vulgare* ssp. *hirtum* plants produce fewer essential oils during the cool and wet vegetative period and more during the warm and dry flowering period, and essential oil yield decreases thereafter, as leaves get older and drier. In addition, the concentrations of *p*-cymene and γ-terpinene fluctuate enormously according to season (Poulose and Croteau, 1978; Skoula *et al*., unpublished data; Skoula and Harborne, 2002). The decline in total essential oil and of thymol or carvacrol, which occurs naturally in the autumn, can be mimicked by growing *O. syriacum* in short days (Putievsky *et al*., 1997). Similarly, *O. majorana* grown in controlled conditions under short days yields fewer essential oils (Circella *et al*., 1995). Kokkini *et al*. (1997) reported high content of *p*-cymene in the essential oils of wild *O. vulgare* ssp. *hirtum* collected in autumn.

Besides the qualitative variation of the volatile compounds at the infrageneric level, there is considerable quantitative variation at the infraspecific level. Remarkable chemical variations have been observed not only between but also within populations and accessions. For example, single plant investigations of a grouping of *O. vulgare* ssp. and their offspring resulted in an unexpected differentiation into chemotypes, including one with a marjoram-like profile, but growth characteristics and winter hardiness of a *O. vulgare* ssp. (Marn *et al*.,1999), whereas carvacrol contents ranging from traces up to 95 % of the essential oil are described (Kokkini *et al*., 1991).

21.2 Production and cultivation

21.2.1 Growth habit of wild oregano populations

As a perennial species, oregano grows spontaneously in areas across the Mediterranean region, particularly in high locations. In these areas, oregano is harvested mainly from wild populations, once or twice a year, at flowering stage. The reported life zone of marjoram (*O. majorana* L.), is 6–28 °C with an annual precipitation of 0.5–2.7 m and a soil pH of 4.9–8.7. The plant is adapted to well-drained, fertile loam soils. The cold-sensitive plant cannot survive northern climates.

O. vulgare L., and the sub-species *O. vulgare* subsp. *vulgare*, *O. vulgare* subsp. *viride* and *O. onites*, originate from the Mediterranean and are closely related to marjoram. They grow to a height of about 20 cm, with woody stems and dark green leaves around 2 cm long. The plants protect the inclined soils, and are quite tolerant to cold and dryness. During the winter, the aerial parts are destroyed, but the roots maintain their vitality for revegetation in spring. Oregano grows in medium soils and in areas with high elevation and cool summer (Makri, 2002). Plants seed in warm soil in late summer and can be moved outdoors after 3–4 months. Oregano is best treated as an annual in cold climates where it will not over-winter well. When grown as a perennial, roots should be divided every 3 years for best growth and

flavour. Older plants will do well as a potted plant as long as they receive sufficient sunlight. As with most herbs, desiccated plant parts should be removed as frequently as necessary (Sarlis, 1994). Commercial material of oregano (*O. vulgare*) is partially collected from wild plants even today. To avoid the disadvantages of exploiting oregano directly from the wild, efforts have been made in its domestication and cultivation. Growing wild oregano is rather easy. It grows well in shade; the cultivated sub-species *O. v. hirtum* does not.

21.2.2 Cultivation

For cultivation, marjoram is both seeded directly and transplanted into fields. Oregano has a spreading root system and is usually propagated by seed or cuttings, the latter being removed in late spring once the leaves are firm enough to prevent wilting when placed in sand (average shoot length: 30 cm). Well-rooted cuttings are placed in the ground about 30 cm apart or planted outside in pots. If seeds are used, they should be sown in a seedbox in spring and planted outside when seedlings are 7.5 cm tall. Old wood that becomes leggy should be cut out at the end of winter and plants should be replaced every 4 years or so to prevent legginess. Pungency declines in rich soils and after flowering.

Ploughing of the soil and fertilization with ammonium phosphate during November–December is sufficient for oregano cultivation; the effect of nitrogen fertilization has been recently reviewed by Sotiropoulou and Karamanos (2011). They found that nitrogen application promoted oregano herbage yield, branching and secondary stems with an optimum at 80 kg N/ha. No effects on oil concentration were observed. Dordas (2009) reported that magnesium and calcium applications promoted growth and essential oil accumulation. Under normal conditions, pest control can be reduced to a simple weeding out (manually or by using pesticides) (Zumelzú *et al*., 1999; Kintzios, 2002a; Makri, 2002) although aphids, thrips and red spider mites may occasionally present a problem (Csizinszky, 1992). In addition, *O. majorana* can be severely affected by *Alternaria* and *Fusarium*. There is scarce documentation on biological pest control in oregano, which needs frequent (e.g. at least four times a year) mechanical weed control (Chiapparo, 1997; Hammer and Junghanns, 1997). The lifespan of oregano is about 5 or 6 years and usually one harvest is done in the first year and two in the following years. On average, the yield ranges from 2.5–3.5 t/ha and the essential oil yield ranges from 0.5–1.5 % of dry weight (Bernath, 1997).

Cultivation practices may differ from one country to another: for example, Hungarian and German farmers prefer to establish oregano plantations by means of seed propagation (Bernath, 1997; Hammer and Junghanns, 1997), whereas their colleagues in the Mediterranean Basin, Slovenia and the Federal Republic of Yugoslavia prefer to use stem cuttings (Macko and Cok, 1989; Baricevic 1997; Putievsky *et al*., 1997). The percentage of seed germination does not exceed 75 % and declines rapidly with time. Germination occurs over a relatively low temperature range, with an optimum temperature around 15–20 °C (Kozlowski and Szczyglewska, 1994; Thanos *et al*., 1995). Seedlings are usually planted with a spacing of 50–60 cm between rows and 20–25 cm within rows (spacing within rows may reach 40–45 cm in dry areas), therefore allowing for a plant density of approximately 3000 plants/ha. In

humid areas, however, or under conditions of frequent irrigation, plant densities up to 63 000 plants/ha have been reported. Irrigation is required only at the time of planting and a few other times in the first year. In the following years, plants have developed an efficient root system, and thus no further irrigation is usually needed.

21.2.3 Harvest

Depending on irrigation frequency and, subsequently, yield, two or three harvests of the crop are allowed annually. Harvesting the leaves and stem tips should start when plants are at the flowering stage, beginning 10 cm from the ground. In dry climates, the best harvest time to collect the highest amount of essential oil is when 50 % of the plants in the field have started flowering. In relatively small fields, harvest is usually done manually, mechanical harvesting being recommended only for large fields.

Harvesting is generally accomplished at full bloom. Plant material is often dried in drying sheets to avoid direct sunlight and thus preserve the green colour and aroma (Sarlis, 1994; Makri, 2002).

After harvesting, plants are dried in the shade. Although drying under natural conditions is a common procedure, drying ovens operating at 30–35 ºC can also be used in commercial-scale production. Moisture content of 7 % (min.) to 12 % (max.) is required (Kitiki, 1997). Leaves should be dried in a warm, dry, shaded place and stored in an airtight container.

Pääkkónen *et al*. (1990) studied the effect of different drying and storage methods on oregano and marjoram. Marjoram was harvested at the time of bud formation and oregano when in bloom. Herbs were either dried immediately after harvesting or were frozen and stored at –20 °C and freeze dried within 2 weeks. For convection drying, temperature was 35–37 °C and for freeze drying 30 °C. The corresponding drying times were 24 and 12 hours. The moisture content of fresh herbs was 85 % for marjoram and 75 % for oregano. After drying with heated air, the moisture content for marjoram was 9 %, and 7 % for oregano, but only 5 % after freeze drying. The drying method did not affect the water-holding capacity of the dried product; neither did it have any effect on either odour or the taste of dried oregano. A detrimental effect of elevated storage temperature was obvious. In another study (Hälvä, 1987), the concentration of volatile oils in oregano decreased from 2.55 % to 1.94 % in the drying process.

21.2.4 Breeding

As already mentioned above, there remains limited knowledge of the biosynthesis of the essential oil compounds and their inheritance, which would be useful for more effective selection and establishing a targeted breeding programme; only some key enzymes have been identified so far for carvacrol, thymol and linalool synthesis in *Origanum* (Croteau and Karp, 1991; Franz and Novak, 2002). For all these reasons, wild collection accompanied by assurance systems (sustainability, good horticultural practice) aimed at maintaining quality and species and/or field production of reliable genotypes are the future methods of choice for quality products. The enormous inter- and intraspecific chemical polymorphism of *Oregano* sp. offers great scope

for selection with a view to producing specific monoterpenes offering fine chemicals, new odour and flavour profiles, etc. Crop improvement is highly recommended considering oregano's widespread use and the great difficulties that non-uniform material may cause to the commercial sector. Taking into consideration both producers' and users' needs, the objective of any oregano breeding programme should be improvement in the following areas: yield-related parameters, e.g. growth habit, leaf/stem ratio, stress (salt, cold) tolerance, resistance to diseases, and quality-related parameters, e.g. better aromatic characteristics, colour (green is preferred to grey), essential oil content (usually more than 2 %) and composition, antioxidant and antimicrobial properties. In particular, and as far as the composition of essential oils is concerned, a high carvacrol or *cis*-sabinene-hydrate content is desired in oregano or marjoram, respectively. Among agronomical traits, yield is one of the most important parameters securing the necessary productivity for competing on the market. The variation between single plants can range from around 10 g dried leaf/flower-fraction per plant up to 250 g (Marn *et al*., 1999). Improvement in yield can also be obtained within a relatively short time of breeding.

To achieve these goals, selection and hybridization methods, combined with analytical controls on the variability encountered in the material, are the most appropriate tools for crop improvement. Local strains of *O. vulgare* sub-species and *O. majorana* (*Majorana hortensis*), as well as spontaneous hybrids (*Origanum* × *majoricum*, *Origanum* × *intercedens*), are traditionally cultivated in many countries. For example, oregano clones are also cultivated in South America (Farías *et al.*, 2010). In addition, several ornamental varieties are also present on the market. Breeding of oregano started in relatively recent times. Breeding work has focused mainly on *O. majorana*, *O. syriacum*, *O. virens*, *O. vulgare* subsp. *Hirtum* and some hybrids, by using chemotaxonomy results and male sterility as tools for controlled crossings (Kheyr-Pour, 1981). In this context, it is worth mentioning that oregano belongs to the species with the smallest fruits, weighing only approx. 60 μ*g* per seed (thousand seed mass = 0.06 g) (Thanos *et al*., 1995). Because of this, direct sowing of oregano is difficult and up to now planting has been preferred (Franz and Novak, 2002). Artificial pollination is also difficult because of the small flower size and the high number of flowers within an inflorescence. The key to improving the direct sowing production technique will be selecting for higher seed weight since seed quality, germinability and vigour depend on it.

Systematic breeding programmes in several, mostly Mediterranean, countries are using indigenous wild species as starting material and have already produced promising results (Franz, 1990; Franz and Novak, 2002). Although few studies have yet been reported on the tissue culture of oregano (Svoboda *et al*., 1995; Matsubara *et al*., 1996; Baricevic *et al*., 1997; Fortunato *et al*., 2006; Özkum and Tıpırdamaz, 2007), such studies enable biotechnological methods to be used for the enhancement of breeding activities (e.g. *in vitro* selection for disease resistance, exploitation of somaclonal variation) (for a review see Kintzios, 2002b). On the other hand, oregano tissue cultures have been used as a model for studying phenolic metabolism in plants. The major part of this work has been carried out by Shetty and co-workers (Shetty, 1997; Yang and Shetty, 1998; Zheng *et al*., 2001; Shetty and McCue, 2003; Shetty, 2004). Following up on these studies, Lattanzio *et al*. (2009) showed that both nutritional

stress and exogenous proline induce a significant increase of the antioxidant activity of oregano shoot extracts, and this increase is essentially related to increased level of rosmarinic acid, whose level is enhanced by nutritional stress (+158 %) and exogenous proline (+234 %) more than total phenolic content (+120 %).

21.3 Main uses in food processing and medicine

Trade in oregano has been established since classic times. Ceramic containers known as *amphoras* were widely used throughout the Mediterranean region as inexpensive, disposable containers for seaborne bulk commodity transport. Hansson and Foley (2008) reported the identification of ancient oregano DNA from two 2400 year-old amphoras excavated from a deep water shipwreck site in the Mediterranean, off the Greek Aegean island of Chios. Apparently, oregano had been mixed with oil as a herbal additive for flavour and preservation. In addition, fragments from contemporary ancient Greek texts describe olive oil, herbs and spices (such as oregano, thyme and cumin) as common ingredients in fourth century BC cuisine (Olson and Sens, 2000).

Oregano is used in meat, sausages, salads, stews, dressings and soups. The food industry uses oregano oil and oregano resin in both foods and beverages, and it is also used in cosmetics. Oregano oil is used in alcoholic beverages, baked goods, meats and meat products, condiments and relishes, milk products, processed vegetables, snack foods and fats and oils. It is the most common spice for pizza. Along with black pepper, it is a common ingredient of dressings and a good substitute for table salt. Marjoram, too, is used in many foods and beverages in food industry; meat sauces, canned foods, vinegar, vermouths and bitters are often seasoned with marjoram. It increases aroma in such vegetable dishes as pea soup and other pea dishes, squash and stews made from mixed vegetables, mushrooms and asparagus.

21.3.1 Dietary value

The dietary value of oregano is quite high: it contains significant amounts of vitamins E, B6, riboflavin, niacin, folate, pantothenate and biotin (Holland *et al*., 1991). Relatively high values (expressed as mg/100 g fresh leaves) have also been reported for vitamin C (45), thiamin (0.07) and carotene (0.81). Lagouri and Boskou (1996) detected α-, β-, γ- and δ-tocopherol in a non-polar fraction of oregano extracts, with the γ-tocopherol content being significantly higher than other tocopherol homologues. Oregano is also rich in mineral elements such as potassium, calcium, magnesium, phosphorus, zinc, manganese, iron, copper, sulphur, chlorine, iodine and selenium, whereas its sodium content is low. However, Brune *et al*. (1989) reported that oregano inhibits iron absorption, that the effect is caused by its galloyl substances and that the inhibition is in proportion to its content of galloyl groups. Oregano also has a relatively modest energy and fat content (66 kcal/100 g and 2 g fat/100 g, respectively). According to Gray *et al*. (1997), the concentration of oregano in food can increase or reduce its palatability and intake compared with an unseasoned control food.

21.3.2 Food-preserving properties

Apart from its dietary value, oregano is an effective antioxidant additive in different types of foods, such as mayonnaise and French dressing (Chipault *et al*., 1956; Nakatani and Kikuzaki, 1987; Baratta *et al*., 1998). This property is usually attributed to the high carvacrol content of the spice (Tsimidou and Boskou, 1994), although additional compounds, such as flavonoids may also be responsible (Vekiari *et al*., 1993; Mexis *et al*., 2009). The particular impact of oregano essential oil and phenolic extracts as a superb additive in meat products is reviewed by Simitzis *et al*. (2008) and Weiss *et al*. (2009).

21.3.3 Medicinal uses

There are various reports on the traditional medicinal uses European oregano has as a carminative, diaphoretic, expectorant, emmenagogue, stimulant, stomachic and tonic. In addition, it has been used as a folk remedy against colic, coughs, headaches, nervousness, toothaches and irregular menstrual cycles. Turkish villagers have traditionally used *kekik* water, the aromatic water obtained after removing essential oil from the distillate of oregano herbs, which has in recent years become a commercial commodity (Baser, 2002; Kintzios, 2002a). Although the monograph documentation of *O. vulgare* was submitted to the German Ministry of Health, the staff responsible for phytotherapeutic medicinal domain – Commission E – evaluated *Origani vulgaris herba* negatively (Banz. No. 122 from 6 July 1988), because of lack of scientific proof for a number of indication areas (Blumenthal, 1998). Nevertheless, many of the studies confirmed benefits of oregano for human health and its use for the treatment of a vast list of ailments, including respiratory tract disorders such as cough or bronchial catarrh (as expectorant and spasmolitic agent), in gastrointestinal disorders (as choleretic, digestive, eupeptic and spasmolitic agent), as an oral antiseptic, in urinary tract disorders (as diuretic and antiseptic) and in dermatological affections (alleviation of itching, healing crusts, insect stings), viral infections and even cancer (for a detailed review, see Baricevic and Bartol, 2002).

21.3.4 Microbiological quality and safety considerations

Although oregano can cause aversion symptoms during pregnancy (Hook, 1980), its consumption is considered safe from the chemical point of view. However, considerations have been frequently raised on the microbiological quality of preserved oregano. For example, Mäkinen *et al*. (1986) and Malmsten *et al*. (1991) tested the microbiological quality of marjoram and of oregano and detected moulds and aerobic spore-formers, especially *Bacillus cereus*, in most samples (although at concentrations not high enough to cause food poisoning). Coliforms and faecal streptococci were found in both freeze-dried and air-dried samples, but only sporadically and at very low counts. Moulds and yeasts were found in almost all samples, while increasing the storage time from one year to two increased tenfold the number of aerobic spore-formers in both freeze-dried and air-dried oregano. However, as demonstrated below, microbial contamination of oregano is not a common source of concern, owing to the antimicrobiological properties of the herb. As with most

spices, being natural agricultural commodities, it is not uncommon to find foreign debris, such as dirt, sand, glass, bird feather, whole insects and insect fragments in the raw product. Therefore, ISO, ASTA and the US FDA have set cleanliness specifications (or guidelines) that all imported oregano should meet.

21.4 Functional properties

21.4.1 Antioxidant properties

Oregano extracts have documented antioxidant and antimicrobial properties (Dorofeev *et al*., 1989; Mirovich *et al*., 1989; Deighton *et al*., 1993), which have been attributed to phenylcarboxylic acids, such as cinnamic, caffeic, *p*-hydroxybenzoic, syringic, protocatecholic and vanillic acids. Dietary supplies of antioxidants from *Origanum* species have been considered as effective scavengers of the free radicals that are generated by metabolic pathways in the body; however, limited industrial applications are often ascribed to the characteristic oregano aroma and flavour that influence the sensorial characteristics of processed food, so deodorization steps would be required (Nguyen *et al*., 1991; Moure *et al*., 2001).

Taking these limitations into consideration, practical investigation into the use of oregano as a stabilizer of edible oils or of finished meat products has been carried out by several research groups (Baricevic and Bartol, 2002). Dry leaves of *O. vulgare* ssp. *hirtum* showed a high antioxidant activity in olive oil and, besides their stabilizing effect, the organoleptic quality of the olive oil was significantly improved by addition of oregano, as assessed by Mediterranean consumer acceptability studies (Antoun and Tsimidou, 1997; Charai *et al*., 1999). A significant increase in the oxidative stability of fried chips, measured as the rate of peroxide formation during storage at 63 ºC, was achieved both by addition of ground oregano or its petroleum ether extracts (Lolos *et al*., 1999). In contrast with the significant antioxidative and stabilizing effects of oregano extracts in lard and various oils, contradictory effects on the quality or shelf-life of the fat obtained from animals fed with oregano additives or of meat and fat-containing foods were observed [for a comparison, see Vichi *et al*. (2001) and Fasseas *et al*. (2008)].

Supercritical fluid extraction has been successfully applied to the isolation of antioxidant moieties from oregano (Cavero *et al*., 2006; Ocaña-Fuentes *et al*., 2010). More recently, Kintzios *et al*. (2010) used a spectrophotometric assay to investigate the antioxidant activity of methanolic and water extracts of *Origanum vulgare* subsp. *vulgare* for free radical-scavenging activity against the $DPPH^{\cdot}$ free radical, while their biological activity was studied with the help of a laboratory-made biosensor based on immobilized fibroblast cells.

21.4.2 Antimicrobial properties

In conjunction with the antioxidant properties of the herb, there are abundant reports on the microbial inhibitory effects of oregano essential oil or its components. These effects are generally classified either as antifungal or antibacterial. The available literature on this subject is quite considerable, so that only some representative cases can be mentioned within the scope of the present chapter. According to

general consensus, there is a relationship between the chemical structure of the most abundant essential oil components and their antifungal and anti-aflatoxigenic potency, which is, in addition, strongly correlated with the concentration of the essential oil or active ingredient and pH of the testing medium *in vitro* (Deans and Svoboda, 1990; Thompson, 1990; Biondi *et al*., 1993; Baricevic and Bartol, 2002). Phenols are believed to be the most potent antimicrobials, followed by alcohols, ketones, ethers and hydrocarbons (Bullerman *et al*., 1977; Hitokoto *et al*., 1980; Hussein, 1990; Daw *et al*., 1994; Charai *et al*., 1996). In more practical terms, ground oregano (at 2 % concentration) was found to possess a strong antifungal potential against several food-contaminating moulds, such as *Alternaria alternata* Keissler, *Fusarium oxysporum* Schlecht, *Penicillium citrinum*, *P. roqueforti*, *P. patulum*, *Aspergillus flavus* and *A. parasiticus* (Azzouz and Bullerman, 1982; Schmitz *et al*., 1993).

Phenolic compounds are probably responsible for the high inhibitory activity of carvacrol/thymol chemotypes of oregano against fungal growth, conidial germination and production of *Penicillium* species, such as *P. digitatum* (Daferera *et al*., 2000). In particular, monoterpene components seem to have more than an additive effect in fungal inhibition. Phenolic derivatives, present in essential oils, may also be involved in inhibition of yeast sporulation through depletion of cellular energy by reduction of respiration (Baricevic and Bartol, 2002). Curtis *et al*. (1996) reported that carvacrol or thymol, when applied in concentrations of more than 100 ppm, led to a complete inhibition of fungal growth *in vitro*.

Although the antibacterial properties of oregano extracts are far less documented, Hammer *et al*. (1999) found that *O. vulgare* (Australian origin) yielded one of the most potent antibacterial agents among 52 investigated essential oils, which considerably inhibited the growth of all tested microorganisms. Other reports (Biondi *et al*., 1993; Izzo *et al*., 1995; Shekarforoush *et al*., 2007) demonstrated the inhibitory effects of oregano extracts against a number of gram-positive (such as *Staphylococcus aureus* and *B. subtilis*) and gram-negative (such as *Proteus vulgaris* and *Escherichia coli*) bacteria. These activities have been mainly attributed to thymol and carvacrol. However, as also shown for the antifungal properties of the species, it seems more appropriate to combine the antimicrobial efficacy of different food-preservative compounds, creating synergistic effects, such as those reported by Pol and Smid (1999) for carvacrol and nisin (a bactericidal peptide, used as a biopreservative in certain foods) against *B. cereus* and *Listeria monocytogenes in vitro*. Similar findings have been more recently reported by Dimitrijević *et al*. (2007). Govaris *et al*. (2010) reported on the antimicrobial effects of oregano oil against *Salmonella* sp., while Gündüz *et al*. (2010) found similar effects against *S. typhimurium*.

The antimicrobial value of oregano may exceed its scope of applications beyond the food industry: a therapeutic potency of essential oil of *O. vulgare* L. subsp. *hirtum* against experimentally induced dermatophytosis in rats (infection with *Trichophyton rubrum*) was found by Adam *et al*. (1998). Other studies demonstrated the promising applications of oregano, its essential oil or isolated compounds, in plant protection, in post-harvest crop/fruit protection or in apiculture, where species-specific fungi endanger the production systems (for an extensive and detailed review, see Baricevic and Bartol, 2002). Activity against *Vibrio cholerae* has been identified by Rattanachaikunsopon and Phumkhachorn (2010).

21.4.3 Other properties

It has been reported that oregano (added to the soil) and its essential oil could be used as natural herbicides (da Mastro *et al*., 2006). An inhibitory activity against rose powdery mildew caused by *Podosphaera pannosa* (Wallr. Fr.) De Bary was determined by Scarito *et al*. (2007).

Oregano has been also demonstrated to affect, in a mostly favourable way, the reproductive system of farm animals (Allan and Bilkei, 2005), possibly by interfering with their endocrine system. A general improvement of the animal health status has also been attributed to oregano consumption, especially in association with the protection of the intestinal and the immune system (Bimczok *et al*., 2008).

21.5 Quality specifications and commercial issues

Although oregano has been known and used for centuries, it has only lately gained mass popularity, largely because of its relationship with marjoram (*O. marjorana*), the popular and botanical terms for both species having long been confused. While sweet marjoram was one of the most popular herbs during the Middle Ages, oregano was scarcely cultivated, probably because of the plant's tendency to compete against other plants growing nearby. On the other hand, wild oregano has been traditionally collected in Mediterranean countries and in Mexico for use in many of the favourite dishes (e.g. for tomato-based sauces, lamb, seafood, chilli peppers and almost any garlic-flavoured dish). The rest of the world discovered oregano after World War II, with the expansion of pizza consumption (and, to a lesser degree, Mexican-style foods). Oregano consumption boomed from almost nil to a consumption volume of over 500 000 tonnes, demonstrating a per capita increase of importation into the USA of 3800 % from 1940–85 (Kintzios, 2002a). The European Union imported more than 1000 tonnes of oregano in 1999 (Tsagadopoulos, 2002). In the USA, the average consumption has increased by over 50 % during the 1990s due in part to the increased consumption of spices used to make foods less bland (Wilson, 2003).

Product prices depend heavily on quality. The overall market of oregano is expanding, and oregano is by far the biggest selling herb today. Latest estimates put worldwide production at about 10 000 tonnes. Turkey has a dominant position in the worldwide trade of oregano (over two-thirds of the total production, with 3392 tonnes exported to the USA in 1995), followed by Mexico, Greece and other Mediterranean countries. Greece has long been a leading source and its product has traditionally commanded the highest prices; nevertheless, it has not always met demand. Although Italy harvests large amounts of oregano, most of it is consumed domestically. The Mediterranean-type of product, as compared with the Mexican, is a smaller leaf of somewhat lighter green colour and milder, sweeter flavour. Compared with sweet marjoram, however, it is much stronger flavoured. The harvesting and processing of oregano are similar in Mediterranean and Mexican areas. It is generally accepted that the Greek oregano has the best essential oil quality, the main constituents of which are carvacrol (the compound responsible for characterizing a plant as of the oregano type) and/or thymol, accompanied by *p*-cymene and γ-terpinene. Mexican oregano oil contains approximately equal amounts of carvacrol and thymol and smaller amounts of 1,8-cineole and other compounds.

The herb is often sold by mesh size, indicating average particle size. In the USA, oregano imports are roughly equal from both Mediterranean and Mexican species. Mexican oregano is a much stronger, more robustly, 'wild' flavoured oregano. After cleaning, the leaves of Mediterranean oregano come into a size of 30 or 60 mesh, with larger leaf particles giving the choicest, more refined appearance. In Mexico, shippers often refer to their most refined product as 'Greek cut'. In the USA, the herb is offered as ground or whole leaf oregano (although not always in the original whole form). Beyond that, various mesh sizes may also be available, each being the most appropriate choice for a particular use. Other important species collected and marketed as European oregano include *Thymus capitatus* (Spanish oregano), *O. syriacum* (*O. maru* Syrian marjoram or *zatar*) and *O. virens*. Additional species used in Mexico include *Lippia palmeri* and *Lippia origanoides*.

The original fresh material is the essential factor determining the quality of the dried herbs. Nevertheless, the drying method, type of packaging and storage conditions also have clear effects on the microbiological quality of the herbs. Blending of oregano with substitute spices is very popular, in particular when high essential oil concentrations (>3 %) are desired. However, a common problem is the occurrence of fraud, realized as the customized adulteration of the spice with foreign materials having a similar fragrance.

Quality evaluation is usually based on colour of the traded spice, while in several instances sensory (organoleptic) tests are carried out. Quality criteria also include the relative contribution of leaves to the dried product, since they contain a large number of glandular hairs. Leaves should be uniform and have a relative moisture content of less than 15 %. The essential oil concentration should not be lower than 0.5 % (w/w). Products should be free of impurities, in particular biologically unsafe components such as insects, animal hair and excretions. That said, impurities up to 2 % (thereby including different plant organs from those specified in the drug definition, as stems) can be considered acceptable, and similar values are tolerated by the ASTA and its European counterpart, the ESA. Lower values are accepted by ISO/FDIS 7925 (a review on the different regulatory thresholds is given by Marieschi *et al*., 2009). A number of advanced techniques, such as optical and electron microscopy, have been applied for the quality assessment of commercial oregano in Greece (Tsagadopoulos, 2002). More recent methods for the fast detection of non-aromatic adulterants are based on molecular markers. In particular, the so-called sequence characterized amplified regions (SCAR) have proven to be of value in the screening of adulterants, such as *Cistus incanus* L., *Rubus caesius* L., *Rhus coriaria* L., *Satureja montana* and *O. majorana* (Marieschi *et al*., 2010, 2011). Another source of concern for product quality is oregano extracts, which are typically used as food additives at levels of less than 0.01 % of the finished food product. A common problem with extracts is the lack of standardization owing to crop year, geographical origin, age and lot-to-lot variation. The amount of residual solvent in the spice oleoresin is also of concern, and its levels are monitored by regulatory agencies.

Growers currently enjoy increased market prices owing to the limited product availability, as a result of the exhaustion of wild oregano populations due to intensive collection. A survey in Greece (Papanagiotou *et al*., 2001) indicated that, for a given average yield of 1850 kg per hectare and an average product price of 4.1 euro per kg, the net profit for the grower is 2500 euro per hectare, a value considerably

higher then for most crop and horticultural species. Labour (1260 man-hours/hectare) was estimated to reach 64 % of the total production cost.

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22

Poppy

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Abstract: This chapter looks at poppy (*Papaver*), concentrating on *P. somniferum* L., the variety which is commercially grown. The chapter describes the origins, geographical distribution and botanical classification of *P. somniferum* before covering its botany and looking particularly at planned breeding for high-yielding cultivars. The cultivation of poppy and production of opium latex for pharmaceutical use are described along with the chemical composition. Products from poppy – opium, poppy seeds, poppy seed oil – are detailed. The chapter discusses applications of poppy in food processing, traditional and modern medicine before looking at quality issues and toxicity.

Key words: poppy, *Papaver somniferum*, planned breeding, opiates, poppy seed, poppy seed oil, quality, toxicity.

22.1 Introduction and description

Poppy is the common name for several species of the genus *Papaver* of the family Papaveraceae. It includes many species which are grown as garden flowers (garden poppies) and the species *P. somniferum* grown for the production of the important narcotic drug opium (the dried latex exudate from the fully grown green capsule) and its edible seeds and seed oil. Opium is one of the oldest known painkillers and is the source of several alkaloids used for analgesic, antitussive and antispasmodic purposes in modern medicine. The opium poppy was cultivated by the ancient civilizations of Greece, Egypt, Italy, Persia and Mesopotamia. Poppy is now cultivated mainly for the production of opium and for the edible seed and seed oil. Poppy seeds are highly nutritive and are used in breads, curries, sweets and confectioneries for culinary purposes, and for the production of seed oil.

22.1.1 Origin

Papaver somniferum (L.) is believed to originate somewhere in the western Mediterranean region, spreading from there throughout Europe and via the Balkan peninsula to Asia Minor as early as the tertiary period (Bazilevskays, 1976; Morton,

1977). Today, the opium poppy is widely distributed across the temperate and subtropical regions of the old world, extending from 60ºN in northwest Russia almost as far as the tropics. Because of its narcotic properties, its growth is restricted in North America, but it can still be found in old gardens and nearby waste areas to which it has escaped (Nova Scotia Museum, 2012).

22.1.2 Classification

The poppy belongs to the genus *Papaver* of the dicot family Papaveraceae. There are about 100 species of *Papaver* distributed all over the world. Feede (1909) divided the genus *Papaver* into nine categories, of which only two – 'Mecones' and 'Mycrantha' (Oxytona) – are economically significant. Valuable alkaloid-yielding and edible seed-producing species such as *P. somniferum* and *P. setigerum* DC belong to the 'Mecones' category, but only *P. somniferum* is commercially cultivated. *P. somniferum* and *P. setigerum* display close similarity and are now believed to have originated from a common ancestral stock (Vesselovskaya, 1976; Husain *et al.*, 1983; Singh *et al.*, 1995a). However, *P. somniferum* does not grow in the wild, unlike *P. setigerum* and other members of this genus in the same category, such as *P. glaucum*, *P. glacile* and *P. dicaisnei*. The species in the Mycrantha/Oxytona category are *P. bracteatum*, *P. orientale*, *P. pseudo-orientale* – these also contain some opium alkaloids. A classification of opium poppy seeds is shown below:

- kingdom: Plantae – plants
- sub-kingdom: Tracheobionta – vascular plants
- super-division: Spermatophyta – seed plants
- division: Magnoliophyta – flowering plants
- class: Magnoliopsida – dicotyledons
- sub-class: Magnoliidae
- order: Papaverales
- family: Papaveraceae – poppy family
- Genus: *Papaver* L. – poppy
- Species: *Papaver somniferum* L. – opium poppy (Kartesz, 2009)

Synonyms for opium poppy according to API (2006) are:

- Sanskrit: Khasatilah, Aphukam, Khakhastilah, Khakhasah
- Bengali: Aaphim, Postadaanaa, Postabeej
- English: Opium poppy seeds
- Gujarati: Khaskhas
- Hindi: Apheem, Postadaanaa, Khaskhas, Khasabija
- Kannada: Gasgase, Aapheen, Aphini
- Malayalam: Avin, Karappu, Kashkash, Aalan
- Marathi: Khaskhas
- Oriya: Aapu
- Tamil: Kasakash, Posttakkaai, Avinee
- Telugu: Gasgashaalu, Nallamandu
- Urdu: Apheem

22.1.3 Botany

P. somniferum is an erect, annual herb, 30–150 cm long with a stem between 0.5 and 1.5 cm thick. The root is either shy branched or much branched, tapering and yellow. The stem is glabrous with a thick waxy coating. The leaves are numerous, alternate and sessile, and spread horizontally; the lower leaves are about 15 cm long, with an oval–oblong shape, and are deeply pinnatisect with acute segments. The upper leaves can reach up to 25 cm in length, gradually widening and cordate towards the base. Finally, the topmost leaves are very broadly ovate and amplexicaul with prominent veins. The midrib is very wide and almost white in colour. Puri (1983) observed that in the race 'Safaid patta', the leaves are variegated with white streaks or blotches. In 'Kutila' or 'Kutapatta', the foliage is deeply cut into more or less narrow segments up to the midrib and primary veins. A wide variation in the degree of serration in the leaves of Indian poppy species was observed by Nigam *et al.* (1989).

The poppy plant has few flowers, which grow individually on 10–15 cm long peduncles. The flower buds are ovate–ovoid and hermaphrodite, with two smooth green caducous sepals and four very large, generally white petals. The stamens are numerous and hypogynous, and are arranged in several whorls; the anthers are linear, attached with a filament, and are cream in colour, becoming pale brown and twisted after dehiscence. The ovary is large, depressed, globular, smooth, and pale green in colour, and is one-celled with large spongy parietal placentae. The stigma is sessile and capitate with 8–20 short, obtuse, oblong rays. The fruit is a capsule that varies in colour, shape and stigmatic rays. The immature capsule is covered with a waxy coating which imparts a greyish blue tinge to the capsule. When mature, the capsule becomes pale brownish in colour and may be variegated. It may be globose or spherical, oblong to ovate–oblong, and depressed in some cases. The capsule has a rounded base but ends abruptly at the apex, opening by pore beneath the stigmatic rays. The number of stigmatic rays varies from 7 to 18. The seeds are numerous and very small; they may be white–grey, violet or black in colour. The testa has a raised reticulated network, and its embryo is slightly curved in the axis of the oily endosperm.

Poppy is generally considered to be a self-pollinating plant, but some studies have also reported certain degree of outcrossing (Singh *et al.*, 1999). Nyman and Hall (1976) in fact reported as much as 97 % outcrossing, while Khanna and Shukla (1983) and Bhandari (1990) observed highly variable degrees of outcrossing in poppy, up to 79 %. More outcrossing as a result of insect activity can be expected in this species. However, planned breeding of opium poppy is very recent, with different selection methods for opium yield and quality and oil seed yield the main objectives of the extensive breeding research carried out by many European and Indian breeders (Hlavackova, 1959, 1978, Bohm, 1965; Johnson and Loof, 1973; Goldblatt, 1974; Sip *et al.*, 1977; Khanna, 1978, 1981; Khanna and Gupta, 1981; Saini and Kaicker, 1982; Sharma *et al.*, 1988; Khanna and Shukla, 1989a, b; Singh *et al.*, 1999).

Singh *et al.* (1995b) reported that poppy exhibits heterosis for many economically significant characteristics. This has allowed a number of high-yielding cultivars of poppy to be created through selection and breeding. The creation of an opium-less variety that produces a high seed yield and high-quality oil for use in food is considered to be a particularly important development, in light of the high nutritional value of poppy seed and oil.

22.2 Production, cultivation and chemical composition

22.2.1 International sources

Poppy is cultivated for the legal pharmaceutical use of opium latex as a rich source of morphine in India, states of the former Soviet Union, Egypt, the former Yugoslavia, Czech Republic, Slovakia, Poland, Germany, the Netherlands, China, Japan, Argentina, Spain, Bulgaria, Hungary and Portugal (Vesselovskaya 1976; Ramanathan and Ramachandran, 1977). Poppy is also grown for its seed and seed oil, particularly in a number of European countries and also in India. Poppy is also grown illegally for the narcotic trade, principally in the following two areas:

- Golden Triangle (Burma, Thailand and Laos region);
- Golden Crescent (Afghanistan, Pakistan and Iran region).

There are no records detailing the extent of illegal poppy cultivation and production.

India is one of the largest producers of opium alkaloids in the world. As well as meeting the domestic demand, India exports opium to other countries. Its production and distribution is controlled by the Narcotics Control Bureau of the Government of India. The areas in which poppy is cultivated are controlled by the Narcotics Department of the Government of India, who give annual renewable licences to the farmers, and are divided into 12 divisions covering the districts of Faizabad, Barabanki, Barelly and Shajahanpur in Uttar Pradesh, Neemuch I and II, Mandsaur I and II and Ratlam in Madhya Pradesh, and Kotah, Chittorgarh and Jhalawar in Rajasthan.

22.2.2 Cultivation

Poppy can be cultivated in well-drained soil in open sunny locations in subtropical regions, and requires irrigation during dry spells. Direct sowing is preferable as transplanted seedlings do not grow well. It is a 6-month crop, with sowing generally carried out in autumn: in India, for example, seeds are sown at the beginning of November and harvested in April the following year. Once harvested, poppy seeds should be stored in cool, damp-free airtight containers; if kept at room temperature, the oil will become rancid quickly and the poppy seeds will taste bitter and unappetizing.

A number of varieties of *P. somniferum* L. are cultivated in India. The races with white flowers are commonly grown in Uttar Pradesh, while those with red or purple flowers were once common in Madhya Pradesh and Rajasthan, but have now been largely replaced by white flower types in these states too. No comprehensive taxonomic treatment of the cultivars of Indian opium poppy is available. Asthana (1954) described the different cultivars grown in India and broadly classified the races of opium poppy into 'Sabzadhari' (green, i.e., non-waxy capsules) and 'Safaidhari' (white, i.e. waxy capsules) types. During the last two to three decades, there has been a great erosion of poppy germplasm in India and many of the races described by Asthana are no longer available today. To determine the different races that have been cultivated in recent years, Khanna and Gupta (1981) carried out a detailed investigation in which they evaluated a large collection of germplasm from the

various states and categorized this into basic cultivars. No more than 20–25 basic cultivars could be recognized. Singh *et al.* (1997) has prepared a guide to these cultivars on the basis of their most salient features, which also addresses some of the confusion caused by differing local names.

22.2.3 Chemical composition and products

As a source of both opium latex and edible seed and oil, cultivated poppy (*P. somniferum*) has great economic value. The capsule is the major source of the opium latex, but the alkaloids are also present in other parts of the plants such as the stem, leaves and roots, as well as in poppy straws. The seeds do not contain any alkaloids, but are rich in high-quality edible oil.

Opium

Opium is brownish in colour when fresh, turning to brownish black when dried, and has a fruity odour. It has a very complex chemical composition consisting of sugars, proteins, fats, water, meconic acid, plant wax, latex, gum, ammonia, sulphuric and lactic acids and numerous alkaloids. The total alkaloid content varies from 5–10 %: approximately 40 of these alkaloids have so far been identified, the most important of which are morphine (10–15 %), noscapine (4–5 %), codeine (1–3 %), papaverine (1–3 %) and thebaine (1–3 %). In Indian species, the major alkaloids present are morphine (7–17 %), codeine (2.1–4.4 %), thebaine (1.0–3.0 %), noscapine (3.0–10 %) and papaverine (0.5–3 %). Poppy straw (the dry capsule with 7.5 cm stem) contains a small quantity of alkaloid.

Poppy seed

Poppy seeds are highly nutritious. They are tiny, kidney-shaped seeds, generally white, and occasionally red or pink to grey. They are attached to the lateral projections from the inner walls of the capsules and are produced in abundance. The seeds have well-developed endosperm filled with aleurone grains. About 3300 seeds weigh 1 g (Husain and Sharma, 1983).

Analysis of Indian poppy seeds showed the following composition: moisture 4.3–5.2 %, protein 22.3–24.4 %, crude fibre 4.8–5.8 %, calcium 1.03–1.45 %, phosphorus 0.79–0.89 % and iron 8.9–11.1 mg/100 g. The seeds also contain thiamine, riboflavin, nicotinic acid and lecithin. Minor minerals in the seeds include iodine (6 μg/kg).

The seeds have a high protein content, the major component being globulin, which accounts for 55 % of the total nitrogen. The amino acid makeup of the globulin is similar to that of the whole seed protein and is as follows: arginine (10.41 %), histidine (2.9 %), lysine (1.5 %), methionine (2.3 %), theonine (4.2 %) and valine (7.1 %). The protein is deficient in lysine and methionine. At 10 % level of intake, the seeds have a biological value of 57.5 % and a digestibility coefficient of 81 %.

Banerji *et al.* (1999) studied and characterized the unsaponified matter of poppy seeds and found a total of 15 constituents of which seven major constituents were identified. Sitosterol was found to be the major constituent (59.2 %) followed by campesterol (14.2 %), avenasterol (7.2 %), cholestanol (4.9 %), stigmasterol (2.5 %), cholesterol (0.6 %) and D7-campesterol (0.9 %).

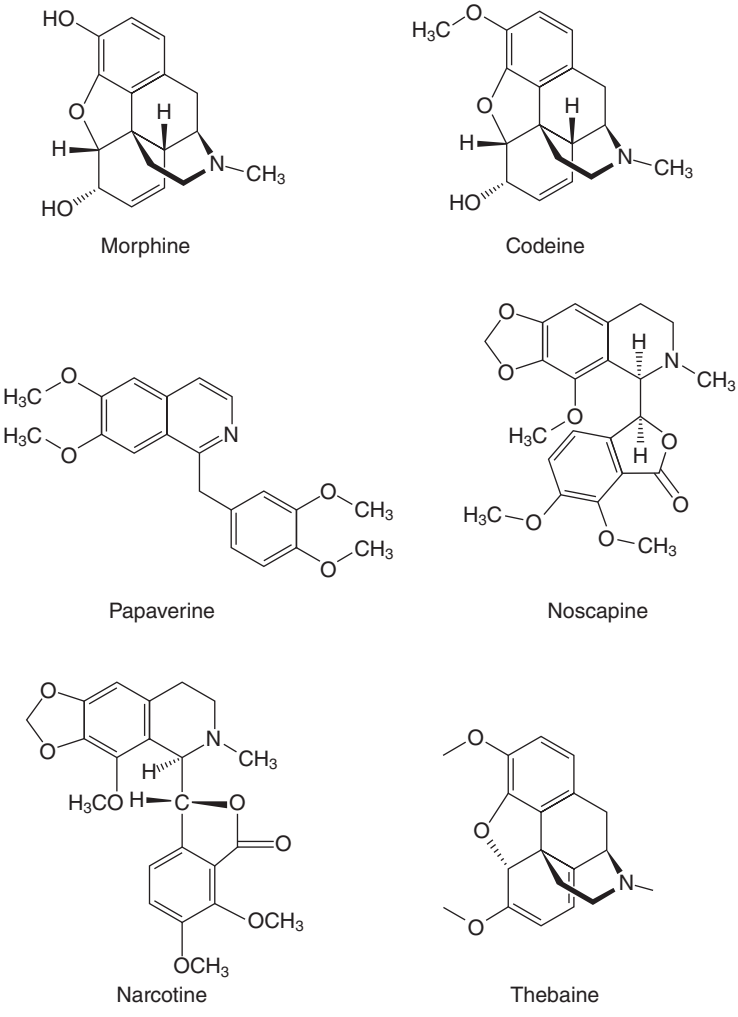

Fig. 22.1 The major alkaloids in opium.

Poppy seed oil

Poppy seeds contain 50 % of edible oil with a pleasant aroma and a taste similar to that of almond oil. Seeds from capsules that have not been used for opium extraction give a higher oil yield. Although both white seeds and black seeds are used for oil pressing, black seed is mostly preferred.

Extraction of poppy seed oil

In India, the oil is expressed by the cold process, the yield being about 90 %. The oil is rendered perfectly colourless by exposure to the sun. In France, three grades of oil are recognized, which correlate with the extraction process used:

1. First cold expression provides a highly superior oil used for table purposes and in the manufacture of very high-quality paints.

2. Second cold expression provides a lower grade edible oil also used for paints and illumination.
3. Third hot expression provides a significantly inferior oil, used principally in soap making.

Cold pressing seeds of fine quality yields 30–40 % of virgin white oil, a transparent limpid fluid with a slight yellowish tinge, bland and pleasant to taste and with almost no perceptible odour. On second pressing, and with the aid of heat, an additional 20–25 % of inferior oil is obtained. This oil is somewhat reddish in colour, with a bitter taste and a linseed-like smell. Poppy seed oil has specific gravity (15˚/25˚C) of 0.924–0.927, refractive index 1.467–1.47, iodine value 132–142, sap value 188–196 and acid value 3.13 %.

Composition of poppy seed oil
Poppy seed oil is a rich source of linoleic acid (68 %) which makes it a good oil for nutritional purposes, as a high percentage of linoleic acid has been shown to lower blood cholesterol. It has a high digestibility coefficient of about 96 % at a daily intake of 50 g. The chemical composition of the seed oil extracted from Indian poppy is reported by Singh *et al.* (1999) as follows:

- palmitic acid (16:0): 8.90–21.48 %
- stearic acid (18:0): 1.40–10.80 %
- oleic acid (18:1): 13.22–36.79 %
- linoleic acid (18:2): 41.00–60.00 %
- linolenic acid (18:3): 0.00–9.40 %
- manganese (29 mg/kg)
- copper (22.9 mg/kg)
- magnesium (15.6 mg/kg)
- zinc (130 mg/kg).

22.3 Main uses of poppy

22.3.1 Applications in food processing

Poppy seeds are used as ingredients in many recipes, and provide not only added flavour but also nutritional benefits, since they are rich in a number of useful vitamins and minerals, including magnesium, calcium and zinc. The seeds are widely used in breads (especially bagels), sweets and confectionery around the world: in Japan, they are widely used in a number of dishes for their nutty flavour; while in India they are used in dips and in carrot, pea and tomato dishes. They are also sprinkled, either raw or toasted, over salads, soups and bakery products. The ground seeds are commonly used as a thickening agent in sauces, such as in the Mughal cooking style of northern India, and may also be added to desserts.

Poppy seed oil is popularly known as khus-khus oil in some Asian countries, and is used to add flavour to a number of food items including breads, rolls, cookies and cakes. It may also be used as a cooking medium or, when mixed with olive oil, as a salad dressing.

Finally, the oil cake, which remains after the extraction of the oil from the seeds, contains about 32.5 % protein and is used as a concentrate in feeding pigs and other animals reared for meat.

22.3.2 Applications in traditional medicine

Walter and Radha (2005) provide a detailed study of the wide range of indigenous uses of *P. somniferum* in traditional medicine. It is used for its aphrodisiac, analgesic, calmative, bactericidal, demulcent, expectorant, emollient, hypotensive, haemostatic, hypnotic, nervine and sedative properties.

The seeds are used to promote good digestive health, and can be used in remedies for constipation, diarrhoea, intestinal inflammatory pain and dysentery. For dysentery, the recommended preparation is $^1/_4$ teaspoon of poppy seeds sautéed in honey until golden brown. This should be taken twice a day, but for no more than 3 days due to its sedative effect (Anon., 2010). Both the seeds and the capsules are used as a cure for insomnia: the capsules are used as a sedative in the form of syrup or extract while, for the seeds, the recommended preparation is 30 g seed milk mixed with sugar. The seeds also have a high carbohydrate content, thus being useful in enhancing energy levels, and have been shown to be helpful in the treatment of various types of cancers, abscesses, ulcers, syphilis, scrofula and leprosy, and in providing relief from conditions such as arthritis, rheumatism and gout (Anon., 2010). Poppy is also used as an antitussive and can help to alleviate the symptoms of asthma and whooping cough. The seed oil is used for the treatment of cardiovascular diseases, stroke and heart attacks and, like the capsule and seeds, can be useful in the treatment of insomnia. A paste prepared from the roots of poppy can be used to relieve burning sensations on the skin (Anon., 2010).

The powdered capsule husk is used in the preparation of a tea called Bonda Chai, which is particularly popular in Punjab and Madhya Pradesh. Poppy tea has been used as a common home remedy for many hundreds of years in Europe. It is considered to be helpful in overcoming heroin addiction.

Use in Ayurvedic medicine

According to Ayurveda, poppy seeds are sweet, mildly astringent and calming. They are used as an aphrodisiac and analgesic, and for their astringent and bactericidal properties. They are used in the treatment of cough, conjunctivitis, hypertension, menstrual pain, rheumatism and fever. Soaked poppy seeds are ground into a fine paste with milk and applied to the skin as a moisturizer.

According to The Ayurvedic Pharmacopoeia of India (API, 2006) the Rasa of the drug is described as Madhura, Guna of the drug is described as Guru, Veerya of the drug is classified as Sita, Vipaka of the drug is classified as Madhura and Karma of the drug is variously described as Vatahara, Rucya, Stambhana, Vedanasthapana, Vrsya, Balya and Varnya. Some important Ayurvedic formulations containing Khakhasa seed are Abhyadi Gutika, Abhrakkadi Vati, and Asvani Kumar Rasa.

22.3.3 Use in the pharmaceutical industry

Opium derived from *P. somniferum* L. is well known as a natural source of important alkaloids such as morphine (a potent narcotic analgesic drug), thebaine, codeine (an

analgesic antitussive drug) and oripavine, and is widely used in the pharmaceutical industry. The opioid analgesics are extremely valuable as they reduce or relieve pain without causing loss of consciousness.

Papaverine, another opium poppy alkaloid, is a smooth muscle relaxant used principally for the relief of cerebral and peripheral ischaemia associated with arterial spasm and myocardial ischaemia complicated by arrhythmias. Apomorphine, a semi-synthetic analogue of morphine, was the first dopaminergic drug used to treat the symptoms of Parkinson's disease. Recently, noscapine, another important alkaloid found in opium poppy, has been found to have interesting properties for chemoprevention and treatment of cancers, especially prostate cancer, and stroke (Li *et al*., 2010).

The global consumption of these products is growing. Indeed, Shukla *et al*. (2006) observed:

> The demand for morphine from the level of 2 MTs in 1981–83 rose to 20 MTs in the period 1998–2000. Similarly the demand for codeine rose from 160 MTs in 1981 to 82 to 169 MTs in 2000. Morphine apart from being used for medical treatment is predominantly converted into other opiates, mainly codeine, and its consumption increased from 200 MTs in 1990 to 265 MTs in 2000, while codeine utilization for the manufacturing of other drugs, mainly dihydrocodeine and hydrocodeine, has increased 5 times to a record level of about 80 MTs in 2000. Thebaine is an important component in the manufacture of oxycodone, oxymorphine, buprenorphine etc. and its utilization has increased from 5 to 8 MTs in 1981 to 45.6 MTs in 2000.

Moreover, more recently still, the demand for thebaine in 2007 rose to 52 MTs (Chaterjee *et al*., 2010).

22.4 Quality issues

22.4.1 Quality specifications

According to the Ayurvedic Pharmacopoeia of India (API, 2006) the quality specifications for poppy seeds are:

- foreign matter: not more than 1 %
- total ash: not more than 8 %
- acid-insoluble ash: not more than 1.5 %
- alcohol-soluble extractive: not less than 7 %
- water-soluble extractive: not less than 13 %
- fixed oil: not less than 19 %

22.4.2 Adulteration of poppy seeds and seed oil

Since poppy seed oil is an expensive product, it is often adulterated with cheap edible oils such as sunflower oil (Krist *et al*., 2006). Poppy seeds themselves are also adulterated using cheap *Amaranthus paniculatas* seeds, which closely resemble poppy seeds. This can be detected qualitatively by two methods: (i) by puffing a sample, causing any amaranth seeds, but not poppy seeds, to puff and reveal their presence; and (ii) by estimating the content of squalene, an unsaturated long-chain

hydrocarbon found in large quantities in amaranth seed oil and only in traces in poppy seed oil (Singhal *et al*., 1997).

22.4.3 Toxicity studies

Although not all poppies contain opium, they are all poisonous. The Nova Scotia Museum (2012) provides a detailed overview of the toxicity of *P. somniferum* as follows:

> Poisoning occurs from ingesting the unripe seed capsules and from the illicit use of opium and its derivatives: codeine, heroin, and morphine. Poppy seeds are harmless and edible, but all other parts of poppy family members contain toxins. Alkaloids, including morphine and codeine, in the opium poppy are combined to produce the resinous, addictive drug, opium. It is harvested commercially in the Middle East and Asia by gathering and processing latex from the unripe seedpods. The illegal drug heroin is a concentrated extract of opium. Many of the same alkaloid toxins found in the poppy family are also present in the otherwise unrelated bleeding heart. Though morphine and codeine have legitimate medical applications, most human poisonings and fatalities are the result of deliberate abuse of opium, heroin, or other poppy products. Occasionally, bloodroot is accidentally ingested, with dangerous results. Opium affects the body by mimicking naturally occurring compounds in the nervous system that function as sedatives, pain suppressors, and mood elevators. Not surprisingly, then, poppy poisoning is marked by erratic behaviour, loss of appetite, stupor, and coma. Overdoses of opium or its derivatives cause death by respiratory failure. Bloodroot poisoning causes vomiting before fainting and potentially fatal coma set in.

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23

Sesame

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Abstract: This chapter looks at the classification and species inter-relationship, morphology and biology of sesame (*Sesamum indicum* L.) before going on to discuss the chemical composition and nutritional components of sesame oil, cake and meal, including their functional properties. Production, cultivation, harvesting and seed storage are all described, and processing, including dehulling, oil extraction and purification, sesame cake and meal and protein concentrates and isolates, reviewed. The chapter discusses the culinary uses of sesame seed together with its use as an animal feed and in industry and its medical applications. Finally, quality issues and future research needs are addressed.

Key words: chemical composition, sesame oil, meal cake, biology, cultivation, quality specification.

23.1 Introduction

Sesame (*Sesamum indicum* L.), also known as sesamum, gingelly, beniseed, *sim-sim* and *til*, is perhaps the oldest oilseed known and used by human beings (Joshi, 1961; Weiss, 2000). It has been cultivated for centuries, particularly in Asia and Africa, for its high content of edible oil and protein. The crop is now grown in a wide range of environments, extending from the semi-arid tropics and subtropics to temperate regions. Consequently, the crop has a large diversity in cultivars and cultural systems. Although considered to have originated in central Africa, most probably Ethiopia, many believe that there is convincing evidence to show that sesame originated in India (Bedigian and Harlan, 1984). Sesame was widely dispersed by people both westward and eastward, reaching China and Japan which themselves became secondary distribution centres.

Sesame is an important cash crop for small and marginal farmers in several developing countries. It is cultivated for its seeds which contain 38–54 % oil of very high quality and 18–25 % protein. The great diversity of sesame types, their wide environmental adaptation and considerable range of seed oil content and characteristics make an exceptional gene pool which must be harnessed to produce better cultivars to extend the range and profitability of sesame growing. The major obstacles to sesame's expansion are its low yields and the absence of non-shattering cultivars suitable for machine harvest.

23.1.1 Classification and species relationship

The genus *Sesamum*, one among the 13 genera of the family Pedaliaceae, comprises about 40 species, 36 in the *Index Kewensis*. Many occur in Africa (18 exclusively), eight occur in the India–Sri Lanka region (five exclusively). The Australian records are probably due to imports by Chinese immigrants in the mid-nineteenth century (Bennett, 1996). The cytogenetic knowledge of the genus is very limited; thus, the chromosome numbers are known only for about one-third of the species.

Sesamum indicum, together with *S. capense* Burn (*S. Alatum* Thonn), *S. malabaricum* Nar. and *S. schenkii* Aschers, has the somatic number 2n = 26; *S. laciniatum*, 2n = 28; *S. angolase* and *S. prostratum* 2n = 32; *S. occidentale* and *S. radiatum* Schum & Thonn, 2n = 64. The Indian *S. mulayanum* is very similar to *S. indicum*, but has the valuable characteristic of being resistant to phyllody and wilt.

The progenitor species of the cultivated *S. indicum* are unknown as no wild species except *S. malabarium*, which produces fertile hybrids with *S. indicum*, are known. The true wild species of sesamum found in tropical Africa and India produce viable F_1 seeds, but F_1 hybrids are sterile, except for *schenkii–indicum* hybrids, which show some end-season fertility.

S. indicum has a number of local cultivars as noted in the literature, but it is claimed the genus *Sesamum* has only one cultivated species, which can be divided by seed colour into white sesame *S. indicum* spp., *indicum*, and variable sesame *S. indicum* spp., *orientale* (Zhang *et al*., 1990). Based on seed colour and inheritance, it is postulated that sesame evolved from symmetric to asymmetric types and from *S. capense* to *S. indicum* in the sequence black, brown, yellow and white seeded types (He *et al*., 1994). Research into *S. indicum*, *S. alatum*, *S. radiatum* and *S. angustifolium* (Olive) Engl. by analysing the fatty acid composition of the total lipids and of the different acyl-lipid classes indicated that *S. radiatum* and *S. angustifolium* are more closely related to the other two species (Kamal-Eldin and Appelquist, 1994).

Polyploidy can be induced, but colchicine-treated plants tend to produce a low yield even when fertility of pollen is high and seeds per capsule not reduced. The number of capsules set is often very low, but seed oil content can be extremely high. The rate of growth and general vigour of tetraploids can exceed that of diploids; the plants are taller at maturity, with longer leaves, larger flowers, capsules and pollen grains. Sterility of pollen grains in tetraploids can vary between 20 and 40 % and that of diploids between 5 and 30 %.

Interspecific hybridization is possible and crosses may produce viable seed (Prabakaran and Rangasamy, 1995). The discovery of genetic male sterility in sesame (Osman, 1981) has eased the production of hybrid seed (Tu, 1993; Ganesan, 1995; Kang and Lee, 1995; Wang *et al*., 1995).

23.1.2 Morphology and biology

There are many hundreds of varieties and strains of *S. indicum*, which differ considerably in size, form, growth, colour of flowers, seed size, colour and composition. Cultivated sesame is typically an erect, branched annual, occasionally perennial, 0.5–2 m in height, with well-developed root system, multi-flowered, whose fruit is a capsule containing a number of small oleaginous seeds.

Sesame has a taproot system with profuse lateral branches. Long-season types, occasionally treated as perennials, have an extensive and penetrating root system and short-season types have less extensive and more shallow roots. Root growth is also influenced by the soil type, season and soil moisture conditions. Root growth is inhibited by excess soil moisture and relatively low salt concentrations, much lower than is tolerated by safflower, for instance.

The stem is erect, normally square in section with definite longitudinal furrows, although rectangular and abnormally wide, flat shapes occur. It can be smooth, slightly hairy or very hairy and these characteristics are used to differentiate the types. Stem is light green to purple, branching angular and straight with an average height of 1–1.5 m and sometimes up to 3 m. The extent and type of branching are varietal characteristics, as is the height at which the first branch occurs. The degree of branching is directly affected by the environment. Short-stemmed, little-branched types are generally early maturing; the taller branched types late-maturing and tend to be more drought resistant.

Leaves on sesame plants are most variable in shape and size on the same plant and between varieties. Usually, lower leaves tend to be broad, sometimes lobal, margins often prominently toothed with the teeth diverted outwards. Intermediate leaves are entire, lanceolate, sometimes slightly separated. The upper leaves are more narrow and lanceolate. Leaf size varies from 3–17.5 cm in length, 1–7 cm in width with a petiole of 1–5 cm in length. The surface of leaves is generally glabrous but in some types may be pubescent. Generally of a dull, darkish green, leaves can be much lighter with occasionally a yellowish tint or bluish when leaves are very hairy. Leaf arrangement may be alternate or opposite or mixed or opposite below and alternate above and varies with varieties. There is a basic difference in the rate of water conductance between leaves of indehiscent and dehiscent sesame, the former being much faster. These varieties are thus less suited to areas with limited water supply.

Flowers arise in the axils of leaves and on the upper portion of the stem and branches, and the node number on the main shoot at which the first flower is produced is a varietal characteristic and highly heritable (Mohanty and Sinha, 1965). Flowers occur singly on the lower leaf axils with multiple flowers on the upper stem or branches. When borne singly, two lateral flowers are observed as rudimentary buds (nectarial glands) at the base of the fully developed ones. They are invariably pilose and show a fair range of variability in size, colour and marking on the inside of the corolla tube. Flowers are borne on very short pedicels. Two short linear bracts arise at the base of the pedicel just below the nectaries which are shed when flowers mature. Calyx lobes are short, velvety, narrow, acuminate and united at the base. The five lobes are of variable sizes, the lower one being the longest and upper one the shortest. The flower is zygomorphic with slightly bilabiate tubular corolla of five lobes. The upper lip of the corolla is entire, the lower divided into three of which the central division is the longest. Corolla is usually white or pale pink but purple is also observed. The inner surface of corolla tube may have red spots or the lower portion only, may be black spotted or, occasionally, with purple or yellow blotches.

Stamens are attached with the tube of the corolla. Of the five stamens, four are functional and the fifth either sterile or completely lacking. The four greenish white

functional stamens are arranged in pairs, one pair being shorter than the other. There are two anther cells, opening longitudinally, connective usually gland tipped.

The ovary is superior, usually two-celled, cells often completely or partially divided by false septa. The style is terminal, filiform and simple. Stigma is usually two-lobed and hairy.

When there are three flowers per leaf axil, the central bud blooms first and the two side buds open several days later. Flowers open early in the morning, 95 % between 5 and 7 a.m, wilt after midday and are usually shed in the evening, majority between 4.30 and 6.00 p.m. Anthers open longitudinally and release pollen shortly after the flowers open, the interval varying with variety. The stigma is receptive one day prior to flower opening and remains receptive for a further day. Under natural conditions, pollen remains viable for approximately 24 hours. Low temperature at flowering can result in sterile pollen, or premature flower fall. Conversely, periods of high temperature, 40 °C or above at flowering, will seriously affect fertilization and reduce the number of capsules produced.

Sesame is considered a self-pollinated crop giving full seed set under isolation. However, the flowers attract insects and their activity can lead to different rates of cross-pollination from a commonly reported few per cent to as high as 65 % (Yeramanos, 1980).

The fruit is a capsule, rectangular in section and deeply grooved with a short triangular beak. Capsule shape is a varietal characteristic with environment a major modifying factor. Capsule length can vary from 2.5–8 cm, with a diameter of 0.5–2 cm, the number of loculi from 4–12, and capsules are usually hairy to some degree. The capsule dehises, splitting along septa from top to bottom or by means of two apical pores. The degree of dehiscence and the above ground height of the first capsule are varietal characteristic (Weiss, 1983).

Sesame seeds are small, ovate, slightly flattened with testa of variable colour varying from black, white, yellow, reddish brown, grey, dark grey, olive green and dark brown. The dry matter content in seed increases most rapidly between 12 and 24 days, in parallel with the rate of oil synthesis and continues to increase slowly until maturity (Aiyadurai and Marar, 1951). There is significant difference between cultivars in respect of capsule length, number of seeds per capsule and seed size. A capsule may contain 50 to 100 or more seeds. Seed weight is around 3 g/1000 seeds. The seeds mature 4–6 weeks after fertilization.

23.2 Chemical composition

Sesame seed has high food value due to its high content of oil and protein. The composition is markedly influenced by genetic and environmental factors (Kinman and Stark, 1954; Lyon, 1972). The seeds contain 6–7 % moisture, 17–32 % protein, 48–55 % oil, 14–16 % sugar, 6–8 % fibre and 5–7 % ash. The proximate composition of sesame seeds is given in Table 23.1.

In general, Indian varieties tend to be lower in protein and higher in oil than Sudanese varieties, such as those generally appearing in the export market and are commercially used in the USA. The hull content averages about 17 % of the sesame seed, and contains large quantities of oxalic acid, calcium, other minerals and crude

Table 23.1 Proximate composition of whole sesame seeds

Constituent (%)	Joshi (1961)	Smith (1971)	Gopalan *et al.* (1982)	Weiss (1983)
Moisture	5.8	8.0	5.3	5.4
Protein	19.3	22.0	18.3	18.6
Fat	51.0	43.0	43.3	49.1
Carbohydrates	21.2	21.0	25.0	21.6
Ash	5.7	6.0	5.2	5.3

fibre. Thus, when using sesame for human food, it is advisable to remove the hull. When the seed is properly dehulled, the oxalic acid content is reduced from about 3 % to less than 0.25 % of the seed weight (Nagaraj, 1995). Screw-pressed, dehulled sesame contains about 56 % protein, while the solvent extracted meal contains more than 60 % protein. This is mostly used in feed except in India where it is used as a food.

23.2.1 Lipids

Content

Sesame seeds contain more oil than many other oilseeds. Oil content varies with genetic and environmental factors. A wide range of the oil content, from 37–63 %, has been reported in sesame seeds (Lyon, 1872; Swern, 1979; Bernardini, 1986). Oil content in seeds also varies considerably among different varieties and also with growing seasons (Lyon, 1972; Yen *et al*., 1986). The oil content is also related to the colour and size of the seeds. White or light-coloured seeds usually have more oil than the dark seeds and smaller seeds contain more oil than larger seeds (Seegeler, 1983). Rough-seeded cultivars generally have a lower oil content than smooth-seeded types (Yermanos *et al*., 1972).

Agronomic factors also influence the seed oil content. It increases with increasing length of photoperiod and early planting dates (Arzumanova, 1963; Abdel-Rahman *et al*., 1980). Likewise, the seeds from plants with a short growing period tend to have higher oil content than those from plants with a medium to long growing cycle (Yermanos, 1978). Heavy application of nitrogen fertilizer reduces oil content of sesame seeds (Singh *et al*., 1960).

Classification

The lipids of sesame seeds are mostly composed of neutral triglycerides with small quantities of phosphatides (0.03–0.13 % with lecithin : caphalin ratio of 52 : 46). The phosphatides also contain about 7 % of a fraction soluble in hot alcohol but insoluble when cold. Sesame oil, however, has a relatively high percentage (1.2 %) of unsaponifiable matter (Johnson and Raymond, 1964; Weiss, 1983). The glycerides are mixed type, principally oleo-dilinoleo, linoleo-dioleo triglycerides and triglycerides with one radical of a saturated fatty acid combined with one radical each of oleic and linoleic acids (Lyon, 1972). The glycerides of sesame oil, therefore, are mostly triunsaturated (58 mol%) and diunsaturated (36 mol%) with small quantities (6 mol%) of monounsaturated glycerides. Trisaturated glycerides are practically absent in

sesame oil. The unsaponifiable matters in sesame oil include sterols (principally comprising of β-sitosterol, compesterol and stigma sterol), triterpenes (triterpene alcohols which include at least six compounds, of which three were identified *viz*., cycloartanol, 24-methylene cycloartanol and amyrin), pigments, tocopherols and two compounds that are not found in any other oil, namely sesamin and sesamolin (Fukuda *et al*., 1981, 1988). Sesame seed contains high levels of sesamin and γ-tocopherol compared with α- and δ- tocopherol, and their concentration is influenced by genetic, environmental and geographical factors (Williamson *et al.*, 2008). Their contents change with seed maturity and will be highest at 30 days after flowering (Yasumoto, 2008). Sesamin and sesamolin are responsible for the characteristic Baudouin or Villavecchia tests of sesame oil. Among the pigments spectroscopically identified, pheophytin A (λ_{max} 665–670 nm) was found to markedly predominate over pheophytin B (λ_{max} 655 nm) (Lyon, 1972). The pleasant aroma and taste principles contain C_5–C_9 straight-chain aldehydes and acetylpyrazine (Swern, 1979).

Fatty acid composition

Sesame oil contains about 80 % unsaturated fatty acids. Oleic and linoleic acids are the major fatty acids and are present in approximately equal amounts (Lyon, 1972). The saturated fatty acids account for less than 20 % of the total fatty acids. Palmitic and stearic acids are the major saturated fatty acids in sesame oil (Table 23.2). About 44 and 42 % of linolic and oleic acids and 13 % saturated fatty acids are found in sesame oil (Smith, 1971). Arachidic and linolenic acids are present in very small quantities (Rao and Rao, 1981) and are least affected from year to year (Were *et al.*, 2006). It was also reported that the number of fatty acids found in early seed development stage is reduced from seven to four or five in mature seeds (Li *et al.*, 2008).

Endogenous antioxidants

Among the commonly used vegetable oils, sesame oil is known to be most resistant to oxidative rancidity (Budowski, 1950). It also exhibits noticeably greater resistance to autooxidation than would be expected from its content of tocopherols (vitamin E). This high stability to oxidation is often attributed to the presence of a large proportion of unsaponifiable matter. Moreover, the unsaponifiable matter itself includes substances such as sesamol and phytosterol, that are normally not found in other oils. Sesamolin, upon hydrolsis, yields sesamol. Sesame oil contans 0.5–1.0 % sesamin (Budowski *et al*., 1951) and 0.3–0.5 % sesamolin (Budowski *et al*., 1950) with

Table 23.2 Fatty acids composition of sesame oil (% of total fatty acids)

Fatty acid	Godin and Spensley (1971)	Yermanos (1978)	Seegeler (1983)	Maiti *et al.* (1988)
Palmitic	7–9	8.3–10.9	8.4–10.3	7.8–9.1
Stearic	4–5	3.4–6.0	4.5–5.8	3.6–4.7
Arachidic	8	–	0.3–0.7	0.4–1.1
Oleic	37–50	32.7–53.9	39.5–43.0	45.3–49.4
Linoleic	37–47	39.3–59.0	41.0–45.0	37.7–41.2

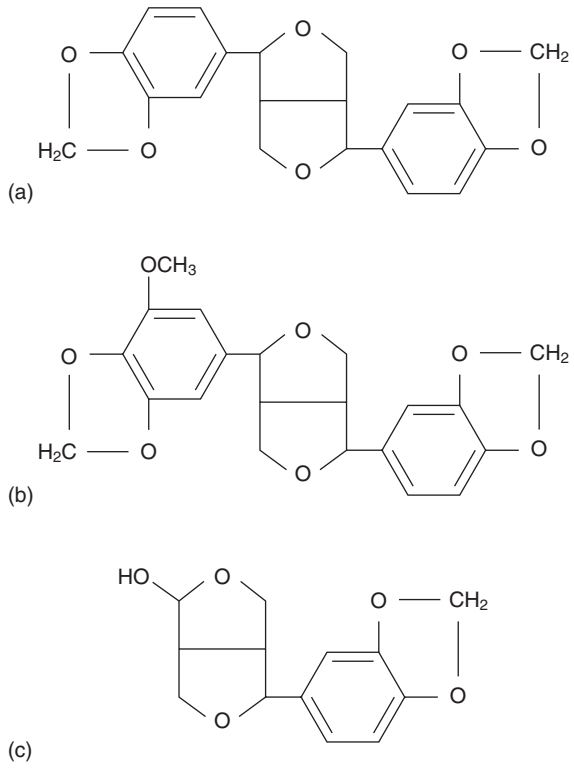

Fig. 23.1 Structures of natural antioxidants found in sesame oil: (a) sesamin; (b) sesangolin; and (c) samin.

only traces of free sesamol (Beroza and Kinman, 1955; Budowski, 1964). Sesamol is released from sesamolin by hydrogenation, by acid or acid bleaching earth or other conditions of processing and storage (Budowski and Markley, 1951; Beroza and Kinman, 1955). Free sesamol is, however, removed by some blending earths or during the deodorization process, which results in decreased stability of sesame oil (Budowski and Markley, 1951; Mathur and Tilara, 1953; Budowski, 1964). Structures of natural antioxidants found in oil from sesame are depicted in Figs 23.1 and 23.2.

Properties of oil

Sesame oil is deep to pale yellow in colour. It is fragrant or scented. It has a pleasant odour and taste. The aroma components were identified as C_5–C_9 straight-chain aldehyde or ketone derivatives (Lyon, 1972). Some of the important characteristics of sesame oil are given in Table 23.3. Sesame oil is dextro-rotatory, which is unusual for an oil devoid of optically active fatty acid glycerides. The unsaponifiable fraction of the oil, however, does contain optically active minor constituents, which are responsible for the optical rotation of the oil.

Nutritional importance

Sesame oil is practically free of toxic components. The oil contains more unsaturated fatty acids than many other vegetable oils. The high proportion of unsaturated fatty

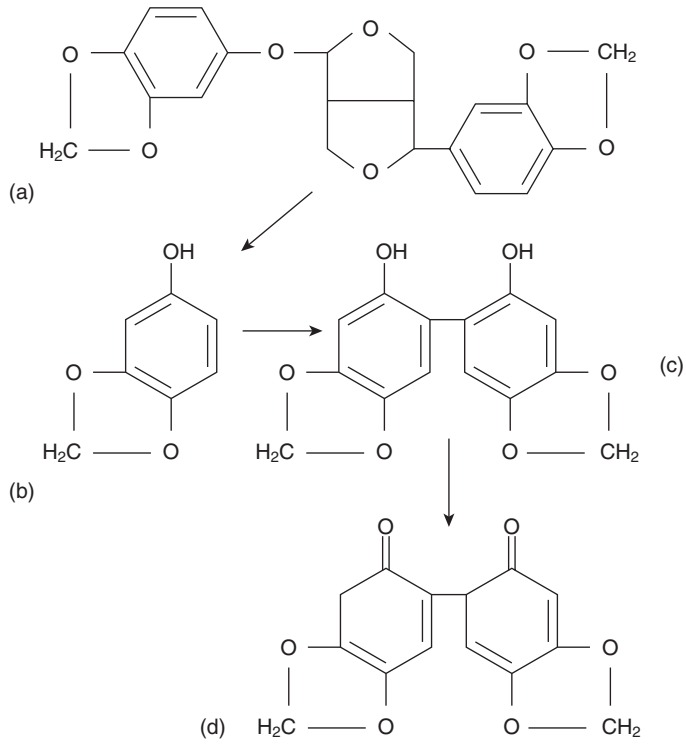

Fig. 23.2 Structures of natural antioxidants found in sesame oil: (a) sesamolin; (b) sesamol; (c) sesamol dimer; and (d) sesamol dimer quinone.

Table 23.3 Characteristics of sesame oil

Character	Andraos *et al.* (1950)	Lyon (1972)	Seegeler (1933)	Weiss (1983)
Specific gravity (25 % / 25 °C)	0.918	0.918–0.921	0.916–0.921	0.922–0.924
Refractive index (n^{50}_{D})	1.463 (25 °C)	1.472–1.474 (25 °C)	1.463–1.474 (25 °C)	1.458 (60 °C)
Smoke point (°C)	165	–	166	–
Flash point (°C)	319	–	375	–
Solidifying point (°C)	–	–	–3 to –4	–3 to –4
Titre (°C)	22	20–25	20–25	22–24
Free fatty acids (as % oleic)	1.0	–	1.0–3.0	1.0–3.0
Unsaponifiable matter (%)	2.3	1.8	0.9–2.3	0.9–2.3
Iodine value	112	104–118	103–130	103–126
Saponification value	186	187–193	186–199	188–193
Reichert-Meissl value	0.51	–	0.1–0.2	0.1–1.0
Polenske value	0.4	–	0.10–0.50	–
Hydroxyl number	5.3	–	1.0–10.0	1.0–10.0
Thiocyanogen value	76	–	74–76	74–76
Hehner value	–	–	96.0	95.7

acids renders sesame oil an important source of essential fatty acids in the diet (Langstraat and Jurgens, 1976). Linoleic acid is required for cell membrane structure, cholesterol transportation in the blood and for prolonged blood clotting properties (Vles and Gottenbos, 1989). Sesame oil is rich in vitamin E but is deficient in vitamin A. The crude oil contains a relatively low amount of free fatty acids. The minor constituents, sesamin and sesamolin, present in sesame oil protect the oil from oxidative rancidity.

23.2.2 Proteins

Content and characterization

Sesame seed contains 17–32 % protein with an average of about 25 % (Joshi, 1961; Lyon, 1972; Yen *et al*., 1986). Protein content tends to decline with increase in productivity level (Caliskan *et al.*, 2004). The proteins in the seed are located mostly in the outer layers of the seed. Based on their solubility, sesame proteins have been classified as albumin (8.6 %), globulins (67.3 %), prolamin (1.3 %) and glutelin (6.9 %) fractions (Rivas *et al*., 1981).

As in most other seeds, globulin is the predominant protein fraction in sesame seeds (Guerra *et al*., 1984). It is composed of two components. α-Globulin is the major fraction and accounts for about 60–70 % of the total seed globulin, while β-globulin is a minor component contributing 25 % to the globulin fraction (Nath and Giri, 1957; Rajendran and Prakash, 1988). α-Globulin is a high molecular weight protein (250 000–360 000 MW) and has a sedimentation coefficient of 11–13 S. It is an oligomeric protein composed of six dimeric units of molecular weight of about 50 000–60 000. The dimeric unit is of the A-B type linked by a disulphide bond (Robinson, 1987). The quaternary structure of α-globulin has been well established (Plietz *et al*., 1988). β-Globulin is the minor component of sesame seed globulins. It has a molecular weight of 150 000 and is rich in acidic and hydrophobic amino acids (Plietz *et al*., 1988).

Nutritional quality

The essential amino acid composition of sesame seed proteins (Table 23.4) indicates that sesame proteins are rich in sulphur-containing amino acids, particularly methionine (Smith, 1971; Brito, 1981; Narasinga Rao, 1985) and also tryptophan (Johnson *et al*., 1979; Yen *et al*., 1986). Sesame proteins are, however, deficient in lysine (Cuca and Sunde, 1967; Evans and Bandemer, 1967; Narasinga Rao, 1985; Sawaya *et al*., 1985; Yen *et al*., 1986) which is unusual for oilseed proteins. Among other essential amino acids, sesame protein is borderline deficient for threonine, isoleucine and valine contents compared with Food and Agriculture Organization (FAO) reference values (Nath *et al*., 1957). During preparation of a protein isolate (>90 % protein), there is some loss of methione, cystine and tryptophan. This may reflect the selective recovery or elimination of certain proteins by the isolation methods employed. The protein nutritive value of sesame is 15–42 relative to casein as 100 (Evans and Bandemer, 1967). Supplementation of sesame seed proteins with 0.2 % lysine significantly increased their protein nutritive value, and nutritive value of sesame protein supplemented with 0.2 % lysine + 0.1 % methionie + 0.1 % isoleucine was almost comparable to that of casein (Evans and Bandemer, 1967). The net protein

Table 23.4 Essential amino acid composition of sesame meal proteins (g/16 g N)

Amino acid	Evans and Bandemer (1967)[a]	Smith (1971)	Rivas *et al.* (1981)	Gopalan *et al.* (1982)	Narasinga Rao (1985)	FAO/WHO (1973)
Arginine	12.0–13.0	11.9	12.5	12.0	–	–
Histidine	2.3–2.8	2.2	2.4	2.7	–	–
Isoleucine	3.3–3.6	4.3	3.9	4.0	4.2	4.7
Leucine	6.5–7.0	6.9	6.7	8.0	7.4	7.0
Lysine	2.5–3.0	2.8	2.6	2.7	2.6	5.5
Methionine	2.5–4.0	2.7	2.5	2.9	2.8	3.5[b]
Cystine	1.1–2.2	–	–	1.9	–	–
Phenylalanine	4.2–4.5	4.7	4.5	5.9	6.4	–
Tyrosine	–	–	3.7	3.7	–	6.0[c]
Threonine	3.4–3.8	3.6	3.4	3.7	3.1	4.0
Tryptophan	2.0–2.4	1.9	–	1.3	1.5	1.0
Valine	4.2–4.4	5.1	4.7	4.6	3.9	5.0

a. Range for five varieties
b. Methionine + cystine
c. Phenylalanine + tyrosine

utilization (NPU) of sesame meal has been reported to be 0.56 as compared to 0.74 for whole egg powder (Fisher, 1973). Supplementation of sesame meal with 0.5 % L-lysine increased the NPU to 0.63. When sesame protein was used at 20 % level as the only source of protein in the chick diet, a good growth was obtained by supplementing it with 0.5 % lysine (Smith, 1971). Sastry *et al.* (1974) reported that supplementation of sesame flour with 1.25 % lysine improved the nutritive value of proteins making them comparable to that of skim milk powder. Supplementation of sesame diet at 18 % protein level with threonine significantly improved the chick growth (Cuca and Sunde, 1967).

The protein efficiency ratios (PER) of sesame seed, meal and isolated protein are 1.86, 1.35 and 1.2, respectively (Narasinga Rao, 1985). Commercially prepared flour and press cake showed PER values of 0.9 and 1.03. Supplementation of sesame seed protein with lysine can increase its PER to 2.9.

The amino acid composition of sesame complements that of most other oilseed proteins. Tryptophan, which is limiting in many oilseed proteins, is adequate in sesame. The availability of amino acids from sesame seed protein is affected by the method of processing. Digestibility is enhanced by heat treatment under moist conditions, while screw pressing for oil recovery apparently has little adverse effect on available lysine. However, *in vitro* digestibility was reportedly the same for the isolated sesame protein before and after autoclaving, indicating a lack of trypsin inhibiters (Kinsella and Mohite, 1985).

The problems encountered during addition of limiting amino acids to achieve nutritional adequacy (Lis *et al.*, 1972) are overcome by covalent attachment and the application of plastin reaction (Fujimaki *et al.*, 1977). Lysine-enriched plasteins have been prepared from sesame protein using *N*-ε-cbz-lysine methyl ester and

Table 23.5 Sugar content of sesame seeds and defatted flour (% on dry weight)

Sugar	Seed[1]	Defatted flour[2]
D-glucose	1.55	3.63
D-galactose	0.65	0.40
D-fructose	0.24	3.43
D-fructose	0.34	0.17
Raffinose	–	0.59
Stachyose	–	0.38
Planteose	0.06	0.23
Sesamose	–	0.14
Other sugars	–	0.16
Total sugars	–	11.26

Sources: [1]Aguilar and Torres (1969); [2] Wankhede and Tharanathan (1976).

also enzymatic hydrolysates of casein or soybean proteins. Plasteins obtained with *N*-ε-cbz-lysine methyl ester had a yield of 40 % for sesame and the lysine content was 16–19 % (Susheelamma, 1983).

The high level of sulphur-containing amino acids in sesame seed proteins is unique. It suggests that sesame protein should be more widely used as a supplement for methionine and tryptophan and should be an excellent protein source for baby and weaning foods. The use of sesame seed protein would eliminate the problems encountered when foods are supplemented with free methione, which is unstable.

23.2.3 Carbohydrates

The carbohydrate content of sesame seeds is comparable to that of groundnut seeds and is higher than that of soybean seeds (Joshi, 1961). Sesame seeds contain 14–25 % carbohydrates. The seeds contain about 5 % sugars, most of which are of reducing type. Defatted sesame meal contains more sugars. The sugar contents of sesame seeds and defatted flour are given in Table 23.5. Sesame seeds are reported to contain 3–6 % crude fibre (Ramachandra *et al*., 1970; Gopalan *et al*., 1982; Taha *et al*., 1987). The crude fibre is present mostly in husk or seed coat (Narasinga Rao, 1985). Wankhede and Tharanathan (1976) reported 0.58–2.34 % and 0.71–2.59 % hemicellulose A and B, respectively, in defatted flour. Hemicellulose A was found to contain galacturonic acid and glucose in the ratio of 1:12.9 while hemicellulose B contained galacturonic acid, glucose, arabinose and xylose in the ratio of 1:3.8:3.8:3.1.

24.2.4 Minerals

Sesame seed is a good source of certain minerals, particularly calcium, phosphorus and iron (Table 23.6). The seeds contain a total of 4–7 % minerals. Deosthale (1981) reported 1 % calcium and 0.7 % phosphorus in the seeds. It also contains sodium

Table 23.6 Mineral content of sesame whole seeds (mg/100 g)

Mineral	Joshi (1961)	Agren and Gibson (1968)		Gopalan *et al.* (1982)	Weiss (1983)
		White seeds	Brown seeds		
Calcium	1000	1017	1483	1450	1160
Phosphorus	700	732	578	570	616
Iron	20	56	–	10.5	10.5
Total	5700	5600	6200	5200	5300

Table 23.7 Vitamin content of whole sesame seeds

Vitamin	Agren and Gibson (1968)		Gopalan *et al.* (1982)	Weiss (1983)	Seegeler (1983)
	White seeds	Brown seeds			
Vitamin A (IU)	–	–	60*	30	Trace
Thiamin (mg/100 g)	0.22	0.14	1.0	0.98	1.0
Riboflavin (mg/100 g)	0.02	0.05	0.34	0.24	0.05
Niacin (mg/100 g)	7.3	8.7	4.4	5.4	5.0
Pantothenic acid (mg/100 g)	–	–	–	–	0.6
Folic acid (µg/100 g)	–	–	–	–	–
Free	–	–	51	–	–
Total	–	–	134	–	–
Ascorbic acid (mg/100 g)	–	–	–	–	0.5

*µg carotene (100 g).

and potassium. Calcium is mostly present in the seed coat which is lost during dehulling. Further, the bioavailability of calcium from sesame is less than that from milk or bread probably because of the high concentration of oxalate and phytate in the seed. Poneros-Schneier and Erdman (1989) reported the bioavailability of calcium from some food products, relative to $CaCO_3$ as non-fat dry milk 100 %; wholewheat bread 95 %; almond powder 60 %; sesame seeds 65 %; and spinach 47 %. Sesame grown on selenium-rich soils also contains high selenium, although most of it is present in the hulls (Kinsella and Mohite, 1985).

23.2.5 Vitamins

Sesame seeds are an important source of certain vitamins, particularly niacin, folic acid and tocopherols (Gopalan *et al.*, 1982; Weiss, 1983). The vitamin A content of seeds is, however, very low (Table 23.7). Vitamin E group includes several tocopherols, isomers and derivatives that differ in their biological activity (Table 23.8). The vitamin E activities of α-, β-, γ- and δ-tocopherols and tocotrienol are in the ratio of 100, 40, 10, 1 and 30 (McLaughlan and Weihraugh, 1979). Sesame oil is rich in tocopherols. However, the proportion of δ-tocopherols is more than that of α-tocopherols. Therefore, the vitamin E activity of sesame oil is less than that of sunflower oil.

Table 23.8 Vitamin E active compounds in sesame and sunflower oils (mg/100 g oil)

Compound	Sesame oil		Sunflower oil (Speek *et al*., 1985)
	Muller-Mulot (1976)	Speek *et al*. (1985)	
α-Tocopherol	1.2	1.0	78.8
β-Tocopherol	0.6	<.05	2.5
r-tocopherol	24.4	51.7	1.9
δ-Tocopherol	3.2	<.05	0.7
Total tocopherol	29.4	52.8	83.9
Vitamin E activity (α-tocopherol equivalent)	–	14.9	79.0

23.2.6 Antinutritional factors

Sesame seed is nearly free of antinutritional factors and is suitable for human consumption as such or after processing. Sesame seeds, however, contain high amounts of oxalate (Deosthale, 1981; Narasinga Rao, 1985) and phytic acid (Prakash and Nandi, 1978; Johnson *et al*., 1979). Sesame seeds contain about 1–2 % oxalic acid. Gopalan *et al*. (1982) reported 1.7 % oxalic acid in the seeds. The high proportion of oxalate reduces the physiological availability of calcium from the seeds. The oxalic acid in sesame seeds is mostly present in the testa or the hull portion. The presence of testa imparts a slightly bitter taste to the whole seed or meal because of chelation of calcium by oxalic acid. Dehulling reduces the oxalic acid content of the seeds. Oxalic acid may also be removed from sesame meal by treating it with hydrogen peroxide at pH 9.5.

Sesame seeds contain a substantial amount of phosphorus. However, most of this phosphorus is tied up in phytic acid or as phytin, a calcium and magnesium salt of inositol hexaphosphate. The seeds have phytate levels among the highest found in nature (De Boland *et al*., 1975). Phytic acid is a strong chelating agent and binds dietary essential minerals such as calcium, iron and zinc to form phytate–mineral complexes (Reddy *et al*., 1982). The formation of such complexes decreases the bioavailability of these minerals (Oberleas *et al*., 1966; Kon *et al*., 1973). The phytate in sesame meal is insoluble in water. O' Dell and De Boland (1976) extracted phytate from the meal by dilute HCl (0.3 M) and precipitated it with NaOH. The insoluble phytate had a composition of NaMg-phytate, suggesting that phytate in sesame meal exists as a magnesium phytate and not as phytin (CaMg-phytate).

Sesame oil contains two minor constituents, namely sesamin (0.5–1.0 %) and sesamolin (0.3–0.5 %). Sesamolin upon hydrolysis yields sesamol (Godin and Spensley, 1971). Although the nutritional significance of sesamin and sesamolin is not clear, sesamol has been reported to be partially responsible for the resistance of sesame oil to oxidation (Weiss, 1983; Kim *et al*., 2006). Sesame plants seem to have an unusual capacity for lead accumulation in the seeds. Yannai and Haas (1973) reported that whole sesame seeds and kernels contained lead at the level of 0.13–0.22 mg/100 g. More consumption of sesame (>200 g/day) is therefore considered to be harmful to humans.

23.3 Production: crop adaptation

Sesame is grown primarily in the tropical and subtropical regions of the world although it can be grown in more temperate climates. Sesame is cultivated in an area of 7.53 million hectares with a production of 3.60 million tonnes and a global productivity level of 478 kg/ha (Table 23.9). India is by far the largest producer accounting for 23 % of the world's area and 18 % of the production (Table 23.10). Other major countries producing sesame are China, Myanmar, Sudan, Nigeria, Mexico and, to a smaller extent, Ethiopia, Uganda, Venezuela, Turkey, etc. Estimates of world sesame production are always somewhat misleading primarily because, in countries where there is a substantial area planted to the crop, a high proportion is consumed by local farmers and is not marketed. The major obstacles to sesame's expansion are its low yields and the absence of non-shattering cultivars suitable for machine harvest. Consequently it requires much manual labour at the harvest season, which is often scarce even in its traditional growing areas.

Table 23.9 Worldwide area and production of sesame seed

Region	Area (ha)	Production (t)	Yield (kg/ha)
Africa	2 869 000	1 181 000	412
North and South America	290 000	201 000	693
Asia	4 375 000	2 220 000	507
World	7 534 000	3 603 000	478

Source: FAOSTAT (2000).

Table 23.10 Major producers of sesame in the world

Country	Area (ha)	Production (t)	Yield (kg/ha)
Bangladesh	34 000	27 000	794
China	521 000	586 400	1126
Ethiopia	185 900	186 800	1005
Guatemala	45 000	23 000	511
India	1 750 000	666 000	381
Korean Republic	31 000	18 000	581
Mexico	48 400	29 700	613
Myanmar	1 580 000	620 000	392
Nigeria	205 000	110 000	537
Pakistan	90 600	41 000	452
Somalia	62 000	30 000	484
Sudan	1 489 100	350 000	235
Syria	5200	3200	622
Tanzania	120 000	48 000	400
Thailand	65 600	44 300	675
Turkey	28 600	20 300	711
Uganda	286 000	173 000	605
Venezuela	40 500	17 000	420

Source: FAOSTAT (2010).

Different varieties of sesame seed (black, white and brown) are cultivated in India both as a rain-fed and as an irrigated crop. The western and southern states produce sesame as a *kharif* crop (June–October/November), while the eastern region cultivates it as a *rabi* crop (November–February/March). With two harvests of *kharif* and *rabi* crops, sesame seed supplies are available year-round in India.

The world trade of sesame is limited to about US $500 million. Sesame demand on a world basis is frequently greater than world production and, except where the crop is deliberately grown as a cash crop for export, there are seldom any large amounts available to world trade. India, China, Myanmar, Sudan and Latin American countries like Mexico are major suppliers of sesame seed. White sesame is preferred in the export markets as commercial bakers and confectioners consider it to be of higher quality than dark coloured sesame. White sesame also commands a higher price.

Sesame is basically considered a crop of the tropics and subtropics, but its extension into more temperate zones would be possible by breeding suitable varieties. The diversity of local ecotypes well adapted to their particular locality is an indication of the plants' potential in this respect. Sesame's main distribution is between 25°S and 25°N, but it can be found growing up to 40°N in China, Russia and the USA and up to 30°S in Australia and 35°S in South America. It is normally found below 1250 m, although some varieties may be locally adapted up to 2500 m. It is grown in Himalayas up to 1250 m and in Nepal up to 2000 m. The high-altitude types are usually small, quick-growing and relatively unbranched with frequently only one flower per leaf axil and low seed yields. Within varieties, yields invariably decrease with altitude. Oil content normally decreases with altitude in the same variety.

Sesame normally requires fairly high temperature during growth to produce maximum yields and 2700 heat units are reportedly required in Israel during the critical 3–4 months growth period (Kostrinsky, 1959). Temperature for optimum growth from seedling emergence to flowering and fruiting has been found to be in the range of 27–33 °C (Kinman and Stark, 1954; Smilde, 1960). Considerable genotypic variation in germination response to temperature has been reported (Sharma, 1997). A temperature of 25–27 °C encourages rapid germination, initial growth and flower initiation. If temperature falls below 20 °C for any length of time, germination and seedling growth will be delayed and below 10 °C, these processes are inhibited (Salehuzzaman and Pasha, 1979). High temperatures, particularly high night temperatures, promote stem growth and leaf production (Smilde, 1960). Temperature above optimum (40 °C or above) at flowering can seriously affect fertilization and the number of capsules set. A frost-free growing period is required for sesame and hard frost at maturity will not only kill plants but will also reduce seed and oil quality. It can also adversely affect minor seed oil constituents, such as sesamolin and sesamin (Beroza and Kinman, 1955).

Sesame is basically a quantitative short day plant and with a 10-hour day will normally flower in 40–50 days, but many varieties have locally adapted to various light periods. Early cultivars are generally less sensitive to day length than late types (Sinha *et al.*, 1973). When varieties are introduced to other areas which have a similar day length but different rainfall or temperature patterns, there is considerable variation in growth and yield from that in their original location. This is because of interaction of photoperiod with factors like light intensity, rainfall and

temperature. Light intensity has a significant morphogenic effect influencing yield and oil content. Considering yield obtained at the optimum planting period as maximum, then yield from sowings after this period decreases as the time from optimum sowing increases. However, rainfall has a major modifying influence on optimum time of planting relative to photoperiod.

The locally adaptable sesame varieties have been well utilized in countries such as India with distinct growing seasons. Varieties adapted to one season give uneconomic yield if grown in another season because of photoperiod and light intensity adaptability. The relationship of time of planting to maximum yield is generally well appreciated although less understood.

The rate of net total dry matter production per unit of ground area is related to the daily amount of photosynthetically active radiation intercepted by the crop. Low yields in *kharif* season in India could result from low radiation levels caused by heavy cloud cover, resulting in reduced radiation input, or from low plant density rendering suboptimal interception by the crop canopy. In the intercropping systems, sesame yields can be reduced due to shading by the companion crop. The stage at which shading occurs has great influence on the level of yield reduction. Recent advances in plant breeding have reduced plant height of the traditional companion crops like sorghum, millets and pigeonpea, putting sesame in a more equitable position in the competition for space and light, thereby improving the potential of sesame as an intercrop in India.

Sesame has great adaptability with regard to rainfall. It will produce an excellent crop with a rainfall of 500–650 mm but down to 300 mm and up to 1000 mm will also produce a crop under certain conditions, particularly under irrigation from newer varieties. For maximum yields, precipitation should be distributed over the period of plant growth as follows: germination to first bud formation 35 %, bud formation to main flowering 45 %, flowering to maturity not more than 20 %, falling as seeds are filling and ceasing as first pods begin to ripen. Heavy rain at flowering will drastically reduce yield and, if cloudy weather persists for any period at this time, yield can be very low. Rainfall when plants are ready for harvest also reduces yield by increasing susceptibility to disease and prolonging the period required for capsules to dry. Sesame is extremely susceptible to waterlogging, and heavy continuous rains at any time during growth will greatly increase the incidence of fungal diseases. Wild plants show a higher degree of resistance to waterlogging than the cultivated types (Nakhtore, 1952). Although sesame is susceptible to fungal diseases in high-rainfall areas, if the soil is permeable and drains fully so that there is no standing water to maintain high humidity, good crops may be obtained that would be impossible on more clayey soils with lower rainfall.

Sesame will have lower net photosynthesis and possibly lower yield potential when grown in an arid environment than when grown in areas which have a higher humidity. This is because, at high humidity gradient between leaf and air, there is reduction in the stomatal aperture. Large humidity gradient may cause midday closure of stomata and depression of photosynthesis. There is also an increase in leaf temperature at high temperature. However, the highest yields are obtained under irrigation in arid regions.

Sesame is considered to be a drought-resistant crop. It is capable of withstanding a higher degree of water stress than many other cultivated plants. However, during

the plant establishment phase, it is extremely susceptible to moisture shortage. Once established, the crop will grow almost entirely on stored soil moisture and, even with only the occasional shower of rain in the early stages, good yields are obtained. This ability to produce a crop under adverse conditions makes sesame an important crop under semi-arid conditions. Waterlogging is highly detrimental to the crop.

Sesame is susceptible to wind damage after the main stem has elongated. In the valleys of Kashmir, very cold winds from mountains during early growth and flowering cause severe injury to plants. Sesame is very susceptible to hail damage at all stages of growth. Prior to flowering, stems can be badly bruised, sometimes broken and terminal shoots so damaged that distorted growth occurs. At flowering, both buds and flowers may be stripped from the plant, or damaged buds produce aborted flowers. Heavy storms can virtually strip plants of all leaves and recovery will be slow.

23.4 Cultivation

23.4.1 Soils

Sesame grows well on wide range of soils from high sandy soils to black cotton and clay soils, but it thrives best in well-drained, moderately fertile soils of medium texture. Shallow soils with impervious subsoil are not suitable. The soils on which sesame is grown range from sandy soils in Sudan, Egypt and Rajasthan (India) to highly sandy-loam in Venezuela and river terraces in Northern Thailand, laterite soils in Uttar Pradesh and Madhya Pradesh (India), typical red earths and clay paddy soils in Karnataka and Maharashtra (India) and the central plain area of Thailand. On lighter, more gravelly or sandy upland soils in drier zones, growth and yield are often depressed due to poor moisture retention and low soil fertility.

Soils with neutral reaction are preferred, although good results have been obtained in slightly acidic and slightly alkaline soils. Sesame does not thrive on acid soils. It will grow in soils of pH 5.5–8.0 but, at higher pH, soil structure becomes increasingly important. However, many soils on which sesame is grown are saline. There is considerable variation among cultivars in the degree of tolerance to salinity (Kurien and Iyengar, 1968).

23.4.2 Cropping systems

Sesame, being a short-duration crop, fits well into a number of sequence and intercropping systems in different parts of India and elsewhere in the world both under rain-fed and irrigated conditions. In India, *kharif* sesame is grown both as pure and mixed with other crops, whereas, the semi-*rabi* and summer crops are taken as pure. The common component crops are pigeonpea, maize, groundnut, castor, pearl millet, mungbean, soybean, cotton, sunflower, sorghum, clusterbean, etc., in different states of India. As a sequence crop, sesame is taken after rice, groundnut, cotton, maize, pigeonpea, chickpea, finger millet, sorghum, wheat, mustard, horsegram, sugarcane, potato, lentil, pea, barley, mungbean, etc., depending on soil moisture availability and irrigation source.

23.4.3 Planting time

Correct time of planting is most important to obtain high yields of sesame. In India, sesame is grown in three seasons, *viz*., *kharif*, semi-*rabi* and summer. The *kharif* crop occupies over 70 % of the area under cultivation, whereas, the semi-*rabi* and summer crops occupy 20 and 10 % of the area, respectively. The *kharif* sesame is sown in June–July with the onset of monsoon and is harvested in September–October. The *kharif* and semi-*rabi* crops are entirely rain-fed whereas the summer crop is grown under irrigation. The yield of the *kharif* crop is poor, whereas, those of the semi-*rabi* and summer crops are high, since they are grown on rich soils and under better management (Hegde and Sudhakara Babu, 2002).

In other parts of the world, sesame is sown from August–November in Venezuela, from March–August in Mexico, in the southern USA when danger of frost is past, and in Africa at the start of the rains. Because huge seed losses occur if rain falls during the harvest season, in most tropical countries the planting is timed to allow harvest in the dry season.

23.4.4 Tillage and planting

Sesame requires a well-pulverised seed bed with fine tilth for good germination of seed and establishment of desired plant stand. The soil is brought to a fine tilth by deep ploughing in summer followed by planking (flattening of the soil). Tilth required for sorghum, wheat or similar small grains is suitable for sesame. Land should be perfectly levelled to ensure that there is no waterlogging and lands may be ridged to assist drainage in those areas where high-intensity storms are common. For a *rabi* crop, two or three harrowings followed by levelling is enough. Immediately prior to planting, lands should be harrowed to kill weed growth, since sesame seedlings make slow initial growth. Weed control while plants are small is difficult, and the aim should be as weed free a seed bed as possible.

In many countries, farmers usually sow sesame by hand and just scatter the seed, which is later hoed in to cover the seeds. For line sowing, seed drills may be used. The seeds should be placed shallow to get proper germination and plant stand. For mechanical planting, equipment may vary from small hand-operated seeder units or animal-drawn drills to tractor-operated, multipurpose, electronically-controlled seeders. Depth of planting varies with soil type and is usually 2–5 cm. Uniform depth of planting ensures regular emergence and crop growth, thus facilitating subsequent tillage operations.

Sesame may be scattered or line sown. For a scattered crop, a seed rate of 4–7 kg/ha is adequate to get the required plant stand. For line sown crop, seed rate may be reduced to 2.5–3 kg/ha. The seed rate in mixed or intercrop depends on the proportion of area occupied by sesame in the system. Spacing depends on growth habit of the variety, season and growing conditions such as rain-fed or irrigated. Row spacing of 25–75 cm is recommended in different countries. Thinning should be done scrupulously to ensure recommended plant spacing within a row. The first thinning is to be done invariably 14 days after sowing and the second thinning 21 days after sowing. Excess population adversely affects growth and crop yield. Early thinning will facilitate good establishment and proper use of fertilizers.

23.4.5 Nutrient management

The average nutrient removal to produce a tonne of sesame is 51.7 kg N, 22.9 kg P_2O_5, 64.0 kg K_2O, 11.7 kg S, 37.5 kg Ca, 15.8 kg Mg, 168 g Zn, 793 g Fe, 115 g Mn and 117 g Cu (Hegde, 1998). The level of nutrient application would, however, vary depending on the variety, crop, season, soil, fertility status, previous crop, rainfall and soil moisture. The application of fertilizers must also be related to plant population, for the optimum amount required by crops of different densities will vary (Park, 1967). Fertilizers also affect other plant characteristics that influence yield, i.e. plant height and number of capsules per plant, but the usual effects produced by added plant nutrients are not always correlated with yield. This is particularly so with nitrogen in the seed bed. In general, fertilizers have little effect on seed composition or oil content, except at much higher rates than are economically justified (Mitchell *et al.*, 1974).

Nitrogen application must be related to phosphate availability for, when this is deficient, nitrogen can depress yield. In addition, there is some evidence to indicate that it may also adversely affect seed oil content. Seed bed applications of nitrogen as part of an NPK mixture frequently give good results, but the ratio of NPK should ideally be locally calculated. In those regions where sesame is planted at the beginning of the rains following a pronounced dry season, the release of increased microbial nitrogen which then takes place may preclude seed bed application. Nitrogen application may vary between 20 and 50 kg/ha depending on the expected production. Application is best done at planting and, if needed, top dressed before the first buds appear. Method of application is also of little importance provided coverage is even and timing accurate.

Phosphate is the most important of the major plant nutrients necessary for high sesame yields, especially when irrigated. Uptake of NPK has been shown to be related to the general growth of sesame plants to approximately 60 days. At this point, the proportion of dry matter supplied by leaves falls and, with it, uptake of nitrogen and potassium, the latter to a lesser degree as it is also related to capsule number. Although the rate of phosphorus uptake also declines, it continues at a higher level than the other two as the number of capsules increases (Bascones and Ritas, 1961). In India, responses of up to 40 kg P_2O_5/ha have been reported (Sharma, 1997). If the previous crop is supplied with large amounts of phosphorus as in potato, sesame does not need any additional application.

Analysis of mature sesame plants usually shows a high potassium content, especially in the capsules, but, unless there is known local deficiency, application of this nutrient other than in small amounts is seldom necessary. In soils low in potassium, 15–30 kg K_2O/ha is recommended to maintain the required nutritional balance (Sharma, 1997).

There are no records of minor element deficiencies occurring in sesame although, at many locations, significant responses to micronutrients like Zn have been reported (Anon., 1998). However, as the responses have been inconsistent, commercial recommendations for micronutrient applications to sesame have not been made. In sulphur-deficient areas, application of 15–20 kg S/ha increases both seed yield and oil content. It is desirable to apply a full dose of phosphorus, potassium and sulphur at the time of planting the crop.

23.4.6 Weeding and interculture

The slow initial growth of sesame seedlings make them poor competitors to many quick-growing tropical weeds. Therefore, the crop is very sensitive to weed competition during the first 20–25 days. A weed-free seed bed is most important, since cultivation of sesame seedlings is difficult as the fine, fibrous roots are easily damaged. It is essential to have a minimum of two weedings, one after 15 days of sowing and another 15–20 days thereafter. Row crops can be weeded with any of the normal inter-row tillage implements such as hand hoe, animal drawn blade harrow, rotary or finger weeders, provided they are set to work as shallow as possible. Sesame plants grow rapidly after they reach some 10 cm in height and few cultivations are then necessary. Planting in narrow rows can assist in reducing late weed growth due to shading effect.

Weeds can also be managed effectively by use of proper herbicides. Diuran @ 400–600 g/ha, Basalin @ 1 kg/ha, Alachlor @ 1.75 kg/ha, Fluchloralin @ 1 kg/ha or Pendimethalin @ 1 kg/ha as pre-emergence treatment have been found effective for controlling weeds. Chemical methods of weed control may be resorted to wherever weed growth is severe and labour is scarce, followed by one hand weeding if required around 30 days after sowing. Band spraying plus inter-row cultivation is a combination that most frequently gives good weed control at relatively low cost.

23.4.7 Water management

In India, sesame during *rabi*/summer season is normally raised under irrigation. The crop during *kharif* season rarely receives any irrigation. Nevertheless, protective irrigation will greatly benefit the *kharif* crop whenever there are prolonged dry spells. Highest sesame yields are obtained when grown under irrigation in arid regions, where the sunny dry climate is very suitable and the low humidity reduces the incidence of fungal diseases.

Sesame is very susceptible to drought in various physiological stages. The crop is also very sensitive to waterlogging which causes premature death of the plant. When grown under irrigation, substantial pre-sowing watering is to be preferred to immediate post-emergence application, but the difficulty of planting in wet fields may require that the seed is dry-planted and then irrigated. Subsequent irrigations may be given at intervals of 12–15 days or more depending on soil type, weather conditions and season. The critical stages for irrigation are four to five leaf stage, flowering and pod formation. The short watering interval has been found to give higher yields than a larger application at longer intervals. A high application rate of water tends to reduce both seed weight and oil content (Kostrinsky, 1959). Free flooding and border strip methods of irrigation are normally employed for irrigating sesame in India.

23.4.8 Pests and diseases

Sesame crop is affected by a number of insect pests and diseases. Development and use of resistant varieties is perhaps the most economical method of reducing the losses due to pests and diseases. Nearly 29 insect pests belonging to eight species are reported to be potential pests of sesame. The leaf roller/capsule borer

(*Antigastra catalaunalis* Dup.) is the key pest along with the gall fly (*Asphondylia sesami*) in India. In Sudan, *Agnoscelis versicola* and the sesame seed bug (*Aphamis littoralis*) attack seed capsules in the fields. Other pests include aphids and thrips which stunt seedlings and injure developing flower buds. One or two sprays of organophosphate insecticides 40–60 days after sowing give effective control of these pests.

There are a number of fungal, bacterial, mycoplasma and viral diseases responsible for reduction of sesame yields. Stem and root rot (*Macrophomina phaseoli* Maubl.), ashby, phyllody (virus, mycoplasma), bacterial leaf spot (*Pseudomonas sesami*, Matkoff), fungal leaf spots (*Cercospora* spp.), *Alternaria* blight and leaf curl are the important diseases of sesame worldwide. These diseases are generally more prevalent in regions of high humidity and excessive rainfall and will give little trouble in arid deserts and dry regions provided disease-free seed is sown (ICAR, 1990).

23.5 Harvesting and post-harvest production

23.5.1 Harvesting and threshing

The optimum harvesting period is of great importance in sesame, since harvesting even a few days earlier or later can cause large yield reductions. Sesame is usually ready for harvest 80–150 days after sowing, most commonly in 100–110 days, but some cultivars also mature 70–75 days after sowing (Montilla *et al*., 1977). The sesame crop should be harvested when the leaves turn yellow and start drooping while the capsules are still greenish. At maturity, leaves and stems tend to change from green to yellowish and then reddish. If the harvesting is delayed and the crop is allowed to completely dry, there is loss in yield due to bursting and shattering of capsules. Capsules ripen irregularly from the low stem upwards, the topmost often being only half matured at harvesting. The drying period before harvesting allows the seed to ripen without loss from mature capsules.

The plants are cut with sickles or uprooted. The harvested plants are carried to the threshing yard and stacked for a week. During this period, the capsules burst open and leaves are shed almost completely. Then plants are dried in the open sun and threshed by gentle beating of plants with sticks. Threshing can also be done by simply turning the plant upside down and shaking or beating lightly. The seeds are cleaned with the help of a special type of sieve designed for this purpose. Later, seed is cleaned by winnowing.

The introduction of non-shattering varieties in India will allow mechanical harvesting, provided the crop is planted in large fields. Machine harvesting can be done with a reaper-binder or combine-harvester. The first method is preferred by many growers, who cut the crop when it is not fully mature and combine from the windrows. They consider that this greatly reduces the risk of seed loss and the straw has better feeding value. Most standard combines fitted with a pick-up reel and with the correct drum settings are suitable. Best samples with low seed loss are obtained from slow working and optimum speeds, once determined, should be maintained. Threshing of sesame requires accurate setting of concave and cylinder, for the seed is easily damaged and even microscopic cracks in the seed are sufficient to affect both viability and oil quality.

23.5.2 Yield potential

The yield of sesame varies with season, method of cultivation and variety and ranges from a few hundred to 3000 kg/ha in different countries. In India, according to season, 375–500 kg/ha during *kharif* and 500–750 kg/ha during *rabi*/summer may be expected. According to method of cultivation, a well-managed crop can yield 500–600 kg/ha under rain-fed condition and 900–1000 kg/ha under irrigated condition.

23.5.3 Seed storage

Bulk storage of sesame seed presents few problems provided the seed is clean and dry. Seed that heats or is contaminated by extraneous material produces discoloured or rancid oil. Sesame seed can be stored more economically than many other oilseeds because of its small size. It can also be moved easily and efficiently by modern conveyers without causing damage to the seeds.

Sesame seeds are cleaned and dried in the sun to bring down the moisture content to 5 % before storage to prevent attack from storage fungi and insect pests. In India, the most commonly used kerosene cans or grease drums with tight-fitting lids are quite convenient to handle and to store the seed in small quantities. In Africa, small lots are stored in earthen jars or wrapped in small banana leaf parcels sealed with dung and hung in the smoke of the fireplace (Salunkhe and Desai, 1986). In parts of east and west Africa, conical mud and wattle granaries holding about 100 kg of seed are constructed, and the narrow openings are then sealed with a mud bung. Several such stores may be grouped together on a platform and protected by a roughly thatched roof (Weiss, 1983). The tolerance level for post-harvest fumigation of sesame seed with hydrogen cyanide has been reported to be 25 ppm. Sesame seed retains its viability well under controlled conditions. When kept in storage at 50 % relative humidity and 18 °C, germination vigour was undiminished after 1 year (Prieto and Leon, 1976). To preserve viability of sesame germplasm collections for long periods, the use of silica gel in sealed containers is recommended (Weiss, 2000).

23.6 Processing of sesame

Sesame seeds are mostly used without removing the cuticle or the seed coat. This is especially the case in areas where sesame is processed for its oil. The cuticle contributes to the colour, bitterness and fibre and oxalate contents of the resultant screw-pressed meal. Such meal is not useful as a source of protein for humans and other monogastric animals and can be used mostly as a cattle feed or manure. Therefore, sesame seed is dehulled in order to improve its quality and utilization as a source of human food. Some of the important operations involved in processing sesame seed are described below very briefly.

23.6.1 Dehulling

Dehulling is an integral part of the modern oil extraction plants. It is also essential to produce high-quality oil and meal. However, dehulling still remains the single

Table 23.11 Effects of dehulling on the chemical composition of sesame seeds

Constituent	Whole seeds	Dehulled seeds
Moisture (%)	5.4	5.5
Protein (%)	18.6	18.3
Fat (%)	49.1	53.4
Total carbohydrates (%)	21.6	17.6
Crude fibre (%)	6.3	2.4
Ash (%)	5.3	5.3
Energy (cal/100 g)	563	582
Calcium (mg/100 g)	1160	110
Phosphorus (mg/100 g)	616	592
Iron (mg/100 g)	10.5	2.4
Vitamin A (IU)	30	–
Thiamin (mg/100 g)	0.98	0.18
Riboflavin (mg/100 g)	0.24	0.13
Niacin (mg/100 g)	5.4	5.4

Source: Weiss (1983).

most important problem worldwide in the processing of sesame seed. Many wet processing methods and mechanical treatments have been tried for dehulling (Sastry *et al*., 1969). The most commonly used method of dehulling is to soak the seeds and remove the cuticle manually by light pounding or by rubbing on a stone or wooden block. Ramachandra *et al*. (1970) have reported a lye treatment process for dehulling of sesame. In this process, seeds are cleaned and given a hot lye (0.6 %) treatment for 1 minute. The seeds are washed with excess cold water. The ruptured seed coats are separated by scrubbing in a suitable equipment. The dehulled seeds (kernels) are then dried.

The removal of hull results in significant change in the chemical composition of the seeds. The dehulled seeds contain significantly more fat and less crude fibre, calcium, iron, thiamin and riboflavin and slightly less phosphorus than the whole seeds (Table 23.11). Oxalic acid, being present mostly in the seed coat, is significantly reduced after dehulling (Narasinga Rao, 1985). The digestibility of proteins improves as a result of dehulling (Sastry *et al*., 1974). Heat treatment during dehulling as well as subsequent processing of the flour will not lower the available lysine. Quality of oil is also not affected by lye treatment before dehulling (Narasinga Rao, 1985).

23.6.2 Oil extraction

The most popular method of oil extraction from sesame seed in India is by *ghani* which is basically a large pestle and morter. In earlier days, *ghani* was made of wood and driven by bullock. Subsequently, power-driven steel *ghani* came into existence. The oil extraction by *ghani* is not complete and the yield of oil is about 40–45 % (Weiss, 1983). In many parts of India, water or jaggery (brown sugar) is added to sesame seed to facilitate oil extraction (Muralidhara, 1981). Following extraction, the oil is removed from the *ghani*, allowed to settle, skimmed and sometimes

strained through a cloth before sale. Sometimes, the residual meal is double-pressed to obtain more oil. The Burmese *hsi-zin* is similar to Indian *ghani*, but it is now replaced by power-driven mills. The oil yield from sesame seed by *hsi-zin* is about 33 % (McLean, 1932). In central Africa, sesame seeds are boiled to make them soft, then squeezed in a sausage made from the fibres to extract oil (Weiss, 1983). Some of these methods are still followed in many countries, sometimes with minor modifications.

Modern commercial methods for oil extraction from sesame seeds employ one of three basic designs: batch hydraulic processing, in which the oil is expressed by hydraulic pressure from a mass of oil-bearing material; continuous mechanical processing in which the oil-bearing material is squeezed through a tapering outlet, the oil being expressed by the increasing pressure; and solvent extraction in which the oil-bearing material is taken into solution with a solvent, which is then separated from the insoluble residue and the oil is recovered from the solvent solution (Godin and Spensley, 1971). The sesame seeds produced by farmers are not of uniform size, colour or maturity, being an admixture. They are also contaminated with soil particles. Because of the small size of the seeds, it becomes difficult to clean the seeds. The oil quality is affected if the seeds are not properly cleaned. Similarly, prolonged storage under unsuitable conditions results in a loss of oil quality (Sharma, 1977).

In Europe and Asia, the oil is usually extracted in three stages. The first pressing is made in cold. The oil contained is of very good quality and high grade. It has a light colour and agreeable taste and odour. The second pressing is made of the heated residue which is subjected to a high pressure. The oil obtained is coloured and is refined before using for edible purposes. The residue is used for the third extraction under the same conditions as for the second. The oil obtained from the third extraction is of inferior quality, not suitable for human consumption and is generally used for the manufacture of soaps.

The recovery of oil from screw or hydraulic pressing is not complete. In Europe, a combination of pre-processing and solvent extraction is used to obtain maximum recovery of oil. Direct solvent extraction is not suitable for sesame seeds due to high oil content.

23.6.3 Oil purification

Crude, cold-pressed sesame oil is used directly in cooking wherever it is produced and is often a favoured oil. Sesame oil does not require extensive purification or refining. The crude oil usually contains a suspended meat ('foot') which is removed by settling, screening and filtering. The filtered crude oil from the extraction plant contains impurities like phosphatides, resins, free fatty acids and colouring matters. Alkali-refining removes gums, free fatty acids and some of the colouring matters. The oil is bleached with a relatively smaller quantity of bleaching earth compared to other vegetable oils. Bleaching produces a light-coloured oil. Deodorization is necessary to produce a bland oil. This is usually done by treating refined oil in vacuum with steam at 200–250 °C. For use as a base of salad dressing, the oil must be stable under refrigeration. For this, winterization treatment is given to the oil. It consists of cooling the oil to remove components with high melting points that may settle out at low temperatures. Sesame oil, however, requires little or no

winterization (Lyon, 1972). The hydrogenation process brings about a considerable increase in stability of the oil.

23.6.4 Cake and meal

The by-product left behind after the extraction of oil is called sesame cake. When it is powdered, the cake is converted into the meal or flour. Powdering of cake into meal or flour will not result in any change in chemical composition (Awais *et al*., 1968). Four types of meals can be obtained from sesame seeds, namely whole seed meal, dehulled seed meal, defatted whole seed meal and dehulled–defatted meal. Of these, the dehulled–defatted meal is the most common and, unless otherwise specified, the term sesame meal refers to the dehulled–defatted meal. The chemical composition of sesame meal varies significantly due to dehulling and the method of oil extraction. The meals or flours obtained from dehulled seeds contain more proteins, phosphorus and less ash, crude fibre, calcium and oxalic acid than those obtained from whole seeds (Table 23.12).

Heat treatment will not affect the amounts of total protein and total lysine (Sastry *et al*., 1974). However, autoclaving for a prolonged period (60 minutes) causes significant decrease in the available lysine and dispersibility of proteins in water and NaOH solutions. Heat treatment of sesame flour will not affect the amino acid composition of its proteins except for a slight decrease in basic amino acids. An increase in the available methione from 1.85 to 2.33 mg/16 g N on treatment to pre-pressed solvent-extracted meal at 121 °C for 1 hour has been reported (Villegas *et al*., 1968). Rooney *et al*. (1972) prepared breads from composite flours containing heated and unheated oilseed meals. They observed that heat treatment of sesame meal resulted in less total and specific loaf volumes.

Table 23.12 Effects of dehulling and the method of oil extraction on the composition (%) of sesame flour/cake

Sesame seed and processing	Moisture	Fat	Protein	Ash	Crude fibre	Calcium	Phosphorus	Oxalic acid
Whole seeds								
Flour	5.2	49.8	19.1	5.7	4.1	1.2	–	2.3
Expeller-pressed flour	6.6	10.7	41.4	8.7	6.8	1.7	–	3.7
Expeller-pressed cake	8.1	13.5	35.1	8.9	5.3	1.8	0.8	3.0
Alcohol-extracted cake	8.6	3.1	38.2	9.4	5.9	2.0	0.9	3.5
Hexane-extracted cake	8.6	0.8	39.6	9.7	6.1	2.1	1.0	3.6
Dehulled seeds								
Flour	4.1	60.2	22.1	3.2	3.1	–	–	0.1
Expeller-pressed flour	5.8	10.0	54.4	6.2	5.1	0.3	–	0.2
Prepress-solvent-extracted flour	6.0	0.4	56.1	6.1	4.9	0.4	1.0	0.4
Expeller-pressed cake	8.3	12.7	41.3	4.8	3.1	0.4	1.1	0.5
Alcohol-extracted cake	8.9	3.4	45.8	5.0	3.1	0.5	1.2	0.5
Hexane-extracted cake	8.8	1.1	46.7	5.2	3.2	0.4	–	0.3

Source: Ramachandra *et al*. (1970).

In India, sesame cake is often used as an animal feed when the oil is extracted at village level. The free fatty acid content of Indian *ghani* cake is high and its keeping quality is poor. Therefore, it must be fed to livestock as soon as possible or it rapidly becomes rancid and unpalatable.

23.6.5 Protein concentrates and isolates

Many processed high-protein products such as flakes, flour, protein concentrates and isolates can be obtained from sesame (Sastry *et al*., 1969). The defatted flour contains more protein than the whole seed meal. The protein concentrate contains more protein (about 70 %) than the flour while protein isolate contains about 90 % or more protein. Unlike many oilseeds, the defatted flour and isolates prepared from sesame do not contain any undesirable pigments, off-flavour or toxins (Johnson *et al*., 1979; Toma *et al*., 1979). Sesame proteins are extracted with various salts and alkaline solutions. The extractibility of proteins varies with the extraction medium, pH and time. Sodium hydroxide solution (0.04 M) appears to be the most suitable solvent, extracting about 90 % of the meal nitrogen (Taha *et al*., 1987). With alkaline medium, the recovery of proteins is maximum when the meal is extracted at pH 10.0. Proteins exhibit minimum solubility at their isoelectric point. Most protein isolates are, therefore, prepared by extracting the proteins in suitable solvent and precipitating at or near their isoelectric point. The isoelectric point of sesame proteins is 4.5–4.9. The proteins extracted with salt and alkali showed minimum solubility in the region of pH 5.7 (Rivas *et al*., 1987). A low-phytate protein was proposed by Taha *et al*. (1987) by dissolving the protein by counter-current procedure and precipitating it at pH 5.4. At this pH, 50 % of the phytate was removed while only 17.5 % of the protein was dissolved. The resulting protein isolate contained 91.4 % protein, and was almost free from phytate.

The protein isolate contains very high levels of protein and is almost free from oil, ash, crude fibre and phytate phosphorus with very low levels of nitrogen-free extract (Rivas *et al*., 1981). The chemical composition of protein concentrate is intermediate between those of defatted meal and protein isolate. The essential amino acid composition of alkali isolate is almost comparable to that of sesame flour (Rooney *et al*., 1972; Prakash and Nandi, 1978). The salt isolate, however, contains more threonine and valine and less lysine and methionine than the other two products.

Sesame flour and protein concentrate exhibit less water absorption than soya flour. They also show higher fat absorption than soya products. The emulsifying capacity and emulsion stability of sesame products are comparable to those of soya products. The foam expansion and foam stability are higher in sesame products than in soya products. Protein extractability and whipping potential of sesame flour extract is low as compared to other oilseeds (Lawsen *et al*., 1972; Dench *et al*., 1981).

23.6.6 Roasting

Sesame seeds are often roasted prior to their use in confections. Roasting reduces the moisture content, develops a pleasant flavour and makes the seed or meal more acceptable for consumption. The reduction in moisture content during roasting of

sesame prevents moulding and reduces staling and rancidity. Sesamol, an antioxidant, was detected only in roasted sesame oil (Fukuda *et al*., 1981). 2-Furfuryl alcohol is considered to be one of the most characteristic components, giving a pleasant roasted aroma to sesame seeds. It is present in higher concentrations in red and white sesame (El-Sawy *et al*., 1988).

23.7 Main uses of sesame seed

The world production of sesame seed is almost wholly utilized for culinary purposes. In India, about 78 % of sesame produced is used for oil extraction, about 20 % is used for domestic purposes such as preparation of sweet meats and confectionery (Maiti *et al*., 1988; Weiss, 1983) and about 2 % is retained for the next sowing.

23.7.1 Human food

Seeds and kernels

Dehulled sesame seeds are sweet and oleaginous and are used directly in different types of foods in various parts of the world. They are used in the manufacture of traditional confections such as *halva*, *laddu* and *chikki* in India. They are also eaten whole after roasting. A confection called *laddus* is prepared from roasted groundnuts and sesame seeds by pounding with jaggery in the proportion of 2:1:2. Small balls are prepared by hand (ICMR, 1977). *Laddus* are also prepared from sesame seeds by mixing them with hot jaggery or sugar syrup. The confection prepared by mixing sesame seeds with jaggery or sugar has an auspicious connotation in many southern states of India. It is distributed or exchanged between people to signify a great deal of sharing of goodwill (Mulky, 1985). *Chikki* is another confection popular in Maharashtra and other western parts of India. It is prepared by pouring sesame seeds in boiling jaggery solution to obtain thick slurry. The slurry is spread uniformly on a metalic sheet or table and cut into small rectangular pieces. Ready-to-eat instant foods using sesame seeds have been developed by the Indian Council of Medical Research (ICMR) for use in rural areas (ICMR, 1977). *Bajra* instant food is prepared by mixing roasted *bajra* (pearl millet) flour (60 g) with roasted green gram *dhal* (15 g), roasted groundnut (10 g) and sesame seeds (5 g). The mixture is pounded to obtain a flour. When required, the powder is mixed with boiling water or milk to the desired thickness. Sugar or salt are added to taste. *Ragi* instant food is prepared in the same way by replacing *bajra* flour with *ragi* (finger millet) flour. The *bajra* instant food gives 18.6 g protein, 389 cal and 8 % NDP cal per 100 g flour. The *ragi* instant food gives 16 g protein, 369 cal and 8 % NDP cal per 100 g (ICMR, 1977).

In the Middle East, dehulled sesame seeds are mainly utilized in the production of *tehineh* (sesame butter) and *halwah* (halva). *Tehineh* is made from a paste of dehulled roasted seeds. *Halwah* is a sweet made up of *tehineh*, sugar, citric acid and *Saponaria officinalis* root extract. *Tehineh* and *halwah* are produced commercially in factories in the Middle East and North Africa. *Tehineh* is used in a variety of food dishes and added to bread and bakery products (Sawaya *et al*., 1985).

Sesame seeds and kernels are used in commercial bakeries for the preparation of quick breads, rolls, crackers, coffee cakes, pies and pastry products (Weiss, 1983; Farrell, 1985). The seeds are lightly roasted and used in salads and salad dressing (Farrell, 1985). Toasted seeds and butter or margarine make a tasty spread for bread.

Oil

Oil is the major product of sesame seed processing. In India, of the total sesame, about 75 % is used for edible purposes as vegetable oil for culinary purposes, 5–10 % goes to the *vanaspati* industry for vegetable *ghee* (a type of shortening) manufacture and 4 % for industrial uses as paints, soaps, perfumes, etc. (Salunkhe *et al*., 1992). Oil is a common constituent of Burmese dishes and is used in frying, roasting and stewing of meat, fish and vegetables. Sesame oil is highly favoured for cooking due to its nutty flavour. The oil has excellent stability and keeps well at room temperature for 2–3 months. It makes an excellent frying medium for chickpea and meat, and is a good replacement for peanut oil (Farrell, 1985). Because of the quality and high price, sesame oil is frequently adulterated with groundnut, rape or cottonseed oils. In India, particularly in some parts of Maharashtra state, groundnut, safflower and sesame seeds are extracted together to produce the so-called sweet oil (Weiss, 1983). This sweet oil is cheaper than sesame oil and has a better stability than groundnut or safflower oils. Sesame oil can be readily hydrogenated to medium melting fats and different textures for use in margarine, shortenings and *vanaspati* (Patterson, 1983). It has mild pleasant taste and is a natural salad oil, requiring little or no winterization (Lyon, 1972).

Cake and meal

Sesame meal has become increasingly important as a human food due to the following unique properties: the presence of a high level of sulphur-containing amino-acids, methionine and cystine (Block and Weiss, 1957; Evans and Bandemer, 1967; Smith, 1971), its lack of trypsin-inhibiting factors and its pleasant flavour. Sesame flour and meal have high protein content and are used to fortify foods (Parpia, 1966; Pomeranz *et al*., 1969; Rooney *et al*., 1972). Its use in the diet of children suffering from kwashiorkor has been found to be beneficial. It has been recommended as a protein supplement for soy and legume proteins (Boloorforooshan and Markakis, 1979; Brito and Nunez, 1982). Compoy *et al*. (1984) have prepared a snack food product using 70 % chickpea and 30 % sesame flour. Supplementation of black bean (*Phaseolas vulgaris* L.) meal with sesame meal significantly improved the PER and NPR of black beans proteins. Maximum PER and NPR were obtained when sesame and black bean flours were mixed in 1 : 1 proportion. The sesame lipids, cholesterol and triglyciride levels were also influenced by supplementation of black bean flour with sesame flour.

A number of ready-to-use infant foods using sesame meal like *cholam* and *samai* porridge have been developed by the ICMR, particularly for use in the rural areas (ICMR, 1977). Sesame flour has been used as a methionine supplement in the preparation of fermented foods, *vada* and *dosa*, the most popular South Indian dishes (Gulati *et al*., 1979; Chopra *et al*., 1982). Sesame flour was used to replace 5–20 % of rice–black gram flour. The sesame-supplemented *dosa* was found to be

acceptable organoleptically and had higher levels of methionine than the plain *dosa* (Chopra *et al*., 1982).

There is an increasing interest in fortification of bread and cookies by replacing a portion of wheat flour with non-wheat flours, especially protein concentrates, isolates and oil meals (Dendy *et al*., 1970). The maximum level of replacement depends upon the type of non-wheat flour, the strength of wheat flour, the baking procedure and dough-stabilizing compounds used (Pringle *et al*., 1970). In most cases, a 10 % replacement of wheat flour is optimum. At higher levels, loaf volume is severely decreased with serious deterioration of crumb colour, grain and texture (Mathews *et al*., 1970). Sesame flour has been used in the preparation of bread and cookies (Hoojjat, 1982). When used as a part replacement of wheat flour, sesame flour performed better than sunflower flour. High-protein biscuits are prepared by mixing wheat flour with roasted chickpea and roasted sesame flour to prepare the dough (ICMR, 1977).

Blends of peanut/chickpea, wheat/chickpea, rice/chickpea, peanut/soybean, sunflower/maize and cowpea/rice have all shown improved nutritional qualities when supplemented with sesame meal (Ensminger *et al*., 1994). Even more significant, however, is the finding that a simple blend of one part each of sesame and soya protein has about the same protein nutritive value as casein, the protein of milk. The high lysine and low methionine content of soya protein is complementary to sesame protein. Sesame meal is sometimes fermented for food in India and Java. In some European countries, it is also used as an ingredient in comminuted meat products.

The use of sesame flour or meal in formulating high-protein beverages has been reported (Tasker *et al*., 1966). Silva and Rivenos (1979) prepared a protein liquid from sesame. A nutritious beverage could be prepared using 70 % soya protein and 30 % sesame protein.

23.7.2 Animal food

In India, defatted sesame meal is traditionally used for animal food. The cake is a valuable stock food (Maiti *et al*., 1988), rich in protein, calcium, phosphorus and niacin. The cake is well liked by the stock and keeps well in storage. It is considered equal to cottonseed cake or soybean meal as a protein supplement for livestock and poultry. It is rich in methionine and is a valuable supplement to soybean meal in livestock diets (Grau and Almqvist, 1944). Sesame meal proteins are, however, deficient in lysine. Lysine-rich materials such as soybean meal, meat scrap and fish meal need to be combined with sesame cake to balance the diet. In the USA, most of the meal is used for livestock food. Inferior quality sesame cake or meal is used as a manure in China and Korea.

23.7.3 Industrial uses

Sesame oil is used to some extent in industries. Only a small proportion of low-grade oil is used for the manufacture of soaps, perfumes, paints, pyrethrum-based insecticides and for various other purposes for which the non-drying oils are generally

adopted (Nayar and Mehra, 1970; Weiss, 1983). Its relative scarcity and high price normally render it uncompetitive for large-scale industrial utilization. Sesame oil forms the basis of most of the fragrant or scented oils as it is not liable to turn rancid or solidify and it does not possess objectionable taste or odour. In the perfumery industry, sesame oil is used as a fixative. Scenting oil can be extracted from wetted sesame seeds that have been covered with layers of scenting flowers and left covered for 12–18 hours. A kilogram of strongly scented flowers is enough to perfume 6 l of sesame oil. Sesame oil has synergistic activity with insecticides such as pyrethrums and rotenone. The presence of sesame oil reduces the concentration of the insect toxin required to produce 100 % mortality. The synergistic activity of sesame oil has been attributed to the presence of sesamol and sesamolin.

23.7.4 Medicinal uses

Sesame seeds, oil, leaves and roots have excellent medicinal value. Sesame plant has played a major role in India's rich and diverse health traditions. The people of India, who live in harmony with nature, have an incredible knowledge of the medicinal value of sesame plant and make use of nature's bounty to achieve the best health traditions. Sesame seeds are regarded as microcapsules for health and nutrition. They are supposed to tone the kidney and liver and relax the bowel. The seeds are an aromatic, digestive emollient that softens the skin, a nourishing tonic, an emmenagogue that stimulates menstruation, a demulcent, a soothing laxative, an antispasmodic, a diuretic and a promoter as weight gain. Seeds are used for the treatment of constipation, tinnitus, anaemia, dizziness, poor vision and many general health problems associated with old age.

A paste of the seeds mixed with butter is helpful in treating bleeding piles. A decoction of sesame seed mixed with linseed is used as an aphrodisiac. The seeds milled and mixed with brown sugar are eaten by nursing mothers to encourage their milk production. Regular use of sesame seeds boosts the development of lustrous hair, particularly in children with poor hair development, a general problem in western countries. Sesame seeds are also used traditionally as a medicine for causing abortion. The seeds are valuable in respiratory disorders such as chronic bronchitis, pneumonia, asthma, dry cough and other lung infections. Seeds also help in correcting irregular menstrual disorders and in reducing spasmodic pain during menstruation. Seeds are also useful in treatment of dysentery and diarrhoea.

Sesame oil has been extensively used for therapeutic and cosmetic purposes in the Indian system of cure and care and it therefore is regarded as a magic botanical potion. Sesame oil is used as a laxative, emollient and demulcent. It has been successfully used in the treatment of backache, tinnitus, blurry vision, migraines, vertigo or dizziness, chronic constipation, haemorrhoids, dysentery, amenorrhea, dysmenorrhea, receding gums, tooth decay, hair loss, weak bones, osteoporosis, emaciation, dry cough, blood in urine, weak knee and stiff joints. It has antibacterial, antifungal and antiviral properties. Because of its easily assimilated calcium content, it nourishes the blood, calms nervous spasms and alleviates headaches, dizziness and numbness caused by deficient blood. It is a tonic, particularly for the aged. Oil of sesame will help burns, boils, ulcers and sunburn and removes freckles and age spots. Due to such innumerable benefits, the oil is used as the base for

several *Ayurvedic* preparations. However, it is poorly documented in the modern scientific literature.

Sesame oil is a preferred vehicle for fat-soluble substances because of its high stability. It is employed in the preparation of liniments, ointments and plasters. In India, it is extensively used for conditioning the skin (Weiss, 1983). Sesame oil is considered as anti-cholesterol and highly beneficial for heart ailments. The oil also reduces stress hormones and strengthens the immune system. It reduces anxiety, depression and pain. It also helps control sugar levels and, therefore, its use is beneficial for people with diabetes. In olden days, sesame seed oil was administered for snake bites. Sesame oil is used in the preparation of iodinol and brominol, which are employed for external, internal or subcutaneous use.

An infusion of leaves in hot boiling water is used as a gargle for the treatment of inflamed membranes of the mouth. The leaves, which abound in gummy matter when mixed with water, form rich bland mucilage used in infantile *cha*, diarrhoea, dysentery, catarrh and bladder troubles, acute cystitis and strangury. Crusted leaves of sesame are considered beneficial in the treatment of dandruff. A decoction made from the leaves and root is used as a hair wash which is said to prevent premature greying of hair and to promote hair growth.

A decoction of the root is used in various traditions to treat coughs and asthma.

23.8 Quality issues

There are no quality specifications for sesame oil for trade with respect to fatty acid composition and other physical and chemical characteristics. However, sesame oil, being expensive compared to many other vegetable oils, is sometimes adulterated with various quantities of cheap vegetable oils. Sesame oil is adulterated in various ways, *viz*. adulteration with lower grade, different origin vegetable oil or misbranding or contrabranding of products.

Individual oil samples are very often required to be examined for various parameters based on physical and chemical properties to ascertain the nature of adulteration. Chemical tests are conducted to identify adulteration. Colour tests are commonly used to detect adulterant in sesame as they are rapid. Testing to check adulteration very often becomes difficult when values for some parameters of sesame oil and the adulterating oil overlap. Then the help of other physical characteristics or special parameters like Reichert-Meissel value, Polensley value, etc. are determined.

23.9 Future trends

Sesame is the oldest oilseed known to human beings. It also has several desirable agronomic characters which can give the crop an edge over the competing crops. It does relatively well on poor lands and is resistant to drought. Availability of cultivars with varying duration helps to fit the crop into different intensive cropping systems under irrigated conditions. Cultivars are also available adapted to varying photoperiods and temperature regimes. The seeds contain more oil than many other oilseeds.

The oil has excellent stability and its protein is rich in sulphur-containing amino acids and tryptophan.

Despite all the above desirable qualities, sesame cultivation is generally confined to countries where labour is comparatively cheaper and plentiful. One of the major drawbacks associated with sesame for its large-scale cultivation is the absence of non-shattering varieties which are amenable to mechanical cultivation and harvesting. There is an urgent need to develop indehiscent sesame cultivars by making use of already available types in wild species and in germplasm collections through appropriate breeding and transgenic approaches.

The production potential of sesame is low compared to many oilseeds like groundnut and soybean. Hybrid technology may help to step up this potential. In countries like China and South Korea, hybrids produced through manual crossing have already proved successful in raising the productivity level. There are also reports of the existence of cytoplasmic genetic male sterility in sesame. This sterility system needs to be perfected to develop commercial hybrids for a major dent on productivity.

Sesame production in many countries is constrained by insect pests like leaf-eating caterpillar and stem and root rot. Imparting resistance to these insect pests and diseases will go a long way in enhancing and sustaining sesame production. Towards this end, resistance breeding against these maladies needs urgent attention.

More research should be focused on increasing the levels of sesamolin and sesamol in the oils for cultivated types and understanding their relationship with seed and oil yield. Although, considerable quantitative variability exists for these two traits, cultivars having very high content of oil as well as sesamolin have not been developed.

There exists an excellent opportunity for improving the extraction methods in many developing countries to get both better quality oil and defatted oilseed meal. Improved and easier methods of dehulling sesame seed are also needed. Further research regarding optimization of oil extraction and protein preparation is required with emphasis on techniques for minimizing the oxalic acid content of the flour. Greater emphasis is also needed on utilization of defatted sesame flour and meal in human nutrition. Development of acceptable products from oilseed cake for human consumption in different countries is a very high-priority research area to overcome chronic malnutrition in many developing countries. Standardization of milder processing methods for processing sesame oilseed cake that will eliminate problems associated with dark colour will also help in preparing acceptable sesame products for human consumption.

Sesame oil is too precious and valuable to be converted to biodiesel. Moreover, in terms of total oil production per unit area and time, there are more competitive crops than sesame. However, sesame cake can be a source for production of ethanol after fermentation of hexane-extracted sesame cake fibre (Balan *et al.,* 2009).

Sesame protein has unique qualities such as lack of trypsin inhibitor activity, high level of sulphur-containing amino acids and tryptophan and is therefore very valuable for use in baby and weaning foods. Its use would eliminate problems encountered when foods are substituted with free methionine, which is unstable and imparts a bitter taste to the food. Therefore, further research to develop nutritious foods from sesame protein is fully justified.

23.10 References

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24

Star anise

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Abstract: Star anise (*Illicium verum*, Hooker f.) is an important oriental spice with a star-like shape. It originated in China and is now produced in China, Vietnam and Laos. Fruits of some related species are poisonous and hence care should be taken to avoid their admixture with star anise. Star anise has a prime place in Chinese five-spice powder mix. It is popular in the culinary art of a few Asian countries and possesses high medicinal value. Shikimic acid extracted from star anise has been used in the preparation of vaccine against bird flu and swine flu. Star anise or its oil is used for flavouring kitchen and bakery preparations and in beverages, cosmetics and aromatherapy.

Key words: *Illicium verum*, Hooker f., essential oil, Chinese five-spice powder, shikimic acid.

24.1 Introduction and description

Star anise (*Illicium verum*, Hooker f.), often called Chinese star anise, belongs to the family Magnoliaceae or Magnolia family. Its fruit is an important spice of the orient. The tree is indigenous to Southeastern China. Star anise was known beyond China long before the Christian era as one of the few familiar spices, like cinnamon and clove. However, it was not until the late sixteenth century that this spice was first brought to Europe by an English navigator, Sir Thomas Cavendish (Kybal and Kaplicka, 1995). It is now a popular spice in some of the Asian countries and, to a certain extent, in Europe and North America.

Star anise tree is evergreen and grows to a height of about 8–10 m. Leaves are aromatic. The commercial part of the tree is the fruit. It is 2.5–4.5 cm in diameter and consists of about eight boat-shaped carpels arranged in the form of a whorl around the central axis. Carpels are about 9–19 mm long and every carpel is internally reddish brown, glossy and contains a single, flat, oval, lustrous, brownish yellow, brittle seed (Anon., 1959). The fruit rightly derived the name, star anise, from the attractive star-like arrangement of carpels. It has liquorice-like bouquet which is more pungent and powerful than anise.

24.1.1 Related species

There are a few species that are related to *I. verum* (Hook.f.). Fruits of *I. anisatum* L. or *I. religiosum* known as Japanese star anise were earlier considered identical

with *I. verum* until Hooker determined some distinctions in 1888. The Japanese star anise is slightly smaller, lacks sweet smell and tastes slightly bitter. Also known as *Sikmi* (*Shikimi*), it is seen mainly in Japan and, to a small extent, in Taiwan. In Japan, the tree grows wild in warm localities of the southern and central parts and in the Loochoo Islands. For a long time, the Japanese have planted *I. anisatum* in temple compounds and in cemeteries in order to protect them from desecration of wild animals. This practice appears to have developed from the fact that fruits and leaves emanate a peculiar odour that is supposed to keep animals away. It is a deep-rooted custom such that even today during funeral services altars are sometimes decorated with leaves of this tree. Dried fruits of *I. anisatum* contain about 1 % volatile oil, which has an unpleasant odour, quite different from that of *I. verum* (Guenther, 1972). Volatile oil contains safrole. Fruits are highly poisonous as they contain anisatin which causes severe inflammation of the digestive organs, kidneys and urinary tract. Cases of poisoning had been reported in Japan and also in the Netherlands as early as 1880. Fatalities in children have resulted from the ingestion of the seeds, with toxic symptoms being vomiting and convulsion resembling that of epilepsy with froth coming from mouth, loss of consciousness, dilated pupils and face becoming excessively cyanotic (Felter and Lloyd, 1898). The American Spice Trade Association (ASTA) in its Executive update of 29 July 2003 published an incident of people in Florida becoming ill after drinking tea prepared with star anise unknowingly admixed with *I. anisatum*, quoting a report from the US Food and Drug Administration (FDA). ASTA on 11 February 2002 had already cited a report from the Netherlands that individuals became seriously ill when they consumed star anise. It is not easy to distinguish fruits of *I. anisatum* from *I. verum* visually, especially when ground, but a simple gas chromatographic test can clearly show significant difference as the latter has a noticeable amount of anethole unlike the former, which has none. Further, bornyl acetate found in the fruits of *I. anisatum* in small amounts is not seen in *I. verum* (Anon., 2003). The water-soluble extract of *I. anisatum* has been found to promote hair growth in mouse and it could be a possible line of exploitation of this species (Sakaguchi *et al.*, 2004)

I. parviflorum Michaux is available in the hilly areas of Georgia, Florida and Carolina in the USA. It has yellow blossom and used for floral decorations. Fruits are eight-carpeled and taste like sassafras but are poisonous. Another species, *I. floridanum* Ellis, is also found in the USA in Florida along the Gulf of Mexico coast to Louisiana. It has a disagreeable odour resembling somewhat that of turpentine. Both fruits and leaves are poisonous. *I. majus* Hooker is a native of the Malay Peninsula. Fruits have 11–13 carpels with blackish brown colour and taste like mace. *I. griffithii* Hooker is seen in the state of Arunachal Pradesh of India and in Bhutan and its fruits consist of compressed, beaked, incurved 13 carpels in a single whorl. Fruits are slightly aromatic, bitter and acrid and reported to be poisonous (Anon., 1959). As of now, no fruit other than that of *I. verum* is used as a spice among the *Illicium* species.

24.1.2 Production

Propagation of star anise is by seed (Koshy, 1991). Seeds are collected from fresh fruits of vigorously growing mature trees known for high yield. Fully matured large

seeds, recognized by their characteristic brown colour, are selected. Seeds are sown 3–4 cm apart in a well-prepared raised bed. Since seeds quickly lose germination power, they have to be planted preferably within 3 days of the harvest of fruits. Layering has been attempted and found successful. After seedlings have produced the fourth leaf, they are transferred to a nursery and planted 25 cm apart. Once they are 3 years old, they are sufficiently grown and strong for planting in the field. Spacing for planting is about 5 m. Young trees do not require special care except weeding and manure application when necessary.

Trees flower normally when they are about 10 years old. The nature of flowering is unusual. There are three seasons for flowering. The first blossom of the year is from March to the end of April. Flowers of this blossom are generally sterile and do not develop into fruits. The second blooming is from July to August but lasts for 2 or 3 weeks only. Flowers of this blossom are larger and fruits are developed, but some are lost at a premature stage during November–January. The third flowering season starts immediately after the second, sometimes partly dovetailing with it. Although flowers of this season are relatively small, they develop into fruits and help to produce a bigger harvest by August–October of the following year. Thus the tree flowers almost throughout the year. Flowers are bisexual, scented and colour ranges from white to red. Fruits are available all the year round with seasonal variations. Normally harvest during August–October accounts for 80 % of the production. Many fruits fall from the trees prematurely owing to strong winds and when there are sudden changes in temperature (Anon., 1959).

Heath (1981) indicated that volatile oil content is about 3.5 % in the fresh fruit. Since the maximum content of essential oil is found just before full maturity and ripening, fruits should be gathered at this stage. Traditionally, children do most of the harvest. They climb the trees and gather fruits using hooks attached to long poles. Sometimes, fruits are harvested by shaking branches. In the initial years, yield of fresh fruits is small, amounting to only 0.5–1.0 kg per tree. Yield increases with age and reaches nearly 20 kg fresh fruits per tree by the 15th year. When a tree is 20 years old, full production is expected and the yield may go up to 30 kg. Harvested fruits are dried in the sun. Recently, a drying machine has been developed by modifying a tea drier with a capacity of 120–160 kg. Optimum drying time is 7.536 hours. One hundred kilogram fresh fruits yield about 25–30 kg star anise of commerce on drying and cleaning (Shiva, 2008). During drying, fruits turn into deep red colour. The characteristic aroma and flavour of star anise are developed during the drying process (Pruthi, 2001).

Commercial production of star anise is limited today to China, Vietnam and Laos. China has the largest area under star anise cultivation. Growing areas in China are in southern and southeastern provinces, particularly mountainous elevations of Yunnan. In Vietnam, star anise is grown in provinces adjoining the Chinese border. Lang Son province is the important area, but other provinces such as Bac Kan, Thai Nguyen, Cao Bang and Quang Ninh also produce this spice. In Lang Son province, cultivation is mostly in the districts of Van Lang, Van Quan, Tay Bac, Cao Loc, Binh Gia, Nam Truong Dinh and Bac Son. Total extent in this province is more than 9000 ha, the dominant district being in Van Quan. In the past, trees mostly belonged to collectives and to state farm enterprises. From 1990 onwards, these were dismantled and trees allocated to household management. The Vietnam

government had plans to bring in an additional 20 000 ha of star anise (Pruthi, 2001). The area in Laos under star anise production is much smaller compared to Vietnam.

Official statistics of production from government agencies in China, Vietnam or Laos are not available and FAO does not compile figures. However, trade estimates of production are available. With more area, production in China is higher than that of Vietnam. It was estimated that production in both these countries together was more than 25 000 Mt in 2000 (George and Sandana, 2000). Rough trade estimates in 2010 indicate that production has reached about 40 000 Mt for the three countries together, according to Frédéric (2010) of Bactatum, a company dealing with star anise in Vietnam.

24.1.3 Storage and transportation

Before storage, the moisture content of fruits should be brought down to less than 12 % to avoid deterioration. Freshness of fruits is very important in commerce and it has to be retained throughout storage. Hence, well-dried fruits should be stocked in a cool dry place to avoid loss of volatile oil and to protect from fungal infection. A favourable storage temperature range is 5–25 °C with a relative humidity of 60–70 %. Since fruits are fragile, care must be taken in all stages of handling. Freshness of fruit can be determined by breaking one segment, squeezing it between thumb and forefinger until the brittle seed pops up and then sniffing for the distinct aroma. If the aroma is weak, fruits have probably passed their optimum storage life or been kept in undesirable hot and/or open conditions. Properly dried fruits can be kept for 3–5 years in airtight containers away from heat, light and humidity (Hemphill, 2000). But the current year's crop is the most sought after by buyers.

Gunny bags with 40 kg capacity are used conventionally for packing cleaned and graded produce, but it is better to fill the product first into polythene bags of 25 kg capacity and then pack in 3-ply corrugated hard board boxes. This prevents loss of volatile oil, reduces breakage during transportation and ensures convenience in handling. These boxes are placed in a container leaving about a 50 cm gap at the top to give sufficient air space. Containers should be loaded onto ships in such a way that they are not exposed to direct sun or heat from the engine.

24.2 Oil extraction

Fruit (without seeds) contains volatile oil, resin, fat, tannin, pectin and mucilage. Seed has little volatile oil, but a high amount of fixed oil (Chembakam and Balaji, 2008). Essential oil is distilled generally from fresh fruits. If there is a large accumulation of fresh fruits they can be kept for about 10 days by spreading them in a thin layer and frequently turning them over to prevent fermentation. Oil can be distilled from dried fruits also, but yield is lower. Generally, yield of oil varies from 3.0–3.5 % from fresh fruits, depending on maturity, location, age of trees, region and soil and climatic conditions. Well-dried fresh fruits may have more than 8 % volatile oil, but it can be lost to some extent due to poor handling and storage.

24.2.1 Extraction of volatile oil

Various methods have been adopted for extraction of oil from star anise. Although steam distillation is easy and the cheapest, maximum oil yield is obtained by other methods, such as extraction with solvents or liquid CO_2.

Steam distillation

Traditionally, stills are used for distillation of star anise oil in China. Each still may hold up to 30 kg fresh fruits per charge. Sometimes, fruits are broken into pieces prior to distillation for better yield and for reducing the time needed for distillation. Whole or broken fruits are fed into a retort and water is poured to cover the material. Heating is done directly, but slowly. Steam embedded with oil rises up and it is collected and condensed, and floating oil is recovered. In the traditional still, fresh fruits require 48 hours and dried fruits 60 hours to complete distillation. Modern stills will not only reduce the time required for distillation to 3 or 4 hours but will also produce high-grade oil (Pruthi, 2001).

Leaves of star anise yield about 0.5 % essential oil on steam distillation. Leaf oil is inferior in quality and unscrupulous traders use it for adulterating the oil from fruits. Decorticated star anise seeds contain 55 % fat and fatty acids: myristic 4.43 %, stearic 7.93 %, oleic 63.24 % and linoleic 24.4 % (Pruthi, 2001).

Solvent extraction

Response surface methodology has been applied to optimize the conditions for the extraction of star anise oil from fruits. The results indicate that for efficient extraction acetone may be used keeping an extraction time of about 2–3 hours. The ratio of solvent to solid is 40:5.4 (v/w) and extraction temperature is 78.6 °C. The predictive maximum yield of star anise oil may be 10.49 % (Lu *et al*., 2008).

Distillation with liquid CO_2

Essential oil can be extracted from fruits with liquid CO_2. The fruit are reduced into pieces of 0.425–0.710 mm and it takes about 90 minutes for the distillation to complete. The yield of extraction is somewhat inversely proportional to particle size; the recommended particle size is 0.57 mm as for steam distillation. In a study by Tuan and Sarath (1997) the yield of oil with liquid CO_2 extraction was found to be 9.8 % higher than that with steam distillation, the quality was better and there was an energy saving. However, the anethole content of oils obtained under liquid CO_2 extraction and steam distillation are not significantly different. Extraction of oil using liquid CO_2 has also been studied by Zhu and Zhu (2007). Using an orthogonal test, the optimal conditions have been found to be pressure 16 MPa, temperature 35 °C, flow rate 30 l/hour and time required 2 hours. Yield of oil is 10.5 % which is 40 % higher, and the aroma is better than that of steam-distilled oil.

24.2.2 Separation of shikimic acid

Shikimic acid ($C_7H_{10}O_5$), which is used in the pharmaceutical industry, can be separated from Chinese star anise with hot water extraction at a temperature of 120 °C to obtain 100 % recovery in 5 minutes. Extraction of shikimic acid close to 97 % can be obtained with water at 70 °C adopting a slightly longer extraction time

(approximately 10 minutes). A semi-batch flow apparatus has been used to study the effect of temperature, average particle size, water flow rate and extraction time on experimental recoveries. For Chinese star anise that contained approximately 8 % shikimic acid, 100 % recovery could be obtained with 60 g water at a higher temperature of 150 °C keeping pressure at 15 MPa for a duration of 4 minutes with particle size ranging from 355–600 μm (Ohira *et al*., 2009). In a comparative study of extraction methods of shikimic acid it has been found that the ultrasonic extraction is as efficient as Soxhlet extraction and more rapid and simple. What is more, the linearity and recovery of shikimic acid are good. This method was tested for analysis of shikimic acid in Chinese star anise from different areas in Wenshan state, Yunnan province. It has been observed that Chinese star anise collected from Pingbian and Funing counties contained more shikimic acid than those obtained from other locations. Fruits of two plants from Pingbian county recorded a very high yield of >15 % shikimic acid (Liu *et al*., 2009).

24.2.3 Preparation of anisaldehyde from oil

Anisaldehyde or anisic aldehyde ($C_8H_8O_2$) has a vanilla-like odour and is used in perfumery and flavouring industries. It is also an intermediate in the production of antihistamines. Star anise oil can be used for the preparation of anisaldehyde by an ozonolysis reaction. The single-factor extraction test and Box-Behnken design were employed to determine the optimum conditions. The parameters were: reaction time 21 minutes, reaction temperature 8.45 °C and two solvents ratio 4.27:1 (g/g). Confirmatory experimental results showed that the yield increased to 73.6 % under the above-mentioned conditions (Gao *et al*., 2008). In order to raise the yield of anisaldehyde produced from anethole, star anise oil has been separated and purified with molecular distillation. The mass fraction of anethol is 93.73 % and that of anisaldehyde 91.05 %. Under microwave condition, peracetic acid has been used as catalyst and hydrogen peroxide as oxidant (Su *et al*., 2009).

24.3 Physical properties and chemical constituents of star anise oil

24.3.1 Physical properties

Tuan and Sarath (1997) studied the physical properties of star anise oil obtained by steam distillation and liquid CO_2 extraction. The oil yield obtained by steam distillation was 10.2 % and its colour was greenish yellow while the yield was 11.2 % and the colour yellow by liquid CO_2 extraction. Both oils have characteristic odour resembling true anise (*Pimpinella anisum*) oil. The anethole concentration varied from 89–92.2 % with the higher level in the steam-distilled oil. Various parameters of physical properties studied and the results obtained are given in Table 24.1. The values were as reported for standard star anise oil in the literature.

Hirasa and Takemasa (1998) also obtained more or less similar values for steam-distilled oil: specific gravity of 0.978 (25 °C) and refractive index of 1.5530 (20 °C). At normal temperature, the oil is in liquid form, but it becomes white crystals at a temperature of 22.5–23 °C. The crystals can be dissolved in alcohol but not in water (Tu and Xu, 2002).

Table 24.1 Physical properties of steam-distilled and liquid CO_2-extracted volatile oil

Character	Steam distilled	Liquid CO_2 extracted
Colour	Greenish yellow	Yellow
Dominant wave length (nm)	571.8	575.2
Specific gravity	0.9873 (25 °C/25 °C)	0.9859 (25 °C/25 °C)
Refractive index	1.5553 (25 °C)	1.5517 (25 °C)
Optical rotation	0.3167 (20 °C)	0.3333 (20 °C)

Source: Tuan and Sarath (1997).

24.3.2 Chemical constituents

Della Porta *et al*. (1998) used supercritical fluid extraction (SFE) for separating essential oil of star anise and found that the best essential oil extraction and fractionation conditions were at 90 bar and 50 °C for extraction and 90 bar, –10 °C in the first separator and 15 bar, 10 °C in the second separator for fractionation. The essential oil obtained was yellow with a strong anise fragrance. Waxes precipitated in the first separator were white, odourless and solid. Results of detailed gas chromatography–mass spectroscopy (GC–MS) analysis of these two fractions of both volatile oil column and waxes column, and HP column of the extract recovered in the second separator on a previously treated material are reported in Table 24.2.

The volatile oil contained 94.2 % anethole (*cis* and *trans*), 1.4 % estragole, 1.7 % limonene, 0.3 % linalool, two terpineol isomers (0.3 %) and 0.3 % linalyl acetate. Caryophyllene (0.5 %) and α-*trans*-bergamotene (0.7 %) were the main compounds among the sesquiterpenes. Anise waxes were formed mainly by *n*-pentacosane (35.7 %), *n*-heneicosane (25.8 %), *n*-tricosane (10.3 %), *n*-docosane (9.0 %) and *n*-tetracosane (6.2 %). The percentage of antioxidant compounds in the supercritical extract was 94.2 %.

Singh *et al*. (2006) carried out GC and GC–MS analysis of star anise volatile oil. This study showed 25 compounds which accounted 99.9 % of its contents. The major components were *trans*-anethole (94.37 %), methyl chevicol (1.82 %) and *cis*-anethole (1.59 %). Fifteen components were identified from its acetone extract accounting for 80.27 % of the total amount. *Trans*-anethole (51.81 %) was the major component along with linoleic acid (11.6 %), 1-(4-methoxyphenyl)-prop-2-one (6.71 %), foeniculin (5.29 %) and palmitic acid (1.47 %).

Using silica gel chromatography, seven compounds were separated from the fruits of *I. verum* of Guangxi in China. The chemical structures of these seven compounds were confirmed as shikimic acid, shikimic acid methyl ester, protocatecheuic acid, quercetin, 4-hydroxyphenyl-β-*D*-glucopyranoside, quercetin-5-*O*-β-*D*-glucopyranoside and magnolol according to graph of spectrum. Among them, 4-hydroxyphenyl-β-*D*-glucopyranoside, quercetin-5-*O*-β-*D*-glucopyranoside and magnolol were isolated for the first time from *I. verum* (Yang *et al*., 2010).

24.3.3 Flavour profile of star anise oil

Flavour components of star anise volatile oil obtained by steam distillation have been studied by Padmashree *et al*. (2007) and the results are given in Table 24.3. The

Table 24.2 Area percentages of the compounds found in star anise extracts

Compound	SFE oil (%)[1]	Waxes (%)[2]	HP (%)[3]
α-Thujene	tr.	–	–
α-Pinene	0.09	–	–
β-Pinene	0.03	–	–
Myrcene	0.04	–	–
α-Phellandrene	0.02	–	–
3-Carene	0.09	–	–
α-Terpinene	0.01	–	–
p-Cymene	0.04	–	–
Limonene	1.74	–	–
cis-Ocimene	tr.	–	–
γ-Terpinene	0.06	–	–
Terpinolene	0.02	–	–
Linalool	0.31	–	–
4-Terpineol	0.20	–	–
α-Terpineol	0.09	–	–
Estragole	1.45	–	–
cis-Anethole	0.15	–	–
trans-Anethole	94.05	–	60.53
Lynalyl acetate	0.11	–	–
α-Cubebene	0.21	–	–
β-Elemene	0.01	–	–
Caryophyllene	0.53	–	0.36
α-*trans*-Bergamotene	0.72	–	0.53
α-Humulene	0.02	–	–
β-*cis*-Farnesene	0.01	–	–
δ-Cadinene	–	–	0.11
Spathulenol	–	–	0.15
$C_{12}H_{15}N_3O_2$	–	–	0.87
Torreyol	–	–	0.07
α-Cadinol	–	–	0.46
$C_{16}H_{14}O$	–	–	2.56
trans-*cis*-Farnesol	–	–	2.11
$C_{15}H_{26}O$	–	–	1.97
$C_{15}H_{20}O_3$	–	–	2.23
Palmitic acid	–	0.72	0.33
Methyl palmitate	–	1.37	0.80
$C_{20}H_{28}O$	–	–	0.53
Methyl linoleate	–	–	0.54
n-Heneicosane	–	25.85	3.15
Methyl heneicosane	–	3.44	–
n-Docosane	–	9.03	7.10
n-Tricosane	–	10.35	7.37
η-Tetracosane	–	6.22	4.27
η-Pentacosane	–	35.71	1.70
η-Hexacosane	–	2.18	1.92
η-Heptacosane	–	1.73	0.34
η-Octacosane	–	0.64	tr.
η-Nonacosane	–	2.77	tr.

Note: Percentages are expressed as gas chromatograph area without any correction factor.
tr. = percentage lower than 0.01; not detectable.
[1] SFE oil column: compounds recovered in the second separator.
[2] Waxes column: compound recovered in the first separator (90 bar and 50 °C for 510 minutes).
[3] HP column: composition of the extract (300 bar and 50 °C for 180 minutes) recovered in the second separator on a previously treated material.
Source: Della Porta *et al*. (1998).

Table 24.3 Flavour profile of star-anise volatile oil

Compound	Identity	Peak %
α-Pinene	KI, MS	0.12 ± 0.020
β-Pinene	KI, MS	0.03 ± 0.020
Myrcene	KI, MS	0.02 ± 0.003
α-Phellandrene	KI, MS	0.04 ± 0.001
3-Carene	KI, MS	0.15 ± 0.020
α-Terpinene	KI, MS	0.02 ± 0.001
p-Cymene	KI, MS	0.05 ± 0.003
Limonene	KI, MS	1.05 ± 0.040
trans-Ocimene	KI, MS	0.09 ± 0.010
cis-β-Ocimene	KI, MS	0.01 ± 0.001
γ-Terpenene	KI, MS	0.04 ± 0.001
Terpinolene	KI, MS	0.03 ± 0.003
Linalool	KI, MS	0.29 ± 0.020
γ-Terpineol	KI, MS	0.12 ± 0.030
4-Terpineol	KI, MS	0.09 ± 0.020
α-Terpineol	KI, MS	0.08 ± 0.010
Estragole	KI, MS	1.05 ± 0.120
cis-Anethole	KI, MS	0.14 ± 0.020
trans-Anethole	KI, MS	93.9 ± 1.560
α-Cubenene	KI, MS	0.10 ± 0.010
β-Clemene	KI, MS	0.01 ± 0.001
Carryophyllene	KI, MS	0.10 ± 0.010
Bergamotene	KI, MS	0.01 ± 0.002
Δ-Cardinene	KI, MS	0.04 ± 0.002
α-Cardinol	KI, MS	0.02 ± 0.001

KI = Kovat's index; MS = mass spectra.
Source: Padmashree *et al*. (2007).

major flavour component in the oil is *trans*-anethole (93.9 %) followed by limonene (1.05 %) and estragole (1.05 %).

24.4 Quality issues and specifications

24.4.1 Adulteration

Japanese star anise (*I. anisatum* L.) has been documented to cause both neurological and gastrointestinal toxicity due to the presence of anisatin and concerns have been raised regarding adulteration of (Chinese) star anise with Japanese star anise. Emergency legislation was adopted by the European Commission on 1 February 2002. Decision 2002/75/EC requires all imports of star anise into the EU from third countries to be subjected to documentary checks to ensure that they have been sampled and analysed, and found not contaminated with Japanese star anise. Following Food Order 2002 (Star Anise from Third Countries) (Emergency Control) (England) (S.I. No. 332), which came into force on 16 February 2002, Environmental Health Officers and Port Health Authorities are instructed to monitor the import of star anise into the UK and ensure that each consignment has the required documentation to

prove that Japanese star anise is not present. Similar legislation is also in force in Scotland, Wales and Northern Ireland. Consequent to an incident in which infants became ill after drinking star anise tea, the US FDA advised in September 2003 against the consumption of teas containing star anise. Strict Federal Regulations on the import of star anise into the USA have also been introduced (Ize-Ludlow *et al*., 2004a).

A short and rapid method using microscopy and GC has been developed to detect *I. anisatum* in the powdered mixture of *I. verum*. There exist clear anatomical differences in the epicarp cells of *I. verum* and *I. anisatum* fruits as defined when examined under fluorescent microscopy and scanned with electron microscopy. A GC method has been developed for quick identification of possible *I. anisatum* adulteration in *I. verum* (Vaishali *et al*., 2005). Identification work has been carried out using combination of thin-layer chromatography (TLC) and high-performance liquid chromatography/tandem mass spectroscopy (HPLC-MS/MS) methods for rational quality control of *I. verum*. Species can be distinguished by their flavonoid pattern. A sensitive and selective HPLC/electrospray ionization (ESI)–MS/MS method has been developed for the detection and quantification of admixtures of *I. anisatum* and of other toxic species of *Illicium* even at a low concentration range using the sesquiterpene lactone anisatin as a marker. The proposed assay included a solid-phase extraction and clean up procedure with high recovery (>90 %). Chromatographic separation of anisatin has also been carried out on a C18 column, followed by MS detection using ESI in negative mode. The precursor/product ion transitions m/z 327 (quantifier) and m/z 327 297 (qualifier) have to be monitored. Statistical evaluations of this multi-reaction monitoring procedure have revealed good linearity and intra- and inter-day precision. The limits of detection and quantification are at 1.2 and 3.9 g/kg, respectively (Lederer *et al*., 2006).

Analysis by LC–MS of the sesquiterpene lactone fraction of *I. verum* samples has been performed to determine the presence of anisatin, neoanisatin and veranisatins A, B and C. Anistatin and neoanisatin are potent neurotoxins found in *I. anisatum*. Although *I. verum* is considered safe for consumption, this species has also been reported to contain toxic compounds, veranisatin A, B and C, but their levels in the fruits are very low. However, relatively small quantities may be sufficient in infants to produce adverse neurologic reactions. Ingestion of star anise should be considered in the differential diagnosis of infants presenting with acute-onset irritability, vomiting and seizures. Based on this finding and also possible contamination with *I. anisatum* or other species particularly in the powder form, it is not advisable to administer (Chinese) star anise to infants according to Ize-Ludlow *et al*. (2004b).

Recently, a comprehensive study has been made by Howes *et al*. (2009) to distinguish different species of *Illicium* using thermal desorption–gas chromatography (TD–GC–MS). The species taken are *I. anisatum* L., *I. brevistylum* A.C. Sm., *I. griffithi* Hook.f., *I. henryi* Diels, *I. lanceolatum* A.C. Sm., *I. majus* Hook.f. & Thomson, *I. micranthum* Dunn and *I. verum* Hook.f.. The volatile oil desorbed from the pericarps of *I. verum* was generally characterized by a high proportion of (*E*)-anethole (57.6–77.1 %) and the presence of foeniculin; the latter was otherwise noticed only

in the pericarps of *I. lanceolatum*. In the pericarps of other species analysed, the percentage composition of (*E*)-anethole was much lower (≤16.0 %). The pericarps of Japanese star anise *I. anisatum* were characterized by the presence of asaricin, methoxyeugenol and two other eugenol derivatives, which were not present in any other species examined. TD–GC–MS was found effective in the direct determination of the volatile components in the pericarps of different species and can be employed in distinguishing the fruits of *I. verum* from other species of *Illicium*, especially from the toxic *I. anisatum*.

24.4.2 Grading and quality specifications

Dried star anise is cleaned first by removing stalk, leaves and other extraneous matter.

Broken bits of fruits are also taken out. Traditionally the main criterion for grading is the size of the fruit based on its diameter. The first quality grade, 'Whole', indicates that 85 % of the fruits have a diameter of at least 2.5 cm. The rest of the fruits with a smaller diameter and partly broken pieces are included in the second quality grade called 'Broken'. Natural colour is a factor in grading (George and Sandana, 2000). The 'Whole' grade of superior quality has further specifications as follows: (i) current year's crop, well dried, clean and not mouldy; (ii) characteristic aroma and flavour; (iii) moisture ≤ 12.0 %; (iv) admixture 1 % (max.); (v) broken 7 % (max.); and (vi) seeds present at least in 80 % of the fruits.

The European Spices Association (ESA) prescribes and updates the quality minima for spices periodically and importers should demand at the minimum of this prescribed level of quality from exporters when purchasing spices for use within the EU. As per the document dated 2 November 2007, star anise should not have more than 3 % ash, 0.5 % acid insoluble ash and 8 % moisture of the product by weight, and volatile oil should be minimum 7 % volume by weight. The document does not allow any kind of adulteration and extraneous matter should not be more than 1 % by weight. Star anise should be free in practical terms from live and/or dead insects, insect fragments and rodent contamination visible to the naked eye (ESA, 2007). The ASTA has not specifically prescribed a quality standard for star anise for use by the US importers.

The International Standardisation Organisation (ISO) under the Aegis of the United Nations prepared an international standard – ISO 11178 – as early as 1995 detailing quality specifications for star anise. It covers both physical and chemical requirements. This standard is voluntary but member countries are recommended to follow it. The physical and chemical parameters of star anise prescribed in the document are given in Table 24.4.

For each requirement, a procedure for determination or testing the sample has been provided in the document or a reference is given to the ISO document that outlines the procedure in question. Recommendations for packaging, labelling, handling, storage and transportation are also mentioned. An example of a typical gas chromatogram of the volatile oil of star anise along with operation conditions and peak identification are furnished in the document. The document is reviewed, endorsed or revised every five years (ISO, 1995).

Table 24.4 Physical and chemical requirements of star anise as per ISO 11178

Physical requirements	
Colour:	Colour should be brownish red or reddish brown
Odour and flavour:	Characteristic odour with aromatic, sweet and anise-like flavour
Freedom from insects, moulds, etc.:	Free from living insects and practically free from moulds, dead insects, insect fragments and rodent contamination visible to the naked eye
Extraneous matter:	Not more than 2 % (m/m). The proportion of stalks not more than 3 % (m/m)
Broken and abnormal fruits:	Fruits with fewer than five follicles are broken and those with three or more underdeveloped follicles, abnormal and underdeveloped. The proportion of broken and abnormal fruits should not be more than 25 % (m/m)
Number of fruits per 100 g:	Not more than 130 per 100 g
Chemical requirements	
Moisture content:	Not more than 10 % (m/m)
Total ash:	Maximum 4 % (m/m) on dry basis
Volatile oil:	Minimum 8 % (ml/100 g) on dry basis

Source: ISO (1995).

24.5 Main uses of star anise

Star anise is one of the spices which has many applications. Earlier it was used mainly for culinary purposes and in Chinese and Ayurvedic (Indian) medicines. Today, star anise is important for certain allopathic preparations, bakery products and cosmetic preparations and in aromatherapy. It is also used for flavouring soft drinks and alcoholic beverages.

24.5.1 Food and beverages

Star anise is one of the signature flavours of Chinese savoury cooking. All over China, five-spice powder mix is common. This mix contains star anise, cassia, clove, fennel and Sichuan pepper in equal parts. Optionally, ginger, galanga, black cardamom or even liquorice are added. These spices are kept as whole on the kitchen shelf and ground when to use. The five-spice powder mix is added to the batter of Chinese-style fried vegetables and meat. Meat is sometimes coated with a mixture of corn starch and this spice mix before deep-frying. The mix is also used for marinating meat before stir-frying. One of the popular Chinese recipes which makes use of the five-spice powder mix is called flavoured pork. Star anise flavour combines well with pork and duck. Star anise is also one of the essential ingredients in Chinese master stock and is a component of the ground spice mix of puréed fruits and tarts.

Besides China, star anise is used on a large scale in North Vietnam. There, it is popular as one of the spices for the five-spice powder mix as in China and for making beef soups. It is essentially used to prepare broth for the Vietnamese noodle soup called *phở*. In India also, star anise is a popular spice. It is one of the ingredients in Indian curry powder, *garam masala*, for cooking meat, particularly in Kerala state. It is also used in preparations such as *biryani* and chicken curry to impart the special flavour. Persian and Pakistani cuisine employs star anise to a certain extent as in

North India. Some of the preparations using star anise were introduced from India to Indonesia in the past; but it remains admired only in the palaces of Sultans still adhering to the Royal Indian cooking style. Among other Asian countries, star anise is employed for cooking in Malaysia and southern Thailand. Thai iced tea (*Cha dam yen*) is brewed from black tea which is flavoured with star anise powder. It finds limited application in western cuisine. This spice is marketed as whole and ground, but it is the powder form which is often used for flavouring any preparation.

Star anise is a good substitute for anise seed in mulled wine and alcoholic beverages. In alcoholic beverages such as Pastis and Absinthes which are popular in France, star anise oil is used for flavouring. The oil is also used for flavouring soft drinks and special desserts. Star anise fruits after grinding or its volatile oil find application as a flavouring agent in chewing gum and chocolates. The essential oil from star anise fruits is also used in the confectionery trade to flavour licorice candy and other candies, and in the baking trade to flavour cakes, cookies and biscuits. The whole fruits are sometimes used in craft works for garnishing dishes or floated on a pot of tea (Shiva, 2008).

Star anise is a powerful antioxidant. The antioxidant property and sensory characteristics of star anise along with four other spices of the Chinese five-spice powder have been studied by Dwivedi *et al*. (2006) in cooked ground beef. Thiobarbituric acid (TBA) values were determined as a measure of antioxidant property. Even with star anise at 0.1 % level in the cooked beef, only lower pooled mean TBA values were recorded. Star anise flavour was inversely correlated with rancid odour. Another study was conducted using powders and ethanol/water extracts of star anise and caraway, all of which exhibited strong antioxygenic activity. However, volatile oil from star anise showed relatively higher antioxygenic activity than that of black caraway (Padmashree *et al*., 2007). In an investigation carried out with 68 Chinese herbs suitable for medical or food uses, star anise and five others were found to have the highest total contents of phenolics and flavonoids and maximum antioxidant activities (Liu *et al*., 2008).

24.5.2 Medicinal applications

Star anise is used traditionally in many Chinese medicines and in Ayurveda. The fruit is antibacterial, carminative, diuretic and stomachic. If taken internally, abdominal pain, digestive disturbances and complaints such as lumbago can be cured (Yeung, 1985). Leaves also have antibacterial activity. Some people chew the fruit after meals for better digestion and pleasing breath (Clevely *et al*., 1997). Star anise oil is often included in cough mixtures, particularly because of its aniseed flavour, and it is an ingredient in some cough drops. The oil can be applied externally to treat rheumatism and scabies. It is considered useful against body lice and bed bugs, and forms an ingredient in cattle sprays against fleas (Parry, 1969). For children it is effective for digestive upsets, including colic pain. Hence it is a traditional practice among Hispanic and Caribbean people to give star anise tea to babies when they cry due to colic pain. The antibacterial effect is somewhat similar to penicillin. It is helpful in soothing inflamed mucous membrane of the nasal passage (Winter, 2009).

Attempts have been made to use star anise in modern medicine by studying its therapeutic values. *In vitro* studies with ethyl alcohol extract of star anise have shown antimicrobial activity, mainly due to anethole present in the fruit, and the extract has been found effective against some common bacteria, fungi and yeast (Minakshi, *et al*., 2002). Shikimic and quinic acids are the established starting materials for Tamiflu, a viral neuraminidase inhibitor that is considered as the first-line treatment against a possible H5N1 (bird flu) pandemic. Other plants also have shikimic acid, but star anise has a high concentration that makes it attractive to drug manufacturers. There are different species of star anise, but only fruits of *I. verum* in particular have the concentration of shikimic acid that can be extracted cost-effectively.

Star anise was studied for its anticarcinogenic potential in *N*-nitrosodiethylamine-initiated and phenobarbital-promoted hepatocarcinogenesis with eight groups of rats. Star anise extract was prepared by adding 0.1 g of powdered material to 5 ml water–ethanol (1 : 1) mixture and centrifuging later to obtain the clear liquid. The extract was administered orally by feeding needle into the mouths of rats at a dose of 10 mg/kg body weight. While the treatment with *N*-nitrosodiethylamine increased liver weight of rats, administration of star anise extract reduced it. Treatment with star anise for 20 weeks or during the promotion stage for 6–20 weeks significantly reduced nodule incidence and nodule multiplicity in rats, while treatment with star anise limited to the initiation phase for the first 4 weeks alone could not show any positive response. Treatment for 20 consecutive weeks also significantly reduced the nodule size and nodule volume and restored the liver and erythrocyte superoxide dismutase activities to normal in the carcinogenesis-induced rats. The results indicate that star anise reduces tumour burden, lowers oxidative stress and increases the level of phase II enzymes, which may contribute to its anticarcinogenic potential (Yadav and Bhatnagar, 2007).

Star anise is not recommended for pregnant or breastfeeding women for want of scientific information. It is reported that star anise may increase the risk of bleeding if taken with herbs and supplements that may enhance bleeding. Patients suffering from dermatitis have also been found to have sensitization at 1 % and 2 % concentration of star anise oil. Patients positive to this oil are normally positive to anethole and to other constituents such as, α-pinene and limonene. Star anise oil at 1 % concentration itself is a strong irritant, but at 0.5 % sensitization has been noticed only in one-fifth of the subjects studied (Rudzki and Grzywa, 1976).

24.5.3 Use in cosmetic preparations and aromatherapy

There is demand for star anise oil for applications in the cosmetics industry. Other applications include natural and spa-at-home products. Star anise powder or essential oil finds use in the formulations of facial cream, gel, paste, talcum powder, toilet soap, etc. Star anise oil nowadays is increasingly used in aromatherapy, and the powdered aromatic bark as incense.

24.6 World trade

International trade is dominated by supplies from China and Vietnam which offer both grades: ‘whole’ and ‘broken’. There is no reliable data on the global

Table 24.5 Import of star anise into India during 2005–10

Year	Quantity in Mt	Value in '000US$
2005–6	2232.4	2747.1
2006–7	3164.5	4026.3
2007–8	1724.0	2358.3
2008–9	1375.0	2163.6
2009–10 (P)	2750.0	4394.1

P = provisional.
Source: Spice Board, Cochin, Kerala, India.

Table 24.6 Export of star anise from India during 2005–10

Year	Quantity in Mt	Value in '000 US$
2005–6	10.4	29.42
2006–7	9.6	41.12
2007–8	12.4	43.07
2008–9	12.6	37.54
2009–10 (P)	53.6	175.39

P = provisional.
Source: Spice Board, Cochin, Kerala, India.

export–import trade. As per the views of Frédéric (2010), total annual export of star anise in 2010 has been more than 30 000 Mt and star anise oil over 100 Mt (extracted from about 2000–2200 Mt dry fruit). India, Singapore, the USA, the UK, France, Australia and Pakistan are the leading importers of star anise. A quantity of about 200–250 Mt star anise is shipped to France for distilling volatile oil and use in the production of Pastis and Absinthe. The European Union is an important buyer of star anise oil. The general trend in demand of star anise oil in Europe is as follows: (i) fragrance industry 60 %, (ii) flavour industry 20 %, (iii) pharmaceutical industry 20 % and (iv) aromatherapy 10 % (Frédéric, 2010).

India is a traditional buyer of this spice as it is used for culinary as well as Ayurvedic preparations. Imports during the last 5 years in India are given in Table 24.5. Imports varied from 1375.0 Mt in 2007–8 to 3164.5 Mt in 2006–7 with an average quantity of 2249.24 Mt per annum during the 5 years. The mean import price has been US $1.40 per kg. India imports star anise from both Vietnam and China, but quantities imported from Vietnam are often much larger than those from China. Very small quantities are imported in certain years from Hong Kong and Singapore which re-export the spice.

India, although not a producer of star anise, re-exports small quantities to a few countries. This is mainly done to satisfy buyers abroad who want star anise along with other Indian spices in diminutive quantities, but it could be ground star anise. The quantity and value of re-exports during 2005–10 are given in Table 24.6. The quantities varied with the largest being in 2009–10 at 53.6 Mt. The average price realized is more than twice that of the import price at US $3.31 per kg.

There was heavy demand for star anise in 2005 for the extraction of shikimic acid, a starting material for preparing Tamiflu vaccine against bird flu (H_5N_1) epidemic. Price of star anise reached a record high in that year. Another spurt in demand and price hike were noticed in 2009 when swine flu (H_1N_1) spread to many countries. Roche Laboratories, which is involved in the vaccine manufacture on a large scale, has subsequently found a cheaper source for shikimic acid production.

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25

Tarragon

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Abstract: This chapter begins by discussing the vernacular names and different varieties of tarragon (also known as *Artemisia dracunculus* L). The cultivation, production and processing of tarragon are then introduced. Recent research into the chemical composition of the essential oils of tarragon is discussed, and, in particular, the presence of various terpenoid compounds. These topics are followed by the functional properties of fresh tarragon and its essential oils, such as antihyperglycaemic, anticoagulant and antifungal activities, along with their uses in the treatment of cardiovascular disease and thrombosis. The issues of quality in terms of grade and standard quality of tarragon leaves are also considered.

Key words: terpenoids, essential oils, antihyperglycaemic, antifungal activity, cardiovascular disease.

25.1 Introduction and description

The scientific name for tarragon is *Artemisia dracunculus* L. There are two main types of tarragon, Russian tarragon and French tarragon. These tarragons are actually varieties of *A. dracunculus* – var. *inodora* and var. *sativa*, respectively. French tarragon is native to southern Europe and the Mediterranean area, while Russian tarragon is native to Siberia and western Asia. The flavour of Russian tarragon is much weaker than that of the French variety (Nichols and Maggie, 2002). The common name tarragon is thought to be a corruption of the Arabic word 'tarkhum', which means a little dragon. The generic name for tarragon, *Artemisia*, is derived from the Greek goddess Artemis, the goddess of the moon. Tarragon's species name, *dracunculus*, is from Latin and means little dragon, possibly because of the shape of tarragon's root. There are various modern words for the herb, including tarragon (English and Hebrew), rakuna (Finnish), tarragona (Spanish), estragon (French), esdragon (Scandinavian and Russian), tarhun (Turkish), t'arkhuna (Georgian), tarkhun (Farsi), tarkhuun (Kurdish), tarhon (Turkish, Romanian), tarkanj (Serbian) and tárkony (Hungarian) (Katzer, 2008). In some languages, vernacular names are used, such as slangekruid, meaning snake herb, and drakebloed, meaning dragon's blood (in Dutch).

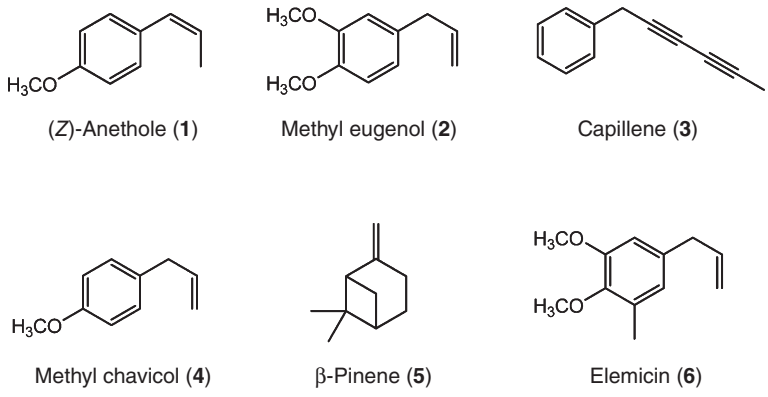

Fig. 25.1 Structures of some volatiles found in essential oils of tarragon.

25.1.1 Chemical composition

Many studies of the chemical composition of tarragon essential oil have been reported. Most have involved the use of the gas chromatographic–mass spectrometric (GC–MS) technique, which provides almost complete information concerning volatile constituents. The structures of the major constituents found in various essential oils of tarragon are shown in Fig. 25.1. The studies described below demonstrate that essential oils from different tarragon plants vary in composition quite significantly. These differences may result from different environmental and ecological conditions during growth, as well as from the existence of different chemotypes of tarragon.

Kowalski *et al.* (2007) determined the content of essential oil of tarragon as well as carrying out a quantitative evaluation of volatile components in hexane and methanol extracts of tarragon oil. They found that the herb of tarragon contained 3.172 % volume/weight (v/w) of essential oil. In this oil, elemicin (48.78 %), sabinene (18.88 %), (*E*)-asarone (*cis*-izoelemicin) (13.32 %) and methyl eugenol (7.63 %) were the major constituents. The compositions of volatile fractions of hexane and methanol extracts were similar to those determined in the essential oil. Tateo *et al.* (1989) reported the identification of 19 compounds in tarragon oil, with methyl chavicol (60 %), γ-terpinene (17 %), ocimene (13.54 %) and limonene (4 %) being the major constituents. Kordali and co-workers (2005) also explored the chemical composition of the oil from tarragon leaves and found that 30 compounds represented 99.5 % of the total oil composition. The predominant compounds of these 30 were (*Z*)-anethole (**1**) (81.0 %), (*Z*)-β-ocimene (6.5 %), (*E*)-β-ocimene (3.1 %), limonene (**3**) (3.1 %) and methyl eugenol (**2**) (1.8 %). Pappas and Stutz (2001) found that the key components of tarragon oil were terpinolene (25.4 %) and (*Z*)-β-ocimene (22.2 %). These authors also noted the presence of two unusual and rarely occurring alkynes in the oil: 5-phenyl-1,3-pentadiyne (11.7 %) and 6-phenyl-2,4-hexadiyne, which is also known as capillene (**3**) (4.8 %). The chemical composition of this oil was slightly different from the typical oil of tarragon in that it only contained a small amount (0.1 %) of methyl chavicol (**4**). Chauhan *et al.* (2010) found 24 compounds using a gas chromatography–flame ionization detector (GC–FID)

and (GC–MS) analysis. The major constituent of essential oil found was capillene (58.38 %). Other constituents were (*Z*)-β-ocimene (8.63 %), β-phellandrene (7.03 %), terpinenolene (5.87 %), camphene (4.16 %), spathulenol (2.02 %) and β-pinene (1.02 %). Sayyah *et al.* (2004) analysed tarragon essential oil and found (*E*)-anethole (21.1 %), (*E*)-α-ocimene (20.6 %), limonene (12.4 %), α-pinene (5.1 %), allo-ocimene (4.8 %), methyl eugenol (2.2 %), β-pinene (0.8 %), α-terpinolene (0.5 %), bornyl acetate (0.5 %) and bicyclogermacrene (0.5 %) as the main components. Lopes-Lutz *et al.* (2008) also reported that tarragon oil contained predominantly phenylpropanoids, such as methyl chavicol (16.2 %) and methyl eugenol (35.8 %). Estragonoside, having the structure of 4′,5,6,7,8-pentahydroxy-3′-methoxyflavone-8-*O*-α-*L*-rhamnopyranoside, and pinocembrin 7-*O*-β-*D*-glucopyranoside, a new flavonoid compound, were isolated from the epigeal part of tarragon for the first time by Kurkin *et al.* (1997). From the aerial parts of tarragon, one known alkamide, pellitorine, two new alkamides, neopellitorine A and neopellitorine B, and one known coumarin 'herniarine' were isolated by Saadali *et al.* (2001). Many chemotypes of tarragon have been reported: a French chemotype rich in methyl chavicol (49–51 %), a Russian chemotype rich in β-pinene (**5**) (23–31 %), a Polish chemotype rich in elemicin (**6**) (38–60 %), an Oregon chemotype rich in terpinolene and (*Z*)-β-ocimene (22 and 25 %), a Tehran chemotype rich in (*E*)-anethol and (*E*)-β-ocimene (21 and 20 %) and a Himalayan chemotype rich in capillene (58 %).

25.2 Cultivation and processing

French tarragon has smooth dark green leaves that have the true tarragon flavour, whereas Russian tarragon has fewer smooth leaves that are a fresher green shade. (Grieve, 1931). Russian tarragon is also taller than French tarragon, but both varieties otherwise are fairly similar in appearance. French tarragon is the variety generally difficult to grow from seed. It is best to cultivate it by root division. This perennial plant normally lies dormant during winter and then requires sunshine without excessive water during the growing season (Nichols and Maggie, 2002). Russian tarragon can be grown from seed. It is a hardier and more vigorous plant, grows better in poor soils and readily tolerates drought and neglect. The recommended pH for growing tarragon ranges between 6.0 and 7.5. Growing tarragon plants can tolerate temperature as low as 4 °C; plants must be protected from lower temperatures. Tarragon shrubs should be replanted every 3 or 4 years because the plants lose flavour and essence over time. The best time for harvesting tarragon plants is before the plants flower. At this time, the plants possess maximum essence and therapeutic value. When harvesting plants, one inch of the stem should be left above ground level to enable new shoots to grow. After harvesting the leaves should be pre-cooled to just above 0 °C (32 °F). At temperatures close to 0 °C, fresh tarragon leaves can be stored at 90–95 % relative humidity for 1–2 weeks. Moreover, storage at 0 °C can minimize effects of ethylene on visual quality (Cantwell and Reid, 1993) and slow the rate of moulds and bacterial decay. Both fresh and dehydrated tarragons are used for culinary purposes. Tarragon essential oil is also obtained from the plant's leaves and flowering tops by steam distillation. Tarragon oil has a herby, spicy, anise-like aroma.

Dried tarragon has a longer shelf-life, is lighter in weight and requires less storage space than fresh tarragon, so incurs lower packing, storage and transportation costs. The moisture content in the dried tarragon needs to remain minimal after drying in order to ensure the absence of mould growth during storage. Some of tarragon's aromatic aspects are lost when it is dehydrated, though, and its colour deteriorates. When tarragon (*A. dracunculus* L.) leaves were dried at a series of temperatures between 40 and 90 °C, the smallest changes in colour were observed at 40 °C, and at 90 °C when drying time was short, whereas the biggest changes were observed at temperatures of 50–70 °C (Arabhosseini *et al*., 2011). Seemingly, the combination of drying time and temperature qualify the change in colour. Overall, most of the colour changes happened before the tarragon reached 35 % moisture content. The essential oil content of tarragon also reduces after dehydration and during storage. Hosseini (2005) found that after 30 days storage of dried tarragon, the essential oil content was reduced by 50 %. An investigation into the effect of storage time on the essential oil content of French tarragon (*A. dracunculus* L.) leaves (Arabhosseini *et al*., 2011) also revealed a reduction in oil content during storage. The largest reduction in oil content was found in the material dried at 90 °C.

25.3 Main uses and functional properties

25.3.1 Uses in food

French tarragon, because of its sweet taste and anise and licorice aromas, is an important culinary ingredient. It is an ingredient in hollandaise, Béarnaise and tartar sauces, Dijon mustard, Montpellier butter and vinaigrettes. Fresh tarragon leaves are added extensively to fish, shellfish, meat and poultry dishes and, in particular, to creamy soups, veal, omelettes and quiche for the flavour and aroma they provide. Tarragon leaves can also be used to enhance vegetable dishes, such as those containing spinach or mushroom dishes. Some steamed vegetables, such as cauliflower, potatoes, zucchini, peas, and summer squash, are frequently topped with tarragon butter. Due to the limited lifetime of flavours and essences released from fresh tarragon leaves, they should be added to hot dishes just before serving. Fresh tarragon leaves may be added to green salads, but they should be added carefully because of tarragon's strong flavour. French tarragon is an essential component, along with chives, chervil and parsley, of the subtle blend of herbs known as *fines herbes*. Essential oils obtained from tarragon leaves are used in the preparation of tarragon vinegar and alcoholic beverages. Tarragon vinegar is a valuable ingredient for salad dressings and mustard. It is used in tartar sauce for flavouring but not in soups since the resulting taste would be too strong and pungent (Gatfield *et al.*, 2004). However, many French cooks mix tarragon vinegar with mustard.

To make tarragon vinegar, freshly picked tarragon leaves are crushed and placed in a one-quart canning jar. Then, white wine vinegar is warmed to a near boiling temperature. This nearly boiling white wine vinegar is immediately poured over the tarragon leaves and stems in the canning jar. The jar is then closed and kept for 10 days in a warm room. This time allows for fermentation to occur. During this fermentation period, the jar is shaken from time to time to ensure the best mixing of

the tarragon leaves and vinegar. After 10 days, when the taste and essence of the vinegar is deemed satisfactory, the vinegar is filtered and then is ready for use.

Tarragon is also considered to be an antioxidant, so capable of protecting foods against oxidative deterioration as reported by Kordali *et al.* (2005). Lopes-Lutz *et al.* (2008), however, determined that the antioxidant and radical scavenging activities of tarragon oil were only weak. Tarragon is also used in perfumes, soaps, and cosmetics and in condiments and liqueurs.

25.3.2 Traditional medicinal uses

Tarragon has been used to relieve flatulence and colic, as well as for treatment of rheumatism. It was believed that tarragon leaf could cure insect stings and snakebites, as well as the bites of rabid dogs. Tarragon is also believed to be a mild diuretic. This herb was used in ancient Greece to relieve toothache and as a local anaesthetic due to its content of eugenol, which is a natural anaesthetic. The herb is thought to mildly stimulate menstruation. It is traditionally administered to women who endure delayed menstrual periods. Tarragon tea is the traditional French drink that is considered very effective in relieving both insomnia and hyperactivity symptoms. It is prepared by adding dry tarragon to boiling water. The tea obtained is then covered and kept for approximately 40 minutes. This tea may be prepared by filtering the liquid in a cup. Homemade vinegar prepared with tarragon is also considered an effective medication for treating digestive disorders. Taking one teaspoonful of this vinegar before every meal is thought to invigorate the digestive system and helps to alleviate several problems related to digestion.

25.3.3 Modern research into functional properties

The biological activities of tarragon have been reported by many researchers. Wang *et al.* (2008) found that an alcoholic extract of tarragon (PMI 5011) could decrease glucose and improve insulin levels in animal models, suggesting an ability to enhance insulin sensitivity. An ethanol extract of Russian tarragon with antihyperglycaemic activity in animal models was reported by Govorko *et al.* (2007). This extract decreased phosphoenolpyruvate carboxykinase (PEPCK) mRNA expression in STZ-induced diabetic rats. Two polyphenolic compounds that inhibited PEPCK mRNA levels were isolated and identified as 6-demethoxycapillarisin and 2′,4′-dihydroxy-4-methoxydihydrochalcone with IC_{50} values of 43 and 61 μM, respectively. The study showed that 6-demethoxycapillarisin exerted its effect through the activation of the PI3K pathway, similar to that of insulin. The effect of 2′,4′-dihydroxy-4-methoxydihydrochalcone was not regulated by PI3K but was dependent on activation of the AMPK pathway. These results indicated that the isolated compounds may be responsible for much of the glucose-lowering activity of the tarragon extract.

Tarragon is used as an anticoagulant in Iranian folk medicine. Shahriyary and Yazdanparast (2007) investigated the effects of tarragon leaf methanol crude extract and its chloroform fraction on platelet aggregation, secretion and adhesion to laminin-coated plates. The methanol extract and its chloroform fraction, at a

concentration of 200 μg/ml, inhibited platelet adhesion to laminin-coated wells by 50 % and 60 %, respectively. In addition to alternation of cell adhesive properties, protein secretion and self-aggregation of the treated platelets all decreased upon treatment with the crude extract and its chloroform fraction. The results showed that the methanol crude extract and chloroform fraction of tarragon could inhibit platelets adhesion, aggregation and secretion. These findings provide some scientific basis for the traditional use of tarragon in Iran to treat cardiovascular disease and thrombosis.

Lopes-Lutz *et al.* (2008) found that tarragon oil had inhibitory effects on the growth of bacteria (*Escherichia coli*, *Staphylococcus aureus* and *S. epidermidis*), yeasts (*Candida albicans* and *Cryptococcus neoformans*), dermatophytes (*Trichophyton rubrum*, *Microsporum canis* and *M. gypseum*), *Fonsecaea pedrosoi* and *Aspergillus niger*. The antibacterial and antifungal activities of tarragon essential oils were evaluated by Kordali *et al*. (2005). They found that tarragon oils exhibited a potent antifungal activity at a wide spectrum on the growth of agricultural pathogenic fungi. Meepagala *et al.* (2002) isolated and characterized antifungal constituents of the steam-distilled essential oil fraction of tarragon. Two components, 5-phenyl-1,3-pentadiyne and capillarin, were found to possess antifungal properties, since they inhibited *Colletrotichum fragariae*, *C. gloeosporioides* and *C. acutatum*. The relative abundance of 5-phenyl-1,3-pentadiyne is about 11 % of the steam-distilled oil as determined by GC–MS. Methyl eugenol was also isolated and identified as an antifungal constituent of the oil.

The antimicrobial activities of the chloroform and acetone extracts, and two different concentrations of methanol extracts of tarragon, were studied by Benli *et al*. (2007). These extracts were tested against nine bacteria and four yeast strains by the disc diffusion method. The results indicated that the methanol extract of tarragon was more effective against the tested microorganisms than the chloroform or acetone extracts. The chloroform and acetone extracts were active only towards *Pseudomonas aeruginosa*, while the methanol extract, diluted with 10 ml of distilled water, showed inhibitory activity against *Shigella*, *Listeria monocytogenes* and *P. aeruginosa*. The methanol extract, diluted with 5 ml of distilled water, showed inhibitory activity against *E. coli*, *Shigella*, *L. monocytogenes* and *P. aeruginosa*. Saadali *et al.* (2001) reported the insecticidal activity of tarragon extract at a 200 mg/ml concentration against *Sitophilus oryzae* and *Rhyzopertha dominica*.

25.3.4 Toxicological studies

Tateo *et al*. (1989) evaluated tarragon oil by the Zimmermann test, and found it to be genotoxic. TARRALIN™, an alcoholic extract of Russian tarragon, however, was examined in a series of toxicological studies reported by Ribnicky *et al*. (2004). The extract was tested in an acute limit test at 5000 mg/kg and no signs of toxicity were noted. In a 14-day repeated dose oral toxicity study, rats appeared to easily tolerate 1000 mg/kg/day. Subsequently, TARRALIN™ was tested in an oral subchronic 90-day toxicity study on rats at doses of 10, 100 and 1000 mg/kg/day. Neither observation, gross necropsy nor clinical chemistry revealed any toxic effects. TARRALIN™ therefore appears to be safe and non-toxic.

25.4 Quality issues

Although, there are no market grades or sizes for fresh herbs, fresh tarragon leaves should follow standard quality requirements. They should appear fresh and green, with no yellowing, decay, insect damage or mechanical damage and the flavour and aroma should be strong. In addition, the EU Marketing Standards for Fresh Horticultural Produce require that fresh tarragon leaves must be free of abnormal external moisture and foreign smell or taste. In terms of labelling requirements, the country of origin must be clearly and prominently displayed on a label of the produce while a quality class and variety are not required.

A bulk of ground dried herbs entering international trade requires specific testing for contaminants or other specific residues. Although the health and safety requirements are issued by some international organizations, such as ASTA Specifications, EU regulations or ESA standards, the actual quality standards required are set by importers and major end-users. However, the main quality factors considered are appearance, flavour, aroma, colour, volatile oil content and cleanliness.

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26

Tamarind

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Abstract: *Tamarindus indica* L., commonly known as the tamarind tree, is one of the most important leguminous tree species. This chapter describes the origin, classification, chemical composition, production, sources, main uses, health aspects and quality issues associated with this crop. The tamarind tree originates from Madagascar. The most valuable and commonly used part is the fruit. The pulp constitutes 30–50 % of the ripe fruit, the shell and fibre account for 11–30 % and the seed about 25–40 %. Tamarind is commonly used as a health remedy throughout Asia, Africa and the Americas. Tamarind products, leaves, fruits and seeds have been extensively used in Indian Ayurvedic medicine and traditional African medicine. Tamarind is reported to have been adulterated with foreign matter which is both organic and inorganic in nature, due to poor post-harvest management practices including processing.

Key words: tamarind, pulp, concentrate, medicinal uses, production.

26.1 Introduction

Tamarind (*Tamarindus indica* L.) is one of the most widespread trees of the Indian subcontinent. It is a large evergreen tree with an exceptionally beautiful spreading crown, and is cultivated throughout the whole of India, except in the Himalayas and western dry regions (ICFRE, 1993; Rao *et al.*, 1999). Tamarind is a multipurpose plant. The pulp of the fruit has been used as a spice in Asian cuisine, especially in the southern part of India, for a long time. Almost all parts of the tree find a use in the food, chemical, pharmaceutical or textile industries, or as fodder, timber and fuel (Dagar *et al.*, 1995; George and Rao, 1997; Rao and Mary, 2001; Pugalenthi *et al.*, 2004).

26.1.1 Origin

Several authors have proposed various geographical areas as the origin of the tamarind tree. Tamarind fruit was at first thought to be produced by an Indian palm, as the name tamarind comes from a Persian word 'tamar-i-hind', meaning 'date of India'. Its name 'amlika' in Sanskrit indicates its ancient presence in the country (Mishra, 1997). As reported by El-Siddig *et al.* (2006), it was mentioned in the Indian

Brahmasamhita scriptures between 1200 and 200 BC. Morton (1987) placed its origin in India, but others considered it indigenous to the drier savannahs of tropical Africa, from Sudan, Ethiopia, Kenya and Tanzania, westward through sub-Sahelian Africa to Senegal (Brandis, 1921; Ridley, 1922; Dalziel, 1937; Dale and Greenway, 1961; Irvine, 1961; NAS, 1979). The tamarind tree is now considered to have originated in Madagascar (Von Maydell, 1986; Hockin, 1993). It is thought to have been introduced to South and Southeast Asia and to have become naturalized in many areas to which it was introduced (Simmonds, 1984; Purseglove, 1987; Coronel, 1991). It is now cultivated throughout semi-arid Africa and South Asia and has been planted extensively in Bangladesh, India, Myanmar, Malaysia, Sri Lanka, Thailand and several African, Australian, Central American and South American countries (Troup, 1921; Sharma and Bhardwaj, 1997).

26.1.2 Classification

The genus *Tamarindus* is a monotypic genus containing the sole species *T. indicus* and belongs to the sub-family Caesalpinioideae of the family Fabaceae (Leguminosae). Tamarind is a large, evergreen tree, up to 24 m in height and 7 m in girth. The morphology of the tree in detail has been described by several authors (Singh, 1982; Prakash and Drake, 1985; George and Radhakrishna, 1993; ICFRE, 1993; Dubey *et al.*, 1997). The most useful part is the pod (also called the fruit). Pods are 7.5–20 cm long, 2.5 cm broad and 1 cm thick, more or less constricted between the seeds, slightly curved, brownish-ash coloured, scurfy. The outermost covering of the pod is fragile and easily separable (Cowan, 1970; Duke, 1981; 1CFRE, 1993; Dubey *et al.*, 1997; Choudhary and Choudhary, 1997; Rao *et al.*, 1999).

26.1.3 Chemical composition

The tamarind fruit consists mainly of pulp and seeds. The fruit, both ripe and dry, contains mainly tartaric acid, reducing sugars, pectin, tannin, fibre and cellulose. The whole seeds also contain protein, fat, sugars and carbohydrates. Both pulp and seeds are good sources of potassium, calcium and phosphorous and contain other minerals like sodium, zinc and iron (Feungchan *et al.*, 1996 a, b; Coronel, 1991; Pino *et al.*, 2004; Soong and Barlow, 2004). The various components of tamarind are detailed in the following sections.

Pulp

The most valuable and commonly used part of the tamarind tree is the fruit. The pulp constitutes 30–50 % of the ripe fruit (Purseglove, 1987; Shankaracharya, 1998), the shell and fibre account for 11–30 % and the seed about 25–40 % (Chapman, 1984; Shankaracharya, 1998). The dried tamarind pulp of commerce contains 8–18 % tartaric acid (2, 3-dihydroxy butanedioic acid–$C_4H_6O_6$, a dihydroxy carboxylic acid) and 25–45 % reducing sugars, of which 70 % is glucose and 30 % fructose (Meillon, 1974; Anon, 1976; Duke, 1981; Ishola *et al.*, 1990; Parvez *et al.*, 2003). The tender fruits contain most of the tartaric acid in free form (up to 16 %). The sweetness of ripe tamarind fruit is, however, outweighed by tartaric acid which has an intensively acidic taste. The tartaric acid and the sugar contents reportedly vary from place to

place. In Thailand, the tartaric acid content varied from 2.5–11.3 % and the sugar from 5.0–40.0 %. The former in sweet tamarind was as low as 2.0–3.2 % and the latter as high as 39.1–47.7 % (Feungchan *et al*., 1966a). In Pakistan, Hasan and Ijaz (1972) found that in sour tamarind the tartaric acid content varied from 8.4–12.4 % and the sugar from 21.4–30.9 %. Tamarind contains other organic acids, such as oxalic acid, succinic acid, citric acid and quinic acid (Lewis and Neelakantan, 1964; Singh, 1973; Anon., 1976). The ascorbic acid content in tamarind is reportedly very low and varies from 2–20 mg/100 g (Lefevre, 1971; Ishola *et al*., 1990). Free amino acids, such as proline, serine, β-alanine, phenylalanine and leucine, were identified in the pulp (Lakshminarayan *et al*., 1954).

Tamarind pulp is rich in minerals such as potassium (62–570 mg/100 g); phosphorus (86–190 mg/100 g); and calcium (81–466 mg/100 g), and iron (1.3–10.9 mg/100 g). According to Parvez *et al*. (2003), magnesium content is high (25.6–30.2 mg/100 g), as is sodium (23.8–28.9 mg/100 g), whereas copper (0.8–1.2 mg/100 g) and zinc (0.8–0.9 mg/100 g) are low. It also excels in riboflavin and is a good source of thiamin and niacin, but is poor in vitamin A and vitamin C (Leung and Flores, 1961; Caluwé *et al*., 2009). The major volatile constituents of tamarind were reported by Pino *et al*. (2004), Askar *et al*. (1987), Zhang and Ho (1990), Sagrero *et al*. (1994) and Wong *et al*. (1998). A review on traditional uses, phytochemistry and pharmacology of tamarind has been published by Caluwé *et al*. (2009).

As mentioned earlier, the most outstanding characteristic of the tamarind fruit is that it is one of the most acidic of all fruits, because of its tartaric acid content which imparts the sour taste and outweighs the high total sugar content. Lee *et al*. (1975) reported that several pyrazines and thiazoles were found in tamarind and that the overall aroma of tamarind is characterized by its warm, citrus-like notes and some roasted undertones. Non-volatile flavour components in the pulp have been identified and analysed by using high-performance liquid chromatography (Khurana and Ho, 1989). Pino *et al*. (2004) reported that major components of the volatiles were 2-phenyl acetaldehyde with a fruity and honey-like odour, 2-furfuryl with a caramel-like flavour and hexadecanoic acid and limonene having a citrus flavour. Volatile components of tamarind fruits were isolated by simultaneous steam distillation/solvent extraction as well (Carter *et al*., 2001; Pino *et al*., 2004; Carasek and Pawliszyn, 2006).

Seeds

The seed consists of the seed coat or testa (20–30 %) and the kernel or endosperm (70–75 %) (Coronel, 1991; Shankaracharyan, 1998). Unlike the pulp, tamarind seed is rich in protein (13–20 %) and oil (4.5–16.2 %). The seed coat is rich in fibre (20 %) and tannins (20 %) as well. Panigrahi *et al*. (1989) reported that whole tamarind seed contains 131.3 g/kg crude protein, 67.1 g/kg crude fibre, 48.2 g/kg crude fat, 56.2 g/kg tannins and trypsin inhibitor activity (TIA) of 10.8, with most of the carbohydrate in the form of sugars. The trypsin inhibitor activity is higher in the pulp than in the seed, but both are heat labile. According to Purseglove (1987), the seeds contain 63 % starch and 4.5–6.5 % of semi-drying oil. According to Ishola *et al*. (1990), the seed also contains 47 mg/100 g of phytic acid, which has minimal effect on its nutritive value. It also contains 14–18 % albuminoid tannins located in the

testa. Following the estimation of the composition of seeds and evaluation of its properties, Marangoni *et al*. (1988), Sano *et al*. (1996), Patil and Nadagouder (1997) and El-Siddig *et al*. (2006) opined that tamarind seeds are potential sources of food or food ingredients.

The chemical composition and nutritive value of tamarind seeds and kernels was determined by several workers (Bose *et al*., 1954; Anon., 1976; Morad *et al*., 1978; Ishola *et al*., 1990; Bhattacharyya *et al*., 1993, 1994a,b; Siddhuraju *et al*., 1995; Patil and Nadagouder, 1997). Fatty acid composition of tamarind kernel oil was reported by several workers (Pitke *et al*., 1977, 1979; Reddy *et al*., 1979; Andriamanantena *et al*., 1983). Among fatty acids, linoleic acid, oleic acid and palmitic acid were the major constituents. Dehusked tamarind seeds have been found to be a rich source of pectin, the jelly-forming constituent of many fruits, vegetables, seeds, etc. (Kumar, 1997; Rao, 1948, 1956).

According to Glicksman (1986), Gidley *et al*. (1991) and Reid and Edwards (1995), tamarind seed polysaccharide (TSP) is the purified product as well as major component of tamarind kernel powder (TKP). Glicksman (1986) reported that TSP had different specifications to TKP. There have been numerous publications in the past 25–30 years concerning the primary structure of TSP (Srivastava and Singh, 1967; Nagaraja *et al*., 1975; Glicksman, 1986; Gidley *et al*., 1991; Manjunath *et al*., 1991; Marry *et al*., 2003; Nitta and Nishinari, 2005; Mishra and Malhothra, 2009). The functional properties of tamarind, such as nitrogen solubility index, water-absorption capacity, emulsifying capacity, foaming capacity and foam stability, were analysed Bhattacharyaa *et al*. (1994 a, b; 1997) and Kumar and Bhattacharya (2008).

26.2 Production and cultivation

At present, tamarind is cultivated in 54 countries of the world: 18 in its native range, including central African countries, and 36 other countries including India and Thailand where it was introduced (El-Siddig *et al*., 2006), and it has become naturalized in several regions. In the American continent, commercial plantations were reported in Belize, Central American countries and in north Brazil (Sharma and Bharadwaj, 1997). The major producing countries are Brazil, Bahamas, Costa Rica, Bangladesh, Cuba, Burma, Egypt, Cambodia, Guatemala, Dominican, Republic, India, Fiji, Indonesia, Gambia, Mexico, Kenya, Nicaragua, Pakistan, Puerto Rico, Senegal, Philippines, Tanzania, Sri Lanka, Vietnam, Thailand, Zambia, Venezuela and Zanzibar. However, tamarind is grown as a major plantation only in a few countries such as India and Thailand.

India is the world's largest producer of tamarind products. Tamarind is abundantly available in the Indian states of Madhya Pradesh, Bihar, Andhra Pradesh, Karnataka, Tamil Nadu, West Bengal, Orissa and Kerala (Jambulingam and Fernandes, 1986; Rao, 1995; Anon., 1997; George and Rao, 1997; Vennila and Kingsley, 2000; http://www.indianspices.com. Figures available for the production of tamarind in India for the years 2007-8 and 2008-9 indicated yields of 188 278 tonnes and 193 873 tonnes from 55 682 ha and 54 222 ha, respectively (Spice Board, 2011a). India exports processed tamarind pulp to western countries, mainly the European and

Arab countries and, more recently, the USA. During the year 2009–10 India exported 12 200 tonnes of different tamarind products valued at Rs 4705.50, lakhs (Spice Buded, 2011b). Tamarind products are exported to around 60 countries (http://www.indianspices.com).

Thailand has become a major producer of tamarind, with its sweet and sour cultivars, particularly the sweet tamarind types, grown there. The total planted area of tamarind in Thailand is 105 785 ha with the area in production being 60 451 ha and the non-production area 45 335 ha as per the reports of Department of Agricultural Extension in 1998. Documents show that Mexico also produced tamarind commercially, with over 4400 ha producing over 37 000 tons of pulp. It exported a small amount of processed pulp to Central and South American countries and to the USA (Hernandez-Unzon and Lakshiminarayana, 1982). Costa Rica, another Central American country, has shown a potential for expansion by producing 200 tonnes annually.

26.2.1 Sources, processing and preservation

A full-grown tamarind tree is reported to yield about 180–225 kg of fruits per season (FAO, 1998). In India, the average production of tamarind pods per tree is 175 kg and of processed pulp is 70 kg/tree, as reported by Kulkarni *et al*. (1993). However, Rao (1997) reported that Periyakulam 1 (PKM1), an improved cultivar in Tamil Nadu, yields about 263 kg/tree.

Tamarind fruits begin to ripen during the months of February–March (Cowan, 1970; Duke, 1981; 1CFRE, 1993; Choudhary and Choudhary, 1997; Dubey *et al.*, 1997; Rao *et al.*, 1999). Lewis and Neelakantan (1964) reported that by mixing the shelled tamarind fruits with a small amount of water and passing them through a pulper, the residual seeds, fibre and other extraneous materials can be removed. Mechanical methods of extracting pulp have been reported (Benero *et al*., 1972), and a tamarind dehuller has also been designed and developed in UAS, Bangalore, India. The machine has a hulling capacity of 500 kg/hour, with hulling efficiency of 80 % for large fruits and 58 % for small fruits (Ramkumar *et al*., 1997). Based on observations on post-harvest physiological and chemical changes in tamarind fruit, Lakshminarayana and Hernandez-Urzon (1983) suggested that maximum yield from tamarind might be achieved by processing within one week of harvest.

Tamarind pulp/concentrate is one of the essential components in Indian culinary habits. It is a common article of trade and is preserved and stored for marketing in a number of ways (Lewis *et al.*, 1957; Lewis and Neelakantan, 1959, 1964; Benero *et al.*, 1972; Patil and Nadagouder, 1997). In most of the tamarind-growing countries, pulp is pressed and preserved in large masses and sold in small shops and markets by weight. Patil and Nadagouder (1997) reported that, the pulp, freed from fibre and seed, is commonly mixed with 10 % salt and beaten down with mallets so as to exclude air and packed in gunny bags, lined with palm leaf matting. In India, the pulp is covered with salt, rolled into balls, exposed to dew and stored in earthenware jars (Chapman, 1984; Morton, 1987, Shankaracharyan, 1998), whereas in Java, the salted pulp is rolled into balls, steamed and sun dried, then exposed to dew for a week before packing in stone jars. In Thailand, the pulp is mixed with salt and compressed and packed in plastic bags to exclude air for storage. In Sri Lanka, the

harvested pods are dried in the sun for 5–7 days to bring all fruits, including the half-mature fruit, to the fully ripe stage. The separated pulp along with the seed is dried in the sun for 3–4 days to remove excess moisture and prevent the growth of moulds, mixed with salt and packed in clay pots for storage (El-Siddig *et al*., 2006).

The freshly prepared pulp is light brown in colour. According to the research findings of CFTRI (Central Food Technological Research Institute), Mysore, India, pulp can be preserved well for 6–8 months, without any treatment, if it is packed in airtight containers and stored in a cool dry place (Shankaracharya, 1997). According to FAO (1989), continuous storage for long periods under extremes of temperature and humidity is a problem because of changes in colour which take place from brown or yellowish brown to black. In the Sri Lankan storage method outlined above, for example, the tamarind could be stored for about a year; however, the colour changed to dark brown or black and changes in flavour occurred (El-Siddig *et al*., 2006). Feungchan *et al*. (1996b) conducted studies on factors related to colour change of tamarind pulp from brown to black to yellow in storage and recommended mixing of 10 % powdered salt and cold storage to prevent this. According to Ramkumar *et al.* (1997), pulp loss during storage was very low in black polyethylene (0.18 %) and plastic (0.17 %) compared to phoenix mat (1.35 %) and metal (1.53 %).

CFTRI developed an improved process for preparation of tamarind paste from good-quality tamarind, free from seeds, fibrous and extraneous matter. The cleaned pulp was subjected to heat processing followed by coarse grinding and was reprocessed to reduce the moisture level to obtain optimum quality tamarind paste (Anon., 2003a). Preservation methods of sweet tamarind fruits and pulp in Thailand have been documented by Chumsai-Silavanich *et al*. (1991) wherein tamarind fruits were steamed for five minutes, followed by drying in a hot air oven at 80 °C for 2 hours and storing in plastic bags at room temperature. Using this method, the fruits were stored for four months without any deterioration in quality. Feungchan *et al*. (1966b), Puranaik *et al*. (2004), Anon. (2003b), Kotecha and Kadami (2003) and Nagalakshmi and Chezhian (2004) found that cold storage of tamarind pulp at various temperatures increased shelf-life. It was reported that the freshly harvested deseeded tamarind pulp can be stored for up to 330 days under refrigeration at 4 ± 2 °C when vacuum packed in 800 gauge poly bags without any colour change in the pulp right from the initial stage of storage (Nagalakshmi and Chezhian, 2004).

26.3 Main uses of tamarind products

26.3.1 Pulp

Tamarind is used in India mainly in the form of pulp. The fruit pulp is the chief agent for souring curries, sauces, chutneys and certain beverages throughout the greater part of India. In India, the immature green pods are often eaten by children and adults dipped in salt as a snack. It is also used in India to make ‘tamarind fish’, a seafood pickle, which is considered a great delicacy. Immature tender pods are used as seasoning for cooked rice, meat and fish and delicious sauces for duck, waterfowl and geese (El-Siddig *et al*., 2006). Tamarind fruit is also reported to be used as a raw material for the preparation of wine-like beverages (Giridhari *et al*.,

1958; Sanchez, 1985; Latino and Vega, 1986; Benk, 1987; Grollier *et al*., 1998). Whilst it is found mainly in Indian regional food, the spice is used in Asian, Latin American and South African dishes extensively. A review on traditional uses of tamarind with reference to sub-saharan Africa has been published by Caluwé *et al*. (2009).

In Sri Lanka, tamarind is widely used in cuisine as an alternative to lime and also in pickles and chutneys (Jayaweera, 1981). In the Bahamas, fully grown but still unripe fruits are roasted in coal, the skin is then peeled back and the sizzling pulp is dipped in wood ash and eaten (Morton, 1987). In Egypt, tamarind is used to make a sour drink during the summer period, and it is also added to a similar lemon-flavoured drink, popular in the Middle East. It is also used for this purpose in Mexico, where the drink is known as well as *agua fresca* (refreshing water) or *agua de tamrindo*, which is sometimes turned into frozen fruit ices. Mexicans also use tamarind as a snack, dried, salted or candied (e.g. Pulparindo). In the Philippines, it is also used to make sweets, but the leaves of the plant are also utilized in the recipe for the famous sinigang soup. In Guadeloupe, the fruit is used to make jam and syrup, whilst in northern Nigeria tamarind is used during breakfast, as it is added to the traditional porridge known as pap or *kunun tsamiya*. Tamarind is also widely used in sauces to give a sour flavour, for example in the popular pad thai from Thailand, or in gravy for assam fish in Singapore and Malaya.

26.3.2 Concentrate

Juice concentrate of tamarind is produced and marketed in India and abroad (Raghuveer, 1997). The product is promoted as being very convenient for culinary purposes and the food industry. The CFTRI, Mysore, has developed processes for the manufacture of juice concentrate and powder of the pulp (Shankaracharya, 1998). All the water solubles were extracted from the fruit pulp by boiling with water then concentrated to about 65–70 % solids and packed in suitable containers. The final product was viscous and set to a jam-like consistency on cooling. Tamarind juice concentrate was found to be more viscous than sucrose solutions (Manohar *et al*., 1991). Formulae for preparing spiced sauces and beverages from the pulp have also been reported (Patil and Nadagouder, 1997). The approximate composition of the concentrate according to a CFTRI report is as follows: total tartaric acid 13 %; invert sugars 50 %; pectin 2 %; protein 3 %; cellulosic material 2 %; and moisture 30 %.

26.3.3 Seeds

Tamarind seed is the raw material used in the manufacture of (TKP), polysaccharide, adhesive, oil and tannin. Tamarind seed used to be an under-utilized by-product of the tamarind pulp industry. However, recent reports (Mishra and Malhothra, 2009) involving utilization of these in the textile, food and pharmaceutical industries show that its potential has been increasingly explored. Tamarind seeds are reported to give amber-coloured oil, free of smell and sweet to taste, which resembles linseed oil. It is reported to be useful in the preparation of paints and varnishes and for burning lamps (Lewis and Neelakantan, 1964; Rao, 1975; Anon., 1976; Salim *et al*.,

1998). The oil is said to be palatable and of culinary quality (Morton, 1987). Tamarind jellose – a pectin-like substance extracted from tamarind seeds – has not been fully exploited but, due to its abundance and cheapness, jellose has great potential for replacing fruit pectins in many industries (El-Siddig *et al*., 2006).

Purified tamarind seed polysaccharide, xyloglucan, has been found to have various applications in food technology, drug-delivery technology and the textile industry (Glicksman 1986; Gidley *et al*., 1991; Mishra and Malhothra, 2009; Gupta *et al*., 2010). Reid and Edwards (1995) pointed out that, even though the tamarind xyloglucan as a viscosifier offered no chemical advantage over guar gum, a galactomannan from cluster beans, a bioprocess to upgrade the tamarind polysaccharide, might be commercially viable as the tamarind flour is cheaper. Purified, refined tamarind xyloglucan is produced in Japan and is permitted as a thickening, stabilizing and gelling agent in the food, cosmetic and pharmaceutical industries (Glicksman, 1986; Gidley *et al*., 1991; Nitta and Nishinari, 2005). It is reported to possess properties like high viscosity, broad pH tolerance and adhesivity. Tamarind xyloglucan imparted more viscous, liquid-like rheological properties and heat stability to gelatinized tapioca starch/xyloglucan mixtures (Pongsawatmanit *et al*., 2006). A recent report by Gupta *et al*., (2010) shows that TSP is a promising polymer in the pharmaceutical industry as a novel carrier of drugs in various bioadhesive and other sustained release formulations. Research on xyloglucan has been extensively reviewed by Mishra and Malhothra (2009).

26.3.4 Kernel powder

TKP was extensively used in the food industry and as a sizing material in the textile industry as well (Rao and Subramanian, 1984; Bal and Mukherjee, 1994; Patil and Nadagouder, 1997). TKP used to be recommended in preparing confectionery, as a stabilizer in ice creams, mayonnaise and cheese (Morton 1987; Patil and Nadagouder, 1997). Use of white TKP in food products such as jellies, jams, marmalades, fortified breads and biscuits was also detailed by Bhattacharya (1997) and Bhattacharya *et al*. (1991, 1994b). Other than the food and textile industries it has been used in cosmetics, pharmaceutical and insecticidal preparations, adhesives, bookbinding, cardboard and plywood manufacture, and in sizing and weighing compositions in the leather industry (Daw *et al*., 1994; Patil and Nadagouder, 1997; Prabhanzan and Ali, 1995).

26.3.5 Seed testa

The testa is reported to contain 40 % water solubles, 80 % of which is a mixture of tannin and colouring matter (FRI, 1955). In the production of TKP or the jellose, large quantities of testa are left as a residual by-product. The use of testa in dyeing and tanning has been suggested (El-Siddig *et al*., 2006). The seed testa contains 23 % tannin which, when suitably blended, is used for tanning leather and imparting colour-fast shades to wool. In leather tanning tests, tamarind tannin gives a harsh and highly-coloured leather which could be used for heavy soles, suitcases, etc. Several authors (Rao and Srivastava, 1974; Glicksman, 1986; Tsuda *et al*., 1994, 1995;

Sankaracharya, 1998) have suggested that seed coat, a by-product of the tamarind gum industries, can be used as a safe and low-cost antioxidant for increasing the shelf-life of foods by preventing lipid peroxidation. Madhulatha and Pitchai (1997) carried out studies on the utilization of spent (detanned) tamarind seed testa as a substrate to grow the edible mushroom *Pleurotus florida*. They claimed that wattle-tamarind seed testa substrate was efficient for growing the mushroom and that the spent mixture was suitable as organic manure as well.

26.3.6 Food colourant

Tamarind brown, the natural food colour from tamarind, is widely used in Japan as a food colourant (Anon., 2000). Leucoanthocyanidin and anthocyanin are the main pigments of the tamarind colour (Shankaracharya, 1997, 1998). Kaur *et al.* (2006) reported that the red pigment (anthocyanin) from the half-matured red variety tamarind could be used to impart a natural and attractive red colour to curries, jam, jelly, etc. and that there is ample scope for red tamarind to be used as a source of natural red food colourant in the near future.

26.3.7 Other uses

Tamarind fruits and other extracts from the tree have a number of reported miscellaneous applications which are still in widespread use. Tamarind pulp mixed with sea salt has been reported to polish brass, copper and silver in Sri Lanka (Jayaweera, 1981), India (Benthall, 1933; Eggeling and Dale, 1951; Coates-Palgrave, 1988), West Africa (Morton, 1987), South Africa and Somalia (Mahony, 1990). In West Africa, an infusion of the whole pod is added to the dye when colouring goat hides. The fruit pulp is used as a fixative with turmeric (*Curcuma longa*) and annatto (*Bixa orellana*) in dyeing, and it also serves to coagulate rubber latex (El-Siddig *et al.*, 2006) and is used for ethanol production (Menon *et al.*, 2010).

The seed husk has also been found to be an effective fish poison (Roy *et al.*, 1987). Jena (1991) reported that powdered seed husks added to water, even at low dosages of 5–10 mg/L, killed several fish species, within 2 hours of its application. The treatment of salted dried fish by TKP was found to be the best in preserving the quality of salted fish (Shetty *et al.*, 1996).

26.3.8 Minor uses

The tender leaves, flowers and the young seedlings are eaten as a vegetable and used in curries, salads, stews and soups in many countries (Benthall, 1933; Coronel, 1991). The leaves are reported to be rich in minerals and vitamin constituents such as calcium, magnesium, phosphorus, iron, copper, chlorine and sulphur; thiamine, riboflavin, niacin and vitamin C (Anon., 1976; Karuppaiah *et al.*, 1997). The flowers are considered to be a good source of honey (Ramanujam and Kalpana, 1992) which is rich golden in colour, but has slight acidity peculiar to its flowers. The tree also yields valuable timber and the wood is used mostly for agricultural implements, tool-handles, wheels, mallets, rice pounders and oil-mills and for turnery (Chaturvedi, 1985; Coates-Palgrave, 1988). Saha *et al.* (2010) and Abhijit *et al.* (2010) suggested

that tamarind fruit shell may be utilized as a low-cost biosorbent for the removal of malachite green from aqueous solutions.

26.4 Functional properties

26.4.1 Medicinal uses of tamarind

Tamarind products are commonly used as health remedies throughout Asia, Africa and the Americas (El-Siddig *et al*., 2006; Anon., 2008). Tamarind products, leaves, fruits and seeds have been extensively used in Indian Ayurvedic medicine and traditional African medicine (Jayaweera, 1981; Parrotta, 1990). The medicinal value of tamarind is mentioned in ancient Sanskrit literature. Tamarind fruits were well known in Europe for their medicinal properties, having been introduced by Arab traders from India (Rao, 1975).

Havinga *et al*. (2009) extensively reviewed the ethnopharmacology of *T. indica* in the African context and suggested differences in the ways tamarind is used in local medicine in different parts of Africa. Anon. (2008) also detailed medicinal uses for tamarind in Africa which include as an anthelminthic (expels worms), antimicrobial, antiseptic, antiviral, sunscreen and astringent and to promote wound healing in the following conditions: asthma, bacterial skin infections, boils, chest pain, cholesterol metabolism disorders, colds, colic, conjunctivitis, constipation (chronic or acute), diabetes, diarrhoea, dry eyes, dysentery, eye inflammation, fever, gallbladder disorders, gastrointestinal disorders, gingivitis, haemorrhoids, indigestion, jaundice, keratitis, leprosy, liver disorders, nausea and vomiting (pregnancy-related), saliva production, skin disinfection/sterilization, sore throat, sores, sprains, swelling (joints) and urinary stones. It was suggested by Sadik (2010) that the consumption of adequate amounts of 'poha beer' a popular tamarind fruit drink of Northern Ghana in Africa, could help reduce the prevalence of iron deficiency anaemia. This was based on the vitamin C content in it which enhances bioavailability of non-haem iron.

Tamarind fruit is commonly used throughout Southeast Asia as a poultice applied to foreheads of fever sufferers (Doughari, 2006). In traditional Thai medicine, the fruit of the tamarind is used as a digestive aid, carminative, laxative, expectorant and blood tonic (Komutarin *et al*., 2004). The laxative properties of the pulp and the diuretic properties of the leaf sap have been confirmed by modern medical science (Bueso, 1980). Tamarind has been used in the treatment of a number of ailments, including alleviation of sunstroke, Datura poisoning and the intoxicating effects of alcohol and 'ganja' (*Cannabis sativa* L.) (Gunasena and Hughes, 2000). It is used as a gargle for sore throats, dressing of wounds (Benthall, 1933; Dalziel, 1937; Eggeling and Dale, 1951; Chaturvedi, 1985) and is said to aid the restoration of sensation in cases of paralysis. Tamarind is also said to aid in the cure of malarial fever (Timyan and Bwa 1996). In Southeast Asia, the pulp is prescribed to counteract the ill effects of overdoses of chaulmoogra (*Hydnocarpus anthelmintica* Pierre), a leprosy medication, and in Mauritius, the pulp is used as a liniment for rheumatism (Morton, 1987). Tamarind seeds have been used in Cambodia and India, in powdered form, to treat boils and dysentery (Rao, 1975; Jayaweera, 1981). Boiled, pounded seeds are reported to treat ulcers and bladder stones and powdered seed husks are used to treat diabetes (Rao, 1975).

Several medicinal properties are claimed for preparations containing tamarind pulp, leaves, flowers, bark and roots (Lewis *et al.*, 1970; Bueso, 1980; Jayaweera, 1981; Ghosh, 1987; Rajan *et al.*, 1989; Lakshmanan and Narayanan, 1990; Mustapha *et al.*, 1996; Doughari, 2006; El-Siddig *et al.*, 2006). These include use as anti-inflammatories in North Africa (Rimbau *et al.*, 1999), use as herbal medicines in Burkina Faso (Kristensen and Lykke, 2003), use against leucorrhoea and skin disorders and folk uses in India (Sen and Behera, 2000; Punjani and Kumar, 2002; Patil and Yadav, 2003; Rajendran *et al.*, 2003).

Improved bioavailability of anti-inflammatory drugs utilizing tamarind xyloglucan was reported by Takahashi *et al.* (2002). Fruit extracts have been shown to enhance the bioavailability of ibuprofen in humans as well (Garba *et al.*, 2003). In rats, tamarind xyloglucan has been found to show a strong antidiabetic effect (Maitin *et al.*, 2004). There is current medical interest in the use of purified xyloglucan from tamarind in eye surgery for conjunctival cell adhesion, corneal wound healing as well (Burgalassi *et al.*, 2000). The tamarind seed polysaccharide appears to be a promising candidate as a vehicle for the topical treatment of bacterial keratitis, a serious infectious ocular disease (Ghelardi *et al.*, 2004). Other medical-related trials have shown that tamarind intake delayed the progression of fluorosis by enhancing excretion of fluoride (Khandare *et al.*, 2004, 2010).

Apart from fruits, tamarind leaves are used to treat conjunctivitis, throat infections, coughs, fever, intestinal worms, urinary troubles and liver ailments, cardiac and blood sugar reducing medicines, in ulcers, and as external applications in boils, rheumatism and external swellings (Rao, 1975; Jayaweera, 1981; El-Siddig *et al.*, 2006). Jayaweera (1981) and Rao (1975) have reported its use in treatment for digestive tract ailments and indigestion in Cambodia, India and the Philippines. The bark as an astringent was being used as a tonic and in lotions or poultices to relieve sores, ulcers, boils and rashes in the Philippines and Eastern Sudan (Dalziel, 1937). Ashes of the burnt shells of ripe fruits are used as an alkaline substance with other alkaline ashes in the preparation of medicine 'Abayalavana' in India, for curing enlarged spleen (Sengupta, 1994). Flowers are used in the treatment of eye diseases in the Philippines and also for jaundice and bleeding piles (Brown, 1954; de Padua *et al.*, 1978). The 'Irula' tribals in Tamil Nadu, India, use tamarind root bark for abortion and for prevention of pregnancies (Lakshmanan and Narayanan, 1990). In some countries, the bark is reported to be prescribed in asthma, amenorrhoea and as a tonic and febrifuge (Anon., 1976). Medicinal and pharmacological uses have been reviewed by Krishnamurthy *et al.* (2008). A recent study by Ranjan *et al.* (2009) revealed a decrease in plasma and bone F levels on ingestion of 100 mg tamarind water extract in rabbits.

26.4.2 Antioxidant activity

Several reports of antioxidant activity in tamarind indicate that fruits contain biologically important mineral elements and have high antioxidant capacity associated with high phenolic content that can be considered beneficial to human health (Gayathri *et al.*, 2004). Tsuda *et al.* (1994) reported that tamarind seed coat contains phenolic antioxidants, such as 2-hydroxy-30, 40-dihydroxyacetophenone, methyl

3,4-dihydroxybenzoate, 3,4-dihydroxyphenyl acetate and epicatechin. Investigations on tamarind seed by Osawa *et al*. (1994) revealed that the seed coat has antioxidative activity as measured by the thiocyanate and thiobarbituric (TBA) method. Ethyl acetate extracts prepared from the seed coat also had strong antioxidant activity. Extraction of antioxidant compounds from the seed coat of sweet Thai tamarind was reported by Luengthanaphol *et al*. (2004) and Lourith *et al*. (2009) as well. Ramos *et al*. (2003) suggested that tamarind seed coat, a by-product of the tamarind gum industry, could be used as a safe and low-cost source of antioxidants, although other herbals could be more effective. Soong and Barlow (2004) reported that seeds of tamarind have higher antioxidant activity than that of the pulp. Martinello *et al*. (2006) observed that the fruit pulp extract of *T. indica*, when administered at a concentration of 5 % to hypercholesterolaemic, hamsters led to a decrease in total serum cholesterol and an increase in HDL, indicating its potential in diminishing the risk of atherosclerosis in humans. Siddaraju (2006) observed radical scavenging activity and good antioxidant activity of tamarind seed coat extracts against linoleic acid emulsion systems. Sudjaroen *et al*. (2005) conducted quantitative analysis of polyphenolic compounds in tamarind seeds and pericarp by analytical high-performance liquid chromatography. The yields of total phenolic compounds were 6.54 and 2.82 g/kg (dry weight) in the seeds and pericarp, respectively.

26.4.3 Antimicrobial properties

The fruits of tamarind are reported to have antifungal and antibacterial properties (Ray and Majumdar, 1976; Guerin and Reveillere, 1984; Bibitha *et al*., 2002; Metwali, 2003; John *et al*., 2004; Doughari, 2006). It is reported to be a potent fungicidal agent to cultures of *Aspergillus niger* and *Candida albicans*. Investigations by Daniyan and Muhammad (2008) revealed antimicrobial properties of tamarind against *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella paratyphi*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* which are aetiological agents in urinary tract infections (UTI), wounds, pneumonia and paratyphoid fever. Extracts from tamarind fruit pulp have also shown molluscicidal activity against *Bulius trancatus* snails. This activity is believed to be due to the presence of saponins in the fruit (Imbabi and Abu-Al-Futuh, 1992). Tamarind plant extracts have been used to purify drinking water in Burkina Faso and Vietnam (Bleach *et al*., 1991).

Several examples have been cited where tamarind extracts were used to control pests and diseases in cultivated plants. Singh *et al*. (1989) reported that extracts obtained from tamarind plant parts have completely inhibited the activity of both cowpea mosaic and the mung bean mosaic viruses in India. The unfolded leaves of tamarind, containing lupeol, are said to be effective in inhibiting viral and fungal diseases in plants. Triterpenoids, phenols and alkaloids in tamarind extracts are being looked at for their use in controlling pests and diseases, e.g. control of citrus canker in Thailand (Leksomboon *et al*., 2001), of root knot nematode (Ranjana and Rajendra, 2001) and of a range of fungi (Neetu and Bohra, 2003; John *et al*., 2004). Tamarind pulp extracts screened for their antimicrobial activities exhibited higher activity against *S. typhimurium* and *S. aureus* and lower activity against *A. niger* (Jadhav *et al*., 2010).

26.5 Quality issues

There are many problems associated with the quality of tamarind products due to their high moisture level and seed, fibre and rind contents. Tamarind is reported to have been adulterated with foreign matter which is both organic and inorganic in nature. Adulteration is considered to be due to poor post-harvest management practices including processing (Rao and George, 1996; George and Rao, 1997). General quality requirements of tamarind have been detailed by Pruthi (1999). The Directorate of Marketing and Inspection and Bureau of Indian Standards have prescribed quality specifications for seedless tamarind (Table 26.1), dry tamarind (Table 26.2) and tamarind seed (Table 26.3) (Anon., 1996). Indian standard specifications are available for tamarind juice concentrate (IS 5955), pulp (IS 6364), kernel oil (IS 9587: 1980) for kernel powder (IS 189: 1977, IS 511: 1962) and for seed testa (IS 9004: 1978).

Table 26.1 Agmark specifications (%/wt max) – tamarind seedless

Character/grade	Special	A	B	C
Moisture	15	17	20	20
Seed content	5	10	15	20
Foreign matter (organic)	4	6	8	10
Foreign matter (inorganic)	1	1.5	2	2

Source: Anon. (1996).

Table 26.3 Agmark specifications (%/wt max.) – tamarind seed

Character/grade	Special	A
Extraneous matter	1	2
Damaged and discoloured	2	5
Wt/lit	900	800
Moisture	9	10

Source: Anon. (1996).

Table 26.2 Agmark specifications (%/wt max) – tamarind dry

Character/grade	Special	A	B
Seed content	35	40	45
Fibres	6	8	10
Rind	3	4	6
Insect damage	2	3	5
Moisture	15	20	25

Source: Anon. (1996).

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27

Other herbs and spices: achiote to Szechuan pepper

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Abstract: The International Standards Organisation (ISO) lists 109 plant species that are used as spices and culinary herbs. Among them, a few are widely used, grown commercially and traded internationally. A few others are less widely used, nevertheless they are known and used widely, while the others are less well known, cultivated only in restricted regions and are classified as under-utilized. These under-utilized herbs and spices are nevertheless valuable in culinary art to impart unique taste and flavour and also as medicinal herbs. This, the first of the two chapters dealing with such herbs and spices, looks at 11 examples, namely achiote (annatto), chamomile, galanga, horseradish, hyssop, juniper berry, kokum, large (or black) cardamom, lemon balm, long pepper and Sichuan pepper are dealt with briefly here. In the case of each, brief description, chemical composition, medicinal uses and uses in food are provided.

Key words: under-utilized spices, achiote (annatto), chamomile, galanga, horseradish, hyssop, juniper berry, kokum, large (or black) cardamom, lemon balm, long pepper, Szechuan pepper.

27.1 Introduction

The International Organization for Standardization (ISO) lists 109 plant species that are used as herbs and spices, while Katzer's spices compendium lists 117 species (Katzer, 2012). Of these, only a few are very widely used, grown commercially and traded internationally. Some others are less widely used but are well known and grown in restricted regions, and are traded at moderate levels on the global market; still others are less well known, or even unknown, outside their production centres and are under-utilized. Such under-utilized herbs and spices are nevertheless valuable, not only as spices for flavouring dishes, but also as medicinal plants of great importance in their respective centres of cultivation. These less well known herbs and spices are also becoming important in the western world as chefs constantly struggle to create exotic dishes with unique and hitherto unknown tastes and flavours. This chapter is the first of two chapters that deal briefly with the more important under-utilized herbs and spices. In this chapter, 11 herbs and spices are discussed,

namely: achiote (annatto), chamomile, galanga, horseradish, hyssop, juniper berry, kokum, large (or black) cardamom, lemon balm, long pepper and Szechuan pepper.

27.2 Achiote (annatto)

Achiote, more commonly known as annatto, comes from a small tree of Central and South American origin, with the botanical name *Bixa orellana* L. (family Bixaceae). The seeds of this tree have a covering of yellowish orange to reddish orange powder (pericarp, also called pulp) that is the source of a widely used food colouring. The seed is also used as a flavouring agent in South and Central American and Caribbean dishes, imparting a mild peppery flavour with a slight nutmeg taste. In South and Central America, the seeds are also used by natives to make body paint and lipstick; hence the achiote tree is also called the lipstick tree. A detailed review of annatto, including its socio-religious connections, has been published by Donkin (1974).

Achiote grows to a height of about 5–6 m and produces pinkish white flowers that develop into clusters of bright red, heart-shaped fruits (capsules) covered with bristles. The fruit is inedible; when ripe the fruit capsules split open exposing an abundance of seeds which are embedded in an orange–red pulp. The following text is reproduced from Iqbal (1994):

> [Annatto is grown in] most tropical countries such as: Brazil, Peru, Mexico, Ecuador, Dominion Republic, Jamaica, India, Sri Lanka, Kenya, and to a lesser extent in the Philippines, Turkey and Angola.… The pigment concentration varies between 5 % in hemispheric fruits, 3 %–3.58 % in conical types and 1.5 %–2 % in oval types. The plant starts bearing at the age of 3, and production continues for a period of 10 to 12 years. Yields vary from region to region from 300 kg to 600 kg per ha, and exceptionally 750 to 900 kg per ha.
>
> Average annual production of annatto seeds is in the range of 10,000 to 11,000 tons, of which 60.2 % comes from Latin America, 27.4 % from Africa, and 12.4 % from Asia. Peru is the largest producer, accounting for 32 % of the world total, followed by Kenya and Brazil.
>
> …Although annatto is produced in developing countries, less than a third (about 27 %) is locally processed. Major processing plants are in Peru, Kenya, India and Brazil. The Peruvian production of annatto seeds is exported worldwide; Kenyan production is almost wholly exported to Japan, while the largest part of Brazilian production is consumed locally.

About 7000–8000 tonnes of annatto enters the international market each year. Jansen (2005) gives the breakdown of the annatto market:

> The main market for annatto is the United States with 3000 t/year, followed by Western Europe (2500 t) and Japan (1500 t). Some 70 % of the product is used in the importing countries to colour cheese. Trade in annatto extracts (instead of dried seeds) has increased since the 1980s, with the water soluble norbixin extract being largest in volume, followed by vegetable oil extracts, and solvent-extracted bixin in third place.

27.2.1 Tribal and non-medicinal uses

In tribal cultures, annatto seeds have a range of non-medicinal uses. For the ancient Mayans and Aztecs, annatto was regarded as a symbolic substitute for blood and

therefore had sacred connotations – many of the ancient Mayan scriptures were written in annatto juice. Indigenous people still use the pulp mixed with oil for cosmetic purposes: as a hair dye, as lipstick, or to decorate the body. When used as body paint, it also repels insects and protects against sunburn due to the UV filtering properties of the carotenoid pigment bixin. Annatto seeds are also used throughout the rainforest to create fabric dyes. In the western world, annatto is used in the cosmetic industry (for nail gloss, lipstick, soap); annatto oil is an emollient, and its high carotenoid content provides beneficial antioxidant properties. In body care products, annatto oil provides antioxidant benefits while adding a rich, sunny colour to creams, lotions and shampoos. Annatto is also used in household products such as floor wax, furniture polish and shoe polish. In the past, it has been widely used as a fabric dye, giving an orange–red colour; however, due to the lack of colour fastness, it has now been largely replaced by synthetic dyes. The dye has also been used to colour wood, rattan and wickerwork.

27.2.2 Traditional medicinal uses

While it is mainly the seeds that are used by indigenous people for non-medicinal purposes, the whole plant is used in traditional medicine. Khare (2007) describes the various medicinal properties that have been ascribed to the different parts of the annatto plant. The whole plant has astringent, antibilious, antiemetic and blood-purifying properties. The young shoots are used as a tea-like drink which is drunk as an aphrodisiac and astringent, and also in cases of fever, dysentery and hepatitis. The leaves have a marked effect on the urinary system and increase the volume of urine in cases of renal insufficiency or cystitis; the leaves are used in an infusion in the treatment of diabetes, jaundice and dysentery, and are also used externally for skin problems and to prevent scarring. The root and bark are used to reduce fever; the seed pulp is believed to have haemostatic, antidysenteric, diuretic and laxative effects; the fruit is used in cases of dysentery. Traditional healers in Colombia also use annatto as antivenom in cases of snakebite. Annatto is commonly used in Peruvian herbal medicine today, where the dried leaves are called *achiotec* and are drunk as a decoction with reported beneficial effects on many different disorders (e.g. prostate problems, arterial hypertension, obesity). The decoction is also used externally as an antiseptic and a wound healer.

The uses of annatto vary around the world. In Gabon, a leaf decoction is used to stop vomiting while in the Seychelles and Mauritius annatto is added to baths to reduce muscular pain. In Ethiopia, the leaves are applied as a wound dressing.

27.2.3 Modern scientific research into medicinal properties

Much research has been undertaken to improve our understanding of the beneficial effects of annatto and to identify the constituents of annatto that are responsible for these effects. Dunham *et al*. (1960) found that a water extract of the root had hypotensive activity in rats (this hypotensive effect has long been recognized in Peruvian herbal systems). The extract also had muscle-relaxing properties and reduced gastric secretions.

Terashima *et al*. (1991) investigated the possible benefits of annatto for diabetes patients; annatto leaves were shown to inhibit the action of aldose reductase, which is thought to play a role in diabetic neuropathy. Otero *et al*. (2000) confirmed, in mice, the effectiveness of a leaf/bark preparation used in Columbia to counteract snake bites. The antibacterial properties of annatto have been demonstrated *in vitro* by Cáceres *et al*. (1995), where several bacterial populations, including *Escherichia coli* and *Staphylococcus*, were adversely affected by annatto flower and leaf extracts; the results support its use as a treatment of gonorrhea and other infections in traditional medicine systems.

The constituents of annatto that have been shown to have beneficial properties include the antibacterial compound – maslinic acid – which has been isolated from the leaves. Annatto seed extract was shown to reduce blood glucose levels in cases of streptozotocin-induced diabetic dogs. Studies have shown that the lowering of blood glucose level is due to the stimulation of peripheral utilization of glucose (Russell *et al*., 2008).

27.2.4 Culinary uses

As a food colour additive, annatto has the E-number E160 b. The fat-soluble portion of crude extract is composed of bixin and the water-soluble part is mainly norbixin, both sharing the same E number as annatto. Annatto seed contains 4.5–5 % pigment, about 70–80 % of which is bixin and the remainder norbixin (Rajendran, 1991; Gabriel and Jack, 2000). Because the extract is soluble in lipids, it is widely used in the food industry to impart red to orange–yellow colours to a variety of foods, e.g. cheese, butter, oils, margarine, ice cream, candy, bakery products and rice. It has been particularly useful in the dairy sector where the available synthetic alternatives have proven to be unstable; annatto has been shown to be non-toxic and to have the additional benefit of high vitamin A content (Jansen, 2005).

Katzer (2012) provides the many culinary uses of annatto around the world:

> In the Caribbean, the seeds are usually fried in (animal or vegetable) fat; after discarding the seeds, the then golden-yellow fat is used to fry vegetables or meat. By this procedure, a golden yellow to golden brown colour is achieved. Mexican cooks often use a paste (*achiote*) of annatto seeds with some preservatives (acetic acid) that dissolves completely in hot fat; it is easy to use and can also be added to marinades and sauces to improve the colour. Similar use is found in Perú and Bolivia in South America.
>
> In South México (Yucatán), meat is often marinated with a spice mixture called *recado* that derives its vibrantly yellow colour from liberal addition of annatto. The annatto seeds may be used ground (often after soaking in hot water to soften them) or in form of annatto oil. *Recado* is made from annatto, dried oregano, ground spices (black pepper, allspice and cumin), garlic and fiery Yucatean chilies. They key flavour is the juice of bitter oranges (sour oranges or Seville oranges) which adds a distinct, acidic fruitiness. *Recado*-marinated meats are wrapped in banana leaves and baked in a hot stone pit. Baking in a hot oven, pan-frying or grilling is also possible. The technique can be applied to poultry and fish, but is most popular for pork, especially suckling pig. Food prepared this way is generally referred to as *pibil*.
>
> By Spanish influence, annatto also has made its way to South East Asia. On the Philippines, the seeds are often ground to a powder and added to soups and stews;

meat is often marinated with annatto-coloured seasonings. The colour obtained here by is brownish-yellow, less vibrant than the colour resulting from usage of annatto oil in the Caribbean. In Vietnam, batters are often prepared with annatto oil to achieve a more attractive colour; annatto oil is also common for improving the colour of coconut-based curries. Lastly, there are Vietnamese variations of Beijing duck that use annatto to colour the bird's skin. In China, annatto seeds are occasionally contained in seasonings or marinades for grilled or fried meats (predominantly pork), resulting in a bright orange meat surface.

Apart from the direct uses, annatto products are also used indirectly in food preparation such as:

- ***Achiote*-coloured oil**: There are bland oils flavoured and coloured with *achiote* seeds. In Mexican and Indian cuisine the oil is used to add a colour and flavour to many foods.
- ***Achiote* paste**: This is a paste made from ground *achiote* seeds, water or vinegar, and then mixed with other herbs and spices (e.g. cinnamon, bay leaf, cilantro, salt, cloves and oregano).
- ***Achiotina***: This is a lard compound that has been flavoured and coloured with *achiote* seeds. It is mainly used in Puerto Rican cooking for the preparation of bean and rice dishes, as well as vegetables, meats, and stews.

27.3 Chamomile

Chamomile (or camomile) is the common name used for several aromatic plants from the aster family (Asteraceae). There are two major types, the German chamomile and the Roman chamomile. German, or blue chamomile is *Matricaria recutita* (syn. *M. chamomilla*), commonly used in tea. Roman chamomile, or 'lawn' chamomile, is *Anthemis nobilis* (syn. *Chamaemelum nobile*). Other species that are also used as chamomile include:

- *Anthemis arvensis*, corn or scentless chamomile;
- *Anthemis cotula*, stinking chamomile or dog fennel;
- *Anthemis tinctoria*, yellow chamomile or golden marguerite;
- *Ormenis multicaulis*, Moroccan chamomile;
- *Eriocephalus punctulatus*, Cape chamomile;
- *Matricaria discoidea*, wild chamomile or pineapple weed.

Roman chamomile is a small perennial herb with a hairy stem, feathery pinnate leaves, and daisy-like white flowers (larger than those of German chamomile); it grows to about 25 cm high. German chamomile, on the other hand, grows to about 60 cm high and has a hairless branching stem bearing delicate feathery leaves and simple daisy-like white flowers on single stems; it is German chamomile that is the more commonly used plant.

These plants are best known for their ability to be made into a tea that is used to help with sleep and is often served with either honey or lemon. Chrysin, a specific flavonoid found in chamomile, has been shown to have anti-anxiety effects in rodents and is believed to be at least partially responsible for chamomile's reputation as a sleep aid. For a comprehensive treatment of chamomile, readers may refer

to Franke and Schilcher (2005). A detailed review can also be found in Ross (2001), in which both traditional and modern uses in various countries are summarized.

27.3.1 German chamomile

Parts of this section are reproduced with permission from an article that first appeared in *Alternative Medicine Review* in 2008 (Thorne Research Inc., 2008).

> Chamomile is a widely recognized herb in Western culture… A common ingredient in herbal teas because of its calming, carminative, and spasmolytic properties, it is also a popular ingredient in topical health and beauty products for its soothing and anti-inflammatory effects on skin. Chamomile has a sweet, grassy, and lightly fruity aroma.
>
> …German chamomile flowers contain 0.24- to 2.0-percent volatile oil that is blue in color.

Over 120 constituents have been identified in chamomile flowers (Pino *et al.*, 2000). The complete chemical constitution is listed in Ross (2001); the most important compounds are discussed below (from Thorne Research Inc., 2008):

> The two key constituents, (–)-alpha-bisabolol and chamazulene, account for 50–65 percent of total volatile oil content. Other components of the oil include (–)-alpha-bisabolol oxide A and B, (–)-alpha-bisabolone oxide A, spiroethers… sesquiterpenes, cadinene, farnesene, furfural, spathulenol, and proazulene (matricarin and matricin). Chamazulene is formed from matricin during steam distillation of the oil. Yield varies depending on the origin and age of the flowers. According to European Pharmacopoeia (1996) chamomile contain no less than 4 ml essential oil /kg.
>
> Chamomile also contains up to 8 % flavone glycosides (apigenin 7-glycoside and its 6′-acetylated derivative) and flavonols (luteolin glucosides, quercetin glycosides and isohamnetin); up to 10 % mucilage polysaccharides; up to 0.3 % choline; and approximately 0.1 % coumarins (umbelliferone and its methyl ether, herniarin). The tannin level in chamomile is less than one percent.

27.3.2 Medicinal uses of German chamomile

Chamomile is used in the traditional medicine in Europe and America, usually as a hot infusion. Khare (2007) reports that chamomile has sedative, anticonvulsing, carminative, antispasmodic, analgesic, anti-inflammatory and antiseptic properties. Key application areas are in the treatment of inflammatory diseases of the gastrointestinal tract and gastrointestinal spasm; it is used externally for treatment of skin, mucous membrane and ano-genital inflammations and for bacterial skin diseases.

Thorne Research Inc. (2008) describes the medicinal properties of chamomile as follows:

> Several pharmacological actions have been documented for German chamomile based primarily on *in vitro* and animal studies. Such actions include antibacterial, antifungal, anti-inflammatory, antispasmodic, anti-ulcer, antiviral, and sedative effects.
>
> The constituents of chamomile thought to have antimicrobial properties include alpha-bisabolol, luteolin, quercetin, and apigenin. Herniarin may also have antibacterial and antifungal properties in the presence of ultraviolet light.…Chamomile oil, at a concentration of 25 mg/ml, demonstrates antibacterial activity against … *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus mutans*, and *Streptococcus salivarius*, as

well as some fungicidal activity against *Candida albicans*. Whole plant chamomile extract at 10 mg/ml demonstrates a similar effect.…Chamomile extract has also been shown to inhibit the growth of poliovirus and herpes virus.…Chamazulene, alpha-bisabolol, flavonoids, and umbelliferone display antifungal properties against *Trichophyton mentagrophytes* and *Trichophyton rubrum.*

The high alpha-bisabolol content in chamomile oil is credited for providing the majority of antibacterial, antifungal, anti-inflammatory, and anti-ulcer activity, although the precise mechanism of action remains unclear.

In vitro, chamomile extract inhibits both cyclooxygenase and lipoxygenase, and consequently prostaglandins and leukotrienes. Other anti-inflammatory effects are thought to occur via the influence of azulenes (chamazulene, prochamazulene, and guaiazulene) on the pituitary and adrenals, increasing cortisone release and reducing histamine release.

Chamomile extracts exhibit antispasmodic properties. Apigenin, alpha-bisabolol, and the cisspiroethers appear to provide the most significant antispasmodic effects.

…*In vitro studies* demonstrate α-bisabolol inhibits gastric ulcer formation induced by indomethacin, ethanol, or stress. Oral administration of chamomile oil to rats at doses ranging from 0.8–80 mg/kg bisabolol demonstrate significant protective effect against gastric toxicity of 200 mg/kg acetylsalicylic acid.

…Apigenin functions as a ligand for benzodiazepine receptors, resulting in anxiolytic and mild sedative effects, but no muscle relaxant or anticonvulsant effects.

Research is exploring the anti-proliferative and apoptotic effects of chamomile extract in various human cancer cell lines. One preliminary study observed that *in vitro* exposure to chamomile results in differential apoptosis in cancer cells but not in normal cells at similar doses; apigenin and apigenin glycosides appear to be the key components responsible for these effects.

Oil of German chamomile, extracted through steam distillation, is an excellent skin tonic and is used to treat skin allergies, eczema, psoriasis and other flaky skin conditions; the high α-bisabolol content promotes granulation (healing) and tissue regeneration.

Further information on the pharmacological properties and potential of chamomile can be found in a recent review by Gupta *et al*. (2010).

27.3.3 Roman chamomile

There are two distinct varieties of Roman chamomile, the single and the double, indicating the number of layers of ray florets. The single-flowered is more powerful having higher content of alkaloids, while the double-layered variety is milder and is preferred for the preparation of herbal teas and other herbal medicines and also in cooking.

Roman chamomile contains mainly the terpenoids chamazulene and bisabolol; other constituents are flavonoids (such as apigenin, luteolin and quercetin), coumarins (scopoletin-7-glucoside) and acids and esters (such as angelic and tiglic acid esters, anthemic acid, choline, phenolic and fatty acids) (Gardner, 1999).

27.3.4 Medicinal uses of Roman chamomile

Roman chamomile has tonic, anodyne and antispasmodic properties. The infusion of flowers in boiling water, known popularly as chamomile tea, has long been known for its soothing and sedative effects. When combined with ginger, the infusion is an

excellent stomachic in cases of indigestion (e.g. flatulent colic, heartburn, loss of appetite) and also for the treatment of gout and headache. The flowers of Roman chamomile are known for their diuretic properties (Anon., 2009).

Chamomile flowers are also extensively used by themselves, or combined with other ingredients, as a poultice and fomentation for the treatment of external swelling, inflammatory pain or congested neuralgia. The whole herb is also used to make a lotion for external application in cases of toothache, earache, neuralgia, etc.

Roman chamomile oil, extracted through steam distillation of flower heads, has a sweet, apple-like fragrance and is very light clear blue in colour. The oil is very effective when administered to children who are teething or are suffering from colic. It is also used to relieve premenstrual symptoms in women, and also for general abdominal pain and throat infections. It can be used to relieve allergies, hay fever and asthma. Like German chamomile, it is effective when used to treat skin conditions such as acne, eczema, rashes, dermatitis and allergic reactions.

27.3.5 Culinary uses for German and Roman chamomile

Both chamomiles are most famous as a herbal tea, either used alone or in combination with true tea. Chamomile is an aromatic herb and is used widely in European cooking to impart additional flavour.

27.4 Galanga

Kaempferia galanga L., commonly known as sand ginger, *kencur* or resurrection lily, is an aromatic, monocotyledonous herbaceous perennial plant from the ginger family (Zingiberaceae). (Galanga should not be confused with galangal or the greater galangal, which is *Alpinia galanga*, and the lesser galangal, *Alpinia officinarum*.) It is found primarily in open areas in southern China, Taiwan, Cambodia and India, but is also widely cultivated throughout South East Asia. Further information on galangal can be found in Ravindran and Pillai (2006).

The plant grows to a maximum height of 30 cm, and has fleshy, cylindrical aromatic root tubers. The rhizome has a camphoraceous odour and a bitter aromatic taste. The *K. galanga* rhizome contains about 2.5–4 % essential oil, the main components of which are ethyl cinnamate (25 %), ethyl-*p*-methoxycinnamate (30 %) and *p*-methoxycinnamic acid and a monoterpene ketone compound, 3-carene-5-one. Other constituents of the oil are camphene, δ-3-carene, *p*-methoxy styrene, γ-pinene, β-myrcene, p-cymene, 1,8-cineole, iso-myrcene, camphor, α-terpineol, *p*-cymene-8-ol, eucarvone, δ-cadinene, etc. The leaves contain kaempferol, quercetin, cyanidin and delphinidin.

K. galanga is cultivated for its aromatic rhizomes and also as an ornamental. It is used extensively as a spice throughout tropical Asia and has a long history of medicinal use, as described below.

27.4.1 Medicinal uses

The galanga rhizomes may be chewed and ingested, and are considered to have many beneficial properties, i.e. stimulatory, expectorant, carminative and diuretic

(Khare, 2007). The rhizomes are used in the preparation of gargles and administered with honey for treatment of coughs and respiratory ailments. In the Philippines, a decoction of the rhizomes is used for dyspepsia, headache and malaria. In Thailand, the dried rhizome of this plant is used as a cardiotonic (CSIR, 1959). In India, the dried rhizomes along with some other plants are used for heart diseases. It is also used for treatment of abdominal pain, vomiting, diarrhoea and toothache with the functions of promoting vital energy circulation and alleviating pain. The rhizome mixed with oil is used externally for healing wounds and applied to warm rheumatic regions. A lotion prepared from the rhizome is used to remove dandruff or scales from the head (Duke, 2003). The leaves are used in lotions and poultices for sore eyes, rheumatism and fever. The juice of the plant is an ingredient in the preparation of some tonic preparations.

Laboratory studies have shown that the oil extracted from the rhizome has several beneficial effects. The essential oil from the root induced glutathione-*s*-transferase activities in the stomach, liver and small intestine of mouse. Ethanol extract of the dried rhizome showed antispasmodic activity following histamine-induced contraction and barium-induced contraction in guinea pigs. An ethanol–water extract indicated smooth muscle stimulant activity. Water extracts of dried rhizomes have also been shown to exhibit anti-tumour activity. Rhizome and root oils showed antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, and antifungal activity against *Cladosporium* sp. (Ravindran and Pillai, 2006). A hypolepidaemic action of the ethanolic extract of *K. galanga* was observed *in vivo*. Oral administration of the extract was effective in lowering the total cholesterol, triglycerides and phospholipid levels in serum and tissues (Achuthan and Padikkala, 1997).

27.4.2 Culinary and other uses

Galanga has a peppery camphorous taste and is used as a food flavouring in South East Asia, particularly for rice. It is also used as a masticatory along with betel leaf and arecanut. Slices of the dried rhizome may be cooked with vegetable or meat dishes, but mostly the spice is used fresh and grated or crushed. It is essential for Javanese cooking and is especially used on the Indonesian island of Bali, where it is known as '*kencur*'. It is also used in Chinese cooking and Chinese medicine and is sold in Chinese grocery stores under the name *Sha Jiang* while the plant itself is referred to as *shan nai*. The rhizomes are used for protecting clothes against insects.

27.5 Horseradish

Horseradish (*Armoracia lapathifolia* Gilib., syn. *A. rusticana* P. Gaertn., B. Mey. and Scherb) belongs to the mustard family Brassicaceae (Cruciferae) and contains the distinctive mustard oils that are common to this family. The plant is also known by various common names such as red cole, creole mustard, German mustard and red horseradish. There are two types of horseradish: the 'common' type, with broad crinkled leaves and roots of high quality; and the 'Bohemian' type which has narrow, smooth leaves and poor-quality roots, but is more disease resistant (NIIR, 2001). Horseradish is native to Europe and Asia (southern Russia, eastern Ukraine), but

has become naturalized in North America and New Zealand, where it can be found growing along roadsides. Cultivation dates back only to about Roman and Greek times, around 2000 years ago (Simon *et al.*, 1984; Phillips and Rix, 1993; Brown, 2002). The crop was introduced into Western Europe in the thirteenth century. The principal production areas are in the USA and, to a lesser extent, in Europe. The crop is cultivated over 600 ha (1500 acres) in the USA. Horseradish is a hardy perennial root crop, grown for its very pungent roots, which grow up to a metre in length and which contain an oil with a strong pungent odour and hot, biting taste. Although the undisturbed root has little odour, pungency develops upon crushing or grinding the tissue. The roots are usually processed under refrigeration immediately after dicing, because of the high volatility of the oil. Distillation of the dried and powdered root gives about 0.05–0.2 % volatile oil. The intense pungency and aroma of horseradish are the result of isothiocyanates released from the glucosinolate sinigrin and 2-phenylethylglucosinolate by the naturally-occurring enzyme myrosinase, in the presence of water. The active constituents are sinigrin (a glycoside, which combined with water yields mustard oils), asparagine and resin (Karnick, 1994a,b). The root is a rich source of vitamin C; the fresh root contains an average of 302 mg per 100 g. Harborne and Baxter (1993) reported the presence of glucoberteroin, glucobrassicanapin, glucocapparin, glucocheirolin, glucochlearin, glucoiberin, glucoiberverin, glucolepidin, gluconapin, glucotropaeolin and sinigrin in horseradish.

27.5.1 Medicinal uses

The following medicinal properties have been reported for horseradish: it is a stimulant for the liver, spleen, pancreas and the digestive and circulatory systems; it acts as a diaphoretic, diuretic, rubefacient, antiseptic and antibiotic; it lowers fever by increasing perspiration; it is used for both urinary and respiratory tract infections; it is used for the treatment of gout; it can increase the excretion of uric acid; and an infusion is used in hepatitis (Karnick, 1994b; Blumenthal *et al.*, 1998; Khare, 2007). Horseradish material has also reportedly been used as a remedy for asthma, blocked sinuses, coughs, colic, scurvy, toothache, ulcers, venereal diseases and cancer. The herb has been shown to control bacterial infection, and this effect is attributed to the presence of allylisothiocyanate. The root is also applied externally as a poultice for infected wounds, inflammation of the pleura, arthritis and inflammation of the pericardium (Phillips and Rix, 1993; Brown, 2002). The peroxidase enzyme extracted from the plant root is widely used as an oxidizer in chemical tests, such as blood glucose determinations. The root also contains useful minerals, including calcium, sodium, magnesium and vitamin C.

Despite the above beneficial effects, it should be noted that horseradish has strong irritant activity and ingestion of large amounts can cause bloody vomiting and diarrhoea.

27.5.2 Culinary uses

Horseradish is generally recognized as safe for human consumption as a natural seasoning and flavouring. It is a pungent herb, with leaves that are used in salads and sandwiches, and roots that are used for sauces or relishes – grated horseradish

is commonly mixed with vinegar and cream and used with roast beef, chicken or hard-boiled eggs. In Eastern Europe, it is used as a condiment in combination with beets. The plant material is also employed as an ingredient in some ketchups and mustards.

27.6 Hyssop

Hyssop (*Hyssopus officinalis* L.) is the flowering top of the evergreen perennial shrub that belongs to the peppermint family, Lamiaceae. Hyssop is native to southern Europe and the temperate zones of Asia. It grows wild in countries bordering the Mediterranean Sea. It is cultivated in Europe, especially in southern France, mainly for its essential oil. In India it is found in the Himalayas from Kashmir to Kumaon at altitudes of 2435–3335 m. The plant grows to a height of 60 cm and flowers in autumn. Whorls of bluish-purple flowers are produced on long narrow spikes (Ravindran *et al.*, 2007).

The herb contains volatile oil, fat, sugar, choline, tannins, carotene and xanthophyll. The flower tops contain ursolic acid (0.49 %) and a glucoside diosmin which, on hydrolysis, yields rhamnose and glucose. The fresh herb contains iodine in a concentration of 14 mcg/ kg. Following steam distillation, the volatile oil yield from the fresh aerial part of the plant is 0.15–0.30 % (0.3–0.8 % from dried material). Hyssop oil is colourless or greenish yellow with an aromatic, camphoraceous odour and a slightly bitter taste. The major component of the volatile oil is ketone 1-pinocamphone. The content of essential oil is rather low (0.3–0.9 %); it is mostly composed of cineol, α-pinene and a variety of bicyclic monoterpene derivatives (*L*-pinocamphene, isopinocamphone, pinocarvone). Hyssop contains large amounts of bitter and antioxidative tannins: phenols with a diterpenoid skeleton (carnosol, carnosolic acid), depsides of caffeic acid and several triterpenoid acids (mainly ursolic and oleanolic acid) (Galambosi *et al.*, 1993; Kerrola *et al.*, 1994; Anon., 2009; Kizil *et al.*, 2010).

27.6.1 Medicinal uses

Khare (2007) mentions the following medicinal properties for hyssop: stimulant, carminative, sedative, antispasmodic, diuretic; it has also been ascribed stomachic and emmenogogue effects. It is used in the treatment of bronchitis, asthma, coughs and colds; it induces heavy sweating in fevers and can increase blood pressure. The antimicrobial activity of the essential oil of hyssop has been investigated and the activity was attributed to the linalool and 1,8-ciniole components of the essential oil (Mazzanti *et al.*, 1998). Hyssop is used externally for bruises, concussion and cuts; a leaf juice preparation is used for the treatment of roundworms. A tea made from the herb is believed to be effective in the treatment of nervous disorders and toothache.

27.6.2 Culinary uses

Hyssop is used as a condiment and the leaves and flowering tops of hyssop are employed in flavourings for salads and soups in European cuisines; it is also used in bitters and tonics, and in the preparation of liquor and perfumes.

27.7 Juniper berry

The juniper (*Juniperus communis* L.) is an evergreen tree native to Europe, northern Asia, and the northern parts of North America. It is a conifer, a gymnosperm, from the family Cupressaceae (Cypress family). The tree produces blue, violet or blackish brown fruits, which are harvested in early autumn for culinary and medicinal use. The berries take 2–3 years to ripen, turning from green to blue to black as they mature. Only the blue, ripe berries are harvested. In Europe, many of the crops are harvested from the wild; once collected, the berries are laid out to dry and during this process they develop the blackish colour seen in the final commercial product. Juniper berries contain up to 2 % volatile oil, which consists of monoterpenes, sesquiterpenes, phenols and phenolic esters, sugar, catechol, tannins, flavonoids, proanthocyanidins and deoxypodophyllotoxin. The main compounds are α- and β-pinenes (16.5–80 %), sabinene (0.2–50 %), limonene (1–12 %) and terpenene-4-ol (up to 5 %); with smaller amounts of α-terpeneol, borneol, geraniol, etc. The flavour profile of young, green berries is dominated by pinenes (Ciesla, 1998).

27.7.1 Medicinal uses

Juniper berries have been in use medicinally for centuries; Ancient Greek and Arab healers, as well as Native American Indians, used them for the treatment of numerous diseases (Adams, 2004). Khare (2007) records that the berries have diuretic, urinary antiseptic, carminative, digestive, sudorific, anti-inflammatory and emmenogogue properties. They are used in the treatment of acute and chronic cystitis, renal suppression, catarrh of the bladder, albuminuria, amenorrhoea, leucorrhoea. The aerial parts of the plant have abortifacient properties.

Traditionally in Western Europe, juniper berries and oil were used to treat inflammatory diseases. It is also widely used for its diuretic effects – a result of the presence of terpinene-4-ol, which is known to increase renal glomerular filtration rates. However, the pinenes in the oils may also cause urinary tract irritation and hence prolonged use of juniper berry is not recommended (Anon., 2009b, 2011). Juniper extract has been used to alleviate menstrual disorders. Recent studies have found that the berries contain compounds that help to foster proper hormone and eicosanoid balances, similar to fish oil and EPA supplements, which may explain its use in menstrual disorders. These compounds also explain the anti-inflammatory and antiplatelet-activating factor activity of juniper tea, which is thought to achieve its effects through the inhibition of prostaglandin biosynthesis (Anon., 2009b, 2011). Juniper berry tea is also listed in the German Pharmacopoeia as a treatment for dyspeptic complaints including gas, heartburn, indigestion and flatulence (Blumenthal *et al.*, 1998; Blumenthal, 2003).

Juniper berries have been used in phytotherapy and cosmetics in the Mediterranean region and in Western Europe; for example in 'juniper baths' for the treatment of neurasthenic neurosis and in the management of scalp psoriasis.

Currently, the juniper berry is being researched as a possible treatment for diet-controlled diabetes, as it releases insulin from the pancreas (hence alleviating hunger). Juniper berry decoction and extract induces uterine contraction and hence should not be used by pregnant women.

27.7.2 Culinary uses

Juniper berry is probably best known as the unique flavouring agent of gin, an important component of the dry martini, a popular intoxicant and a putative calmative revered by western culture for over 300 years. Other juniper-flavoured beverages include the Finnish rye-and-juniper beer known as *sahti*, which is flavoured with both juniper berries and branches. American native tribes are also reported to have used the juniper berry as an appetite suppressant in times of hunger and/or famine.

In northern Europe, and in particular Scandinavia, juniper berries are used in cooking to give a sharp, clear flavour to meat dishes, especially wild birds and game. The berries are also used to season pork, cabbage and sauerkraut dishes.

27.8 Kokum and Malabar tamarind

Kokum and Malabar tamarind belong to the genus *Garcinia* which belongs to the family Clusiaceae (Guttiferae, mangosteen family). It is an important genus distributed in the tropical regions of south Asia, South East Asia and Indonesia. The most famous member of the genus is the mangosteen, *G. mangostana*, commonly known as the queen of the tropical fruits. Kokum (*G. indica* Choisy) and Malabar tamarind (*G. gummi-gutta* (L.) Roxb. (syn. *G. cambogia* Desr.) yield fruits used as spices and also for the manufacturing of soft drinks and concentrates. Raju and Reni (2001) present a brief review of kokum and Malabar tamarind.

Both kokum and Malabar tamarind are evergreen trees reaching a height of about 15 m. They are mainly found in the Western Ghats and adjoining areas, in the states of Maharashtra, Karnataka, Kerala and Tamil Nadu in India; they are also grown in Indonesia. Kokum and its products are exported to the UK, USA, Italy, Canada, Australia, Hong Kong and the Middle East, with Zanzibar being the chief importer. This spice used commercially is the dried, processed rind of the fruit.

Kokum concentrate is prepared from the juice of fresh, ripened fruits. Both juice on its own and juice syrup (with sugar) are manufactured. Rind-sugar candy is prepared from the rind after the extraction of juice; this product is said to be an excellent cure for hyperacidity problems. The oil in the seed is extracted using either a standard extraction procedure (common oil mill, boiling) or by solvent extraction (Ravindran *et al.*, 2007).

The processing of Malabar tamarind is similar to that of kokum described above. In this case also the pericarp from the ripe fruit is cut into pieces and dried in the sun or using smoke (or a combination of the two). The syrup and the concentrate are manufactured from the dried rind. The fruit rind of kokum contains a polyunsaturated phenolic pigment, garcinol and its isomer isogarcinol, along with (–)-hydroxy citric acid (HCA), cyanidin-3-glucoside and cyanidin-3-sambibioside. The fruit rind of Malabar tamarind contains the isoprenylated polyphenols, cambogin and camboginol (Patil and Kattimani, 2009).

The recent excitement concerning kokum and Malabar tamarind is due to the occurrence of HCA, which is reported to be an anti-obesity compound. HCA is in fact very rare in the plant kingdom, but is present in kokum and Malabar tamarind

at levels of 10–30 % and many reportedly anti-obesity formulations have been developed based on HCA (see below). The HCA is extracted from the dried rind by aqueous extraction, followed by treatment with alkali to convert any lactone present into acid, and precipitating the acid with the addition of calcium chloride. The HCA is precipitated as calcium salt.

In addition to the HCA, the fruit rind contains about 10 % malic acid, together with smaller quantities of citric and tartaric acids. Kokum rind has the following approximate composition (%): protein, 1.92; crude fibre, 14.28; total ash, 2.57; tannins, 2.85; pectin, 5.71; starch, 1; crude fat, 10.0; pigment, 2.4; ascorbic acid, 0.64; HCA, 22.80 (Sampathu and Krishnamurthy 1982). Kokum is one of the richest sources of the natural red pigment, anthocyanin. Kokum rind contains 2–3 % of anthocyanin, mostly in the forms cyanin-3-sambubioside and cyanin-3-glucoside. The rind of Malabar tamarind also contains a yellow pigment, garcinol.

27.8.1 Medicinal properties

Khare (2007) reports that the kokum fruit (*G. indica*) has antiscorbutic, cholagogue, cooling, antibilous, emollient and demulcent properties. The bark is known to have astringent properties while the oil is used in the treatment of dysentery and diarrhoea. It is also used in skin ointments and lotions. A syrup from the fruit is given in cases of bilious afflictions.

The dejuiced rind, when made into candy with sugar, is a very effective local remedy for hyperacidity problems. The dried rind has strong acidity and therefore also functions as an antiseptic. The yellow resin obtained (called cambodge) from Malabar tamarind and other related species such as *G. kowa*, *G. morella*, etc. is used in medicinal preparations as an effective purgative, and is also used in veterinary medicine.

The recent demand for the *Garcinia* fruits and fruit products is as a food supplement in the management of obesity-associated problems. The physiological basis of the anti-obesity effect is the blocking of the enzyme ATP citrate lyase involved in the conversion of sugar into glycogen and cholesterol. The (–)-HCA blocks the enzyme competitively, making it ineffective, thereby preventing the production of body fats. Along with the inhibition of lipid production, energy is diverted to the production of glycogen and the increased glycogen production stimulates the glucoreceptors in the liver and sends satiety signals to the brain, which in turn suppresses the desire for food. Besides enhancing glycogen production, HCA also signals β-oxidation which burns the body's stored fat (Badmaev *et al*., 1999). Kokum extract, as well as HCA, has proven effective in significantly reducing fat synthesis in the body. *Garcinia* fruit lowers blood lipids such as cholesterol and triglycerides by triggering fatty acid oxidation in the liver via thermogenesis (Muthulakshmi and George, 1999). Some of the formulations containing HCA or its calcium salt that are available in the market include Citrin®, CitriMax®, Super CitriMax® and APPEsat™, etc.

The seed oil is used in the preparation of liniments, ointments and suppositories. The oil is also used for topical application in cases of ulcerations, or as a lip ointment (Nawale *et al*., 1997).

27.8.2 Related species

Some important related species with medicinal properties include: *G. mangostana*, *G. cowa*, *G. morella* and *G. xanthochymus*. *G. cowa* is used in traditional medicine and the fruit rind is used by local people as a substitute for tamarind. The latex of the plant is used as febrifuge in Thai folk medicine (Anon., 2002). The latex contains cowanin, cowanol, cowaxanthone and norcowanin; cowanol and cowaxanthone are reported to exhibit moderate antimicrobial activity against certain bacterium. The gum from *G. morella* is used as a cathartic and as an antibacterial agent; it contains morellin, neo-morellin, β-guttiferin and α-guttiferin. *G. xanthochymus* contains xanthochymol and isoxanthochymol. These two compounds in the ratio 1:2 showed high cytotoxicity against three cancer cell lines, namely colon (COLO-320-DM), breast (MCF-7) and liver (WRL-68) (Kumar *et al*., 2007).

27.8.3 Culinary and other uses

The processed rind of kokum and Malabar tamarind is used as spice, especially in the preparation of fish dishes, where they not only substitute tamarind but also add a unique flavour to the fish curry. The famous fish curry of Goa, India, owes its unique taste and flavour to this spice. In Sri Lanka also, the Malabar tamarind is used widely as a spice and in the curing of fish. Kokum fruit serves as a flavouring and as an acidulent. Kokum syrup and kokum *sharbat* (a soft drink) are very popular in India. The *sharbat* makes a very pleasant and refreshing drink, especially popular in the Konkan region on the west coast of India.

Kokum butter is often converted by chemical processes to a product having properties similar to cocoa butter. This product is used in pharmaceutical preparations and beauty care products. The kokum butter is edible; it is nutritive, demulcent, astringent and emollient (Pruthi, 1979). It is also used as confectionery butter, in candle manufacture and in the soap industry. It can form a source for the production of stearic acid with a yield of 45.7 %. It is also used in the sizing of cotton yarns. This yellow resin, when dissolved in turpentine, forms a good varnish and it is used in the production of natural colour paints and in the manufacture of lacquer (Drury, 1991).

27.9 Large cardamom

Large cardamom (*Amomum subulatum* Rox.) is also known as black cardamom and Nepal cardamom, and is a member of the ginger family (Zingiberaceae). It is cultivated commercially in the sub-Himalayan regions, mainly in the state of Sikkim (India) and in Nepal and Bhutan and, to a smaller extent, in north Bengal (Darjeeling district). Annual production is around 5000 tonnes, most of which is consumed in India with smaller quantities exported to China, Pakistan and the Middle East. The large cardamom plant is a herbaceous perennial like the true cardamom (*Elettaria cardamomum*). The plant produces flower bunches (botanically condensed spikes) from the base of the plant which eventually produce capsules that are much larger than the true cardamom (hence the name ‘large’ cardamom). On maturity, the capsules are harvested and dried artificially. The processed commercial product

looks almost black, hence the name 'black' cardamom. Each capsule contains a large number of black seeds having a true cardamom-like aroma and taste, although much inferior in quality.

Large cardamom seeds contain about 3 % volatile oil, which is extracted through steam distillation and marketed as large cardamom oil. To improve the quality, it is often blended with true cardamom oil. The major component of large cardamom oil is 1,8-cineole, which contributes a very sharp, undesirable taste to the oil. Minor components are α-terpenyl acetate and bornyl acetate. The oil quality can be improved by the partial removal of 1,8-cineole and blending with α-terpenyl acetate to get a sweeter aroma (Rao and Madhusoodanan, 2006). The value-added products of large cardamom include extracted oil, oil blended with true cardamom oil, enriched oil (lower content of 1.8-cineole and higher content of α-terpenyl acetate), solvent-extracted oleoresin, powder and oil mixed with carriers such as starch, sugar, etc. and encapsulated oil.

27.9.1 Medicinal uses

From ancient times, large cardamom has been used in local and traditional medicine. It is considered a tonic for the liver and heart in the Ayurveda and Unani systems of medicine originating in India (Mukherji, 1972). According to Khare (2007), large cardamom seeds have stomachic, antiemetic, antibilious, astringent and alexipharmic properties; they are used for the treatment of indigestion, biliousness, abdominal pain, vomiting and congestion of the liver. An infusion of the seeds is used as a mouth gargle to cure inflammations in the oral cavity. In the producing areas, large cardamom seeds are used in the treatment of gonorrhea, kidney stones and disorders of the digestive system, and as a carminative (Rao and Madhusoodanan, 2006). In modern medicines, large cardamom oil is used in carminative mixtures as well in various syrups and chewable tablets as an adjunct to give a pleasant taste.

27.9.2 Culinary uses

Large cardamom is widely used in Indian, Pakistani, Chinese and Middle East cooking. Its use is most popular in Northern Indian and in the North-East Indian regional dishes where it is the substitute for the much costlier true cardamom. It possesses a sharp taste and flavour, and hence is capable of masking undesirable odours and tastes in various dishes, in addition to adding a pleasant flavour and taste to the dish. It is used in spice mixes and in rice dishes.

27.10 Lemon balm

Lemon balm (*Melissa officinalis* L.), also known as balm, English balm, garden balm, balm mint, common balm, melissa, sweet balm, heart's delight, is an aromatic herb from the mint family (Lamiaceae). It is a native of Europe and central Asia, and is now cultivated in most temperate and subtropical regions all over the world. The information in this section is reprinted, with permission, from The Herb Society of America guide to Lemon balm (The Herb Society of America, 2007).

There are two subspecies, *Melissa officinalis* subsp. *officinalis*, the common cultivated lemon balm; and *M. officinalis* ssp.*altissima*, naturalized in New Zealand and known as bush balm…Physically, lemon balm is an erect herbaceous perennial with opposite pairs of toothed, ovate leaves growing on square, branching stems…

Although over 100 chemicals have been identified in *M. officinalis*, the main components of the essential oil are citral (neral and geranial), citronellal, linalool, geraniol and β-caryophyllene-oxide.

Lemon balm's lemony flavor and aroma are due largely to citral and citronellal, although other phytochemicals, including geraniol (which is rose-scented) and linalool (which is lavender-scented), also contribute to lemon balm's scent.

Lemon balm is high in flavonoids, which can have an antioxidant effect. Other phytochemicals in lemon balm which may provide antioxidant activity include phenolic acids, terpenes, rosmarinic acid and caffeic acids.

27.10.1 Medicinal uses

Khare (2007) records that lemon balm has antidepressant, antispasmodic, antihistaminic and antiviral properties. It is used in cases of anxiety, neurosis and nervous excitability, palpitation and headache, and also in hyperthyroidism. Hot water extracts exhibit antiviral properties, mainly due to rosmaric acid and other polyphenols. Cream containing lemon balm is used in the treatment of cutaneous lesions. Lemon balm is also thought to have beneficial effects for individuals with Alzheimer's disease – a dose of 60 drops per day of standardized extract of lemon balm has been investigated for this purpose (Khare, 2007). Various other medicinal uses are described in The Herb Society of America guide to Lemon balm (2007):

Lemon balm has a long-standing reputation as a calming and uplifting herb…The hydro-alcoholic extract exhibited sedative effects on the central nervous system in animal studies. A study…showed that a 600 mg dose of standardized *M. officinalis* extract improved mood, calmness and alertness, and a 300 mg dose increased the subjects' mathematical processing speed. [Another study indicated that] a 600 mg dose of a standardized product containing *Melissa officinalis* and *Valeriana officinalis* reduced anxiety in human subjects…

Historically, lemon balm was believed to sharpen memory…a study published in 2003 showed that 1600 mg of dried leaf improved memory and calmness…

[Lemon balm] is one of several plants which may be useful in the prevention and treatment of Alzheimer's disease due to its ability to inhibit acetylcholinesterase and its antioxidant activity…

Lemon balm is approved by the German Commission E for nervous sleep disorders and 'functional gastrointestinal complaints'. ESCOP (European Scientific Cooperative on Phytotherapy) recommends the external use of lemon balm for cold sores and the internal use for tenseness, restlessness, irritability, digestive disorders and minor spasms. Lemon balm is also used in homeopathic medicine for menstrual irregularities. Medicinal lemon balm preparations include teas/infusions, tinctures, syrups, baths/foot baths, capsules, pills, powders, poultices, salves, steams, fomentations, oil, liquid and dried extracts.

The *Botanical Safety Handbook* gives lemon balm a 'class 1' rating, assigned to 'herbs which can be safely consumed when used appropriately,' and Dr James Duke (2003) categorizes lemon balm as 'safer than coffee,' (+++) which is his highest safety rating.

27.10.2 Culinary and other uses

The Herb Society of America guide to Lemon balm (2007) records the following culinary (and household) uses for lemon balm:

> Lemon balm is a surprisingly versatile culinary herb which can be used to flavor many different types of dishes, from beverages, to appetizers, main courses and desserts. It can be added to salads, sandwiches, soups, stews…jams…sauces, marinades, dressings…cakes…ice cream, cookies…pies…
>
> Lemon balm complements many fruits, including honeydew, cantaloupe, pineapple, apples and pears…
>
> For culinary purposes, fresh leaves are most flavorful. Chopped, fresh leaves can be added to baked goods but whole leaves can be used in many other types of dishes…
>
> One of the most popular ways to use lemon balm is in tea. Leaves can be combined with Earl Grey, green or black tea and a handful can be added to a pitcher of iced tea. Fresh leaves are best for tea, but dried leaves can also be used…
>
> Lemon balm flowers also have culinary use. They can be candied or used to garnish fruit salad, beverages or rice…
>
> In the commercial food industry, lemon balm oil and extract are used to flavor alcoholic and nonalcoholic beverages, candy, baked goods, gelatin, pudding and frozen dairy desserts. Lemon balm is an ingredient in liqueurs like Benedictine and Chartreuse.

Lemon balm has been used historically as an insect repellent. It has also been shown to stop the growth of the food spoilage yeasts, *Torulasopra delbrueckii*, *Zygosaccharomyces bailii*, *Pichia membranifaciens*, *Dekkera anomala* and *Yarrowia lipolytica*.

27.11 Long pepper

The long pepper of commerce comes from two species of *Piper*, both from the Piperaceae family. Indian long pepper comes from *Piper longum* L. and the Javan or Balinese or Indonesian long pepper comes from *P. chaba* Hunter (syn. *P. retrofractum* Vahl). The two are not clearly distinguished in the market. Indian long pepper grows widely all over India and Sri Lanka while the Java long pepper occurs naturally in the Java region of Indonesia, and in the Indo-Malayan region. A third species that is also marketed as Indian long pepper is *P. peepuloides* Wall; this is found in the north-east regions of India.

Indian long pepper is a trailing plant, climbing on small shrubs, boulders, etc., while the Java long pepper is a climber climbing on support trees with the aid of clinging roots. The venation pattern is very distinctly different in the two species. The fruit of the Java long pepper is longer, larger and more pungent than that of the Indian long pepper. Both long peppers are similar to black pepper in chemical composition. The fruits contain volatile oil and alkaloids; the flavour is contributed by the essential oil which occurs at levels of 7–8 %. The pungency of the long pepper is contributed by alkaloids, mainly piperine but also piperlonguimine and pipalartine. The volatile oil of long pepper consists mainly of sesquiterpenes, hydrocarbons and ethers (α-bisabolene, β-caryophyllene, β-caryophyllene oxide, α-zingiberene, etc.), and saturated aliphatic hydrocarbons (pentadecane, tridecane, heptadecane, etc.) (Ravindran, 2000; Nirmal Babu *et al.*, 2006).

27.11.1 Medicinal uses

Long pepper, known as *pippali*, is the most widely used medicinal herb in the traditional Indian system of medicine, Ayurveda, and goes into almost 220 various medicinal formulations. Its medicinal properties are due to the actions of piperine and pipalartine. It is one of the herbal mixes known as *trikatu* and forms an essential ingredient in most medicines intended for oral intake. Long pepper is used in diseases of the respiratory system (cough, bronchitis, asthma), as a sedative in cases of insomnia and epilepsy, as a cholagogue (in patients with obstruction of the bile duct and bladder), and also as an emmanogogue, digestive, appetizer, carminative and general tonic. Long pepper is reported to be antipyretic, hypotensive and to stimulate the central nervous system; it also enhances bioabsorption and drug availability (Khare, 2007).

27.11.2 Culinary uses

Long pepper – both Indian and Java – was once widely used in cooking to impart a pungent taste to various dishes, in European as well as in Indian/Indonesian cuisine. The importance of long pepper declined as the use of black pepper and red pepper (chilli pepper, *Capsicum* spp.) became more widespread. Today, long pepper is only rarely used in cooking, and its use is restricted to parts of India and Indonesia (Bali islands) and parts of Africa, especially north and east Africa. However, there seems to be a renewed interest in long pepper as chefs all over the world search for unusual flavours and tastes to create new and exotic dishes. Long pepper spikes need to be ground into a coarse powder or broken into small pieces before adding to a dish in order to release the flavour and pungency. The flavour of long pepper and also Balinese long pepper is deep and complex, simultaneously releasing an earthy pungency, a sweet note similar to cardamom and nutmeg, and the spicy heat of chilli. Ground or crushed long pepper can be added to soups and stews, especially to those containing lamb or mutton. In such dishes long pepper helps to mask any undesirable flavours of the lamb in addition to adding pungency. Long pepper can also be used as a substitute for the costlier black pepper in all dishes.

27.12 Szechuan pepper

The term Szechuan pepper or Japanese pepper refers to a spice obtained from a group of closely related plants of the genus *Zanthoxylum*, belonging to the citrus family (Rutaceae) that consist approximately 200 species with a pan-tropical distribution. Szechuan pepper (also known as Japanese pepper and Chinese pepper) is very important in the cuisine of central China and Japan, but it is also known in parts of India, especially in the Himalayan region, and in certain regions of South-East Asia, especially in Sumatra. The genuine source of Szechuan (also spelled Sichuan) spice is the fruit of *Z. piperitum*, the other sources being *Z. bungeanum*, *Z. rhetsa*, *Z. acanthopodium*, *Z. schnifolium*, etc. The spice, which is the ground husks of the berries, is common in the Szechuan region of China, and the leaves of the plant are also used in Japan as a spice (Ravindran *et al.*, 2007).

Most *Zanthoxylum* species produce pungent alkamides derived from polyunsaturated carboxylic acids, stored in the pericarp. The commonly found alkamides are α-, β- and γ-sanshool and hydroxy sanshools. The total amide content in *Z. piperitum* is as high as 3 %. Non-volatile constituents such as flavonoids, terpene alkaloids, benzophenthredine alkaloids, pyranoquinoline alkaloids, etc. have also been identified. The leaf oil contains glycosides such as (*Z*)-3-hexenol, C-6 compounds, citronellal, citronellol, geraniol and 2-phenylethanol (Kojima *et al.*, 1997). Xanthoxylin and (–)- sesamin have also been isolated from *Z. piperitum* (Harborne and Baxtor, 1993). α-sanshool and β-sanshool, unsaturated aliphatic acid amides isolated from the pericarp, were found to relax the circular muscle of the gastric body, as well as contract the longitudinal muscle of the ileum and distal colon in an experimental system using the gastrointestinal tract isolated from a guinea pig (Hashimoto *et al.*, 2001). Epple *et al*. (2001) investigated the effects of a total extract from *Z. piperitum* fruit on food intake in rats and found that food intake was inhibited in the rats, and that they failed to habituate to the stimulus.

27.12.1 Medicinal uses

Szechuan pepper is a medicinal plant used in local and traditional medicine in India and China. Both bark and berries are used in traditional medicines and herbal cures to purify the blood, promote digestion and as an antirheumatic. The Indian species *Z. rhetsa* (*Z. budrunga*) is used in Ayurvedic medicine in the treatment of diarrhoea, dyspepsia, asthma, bronchitis, rheumatism and diseases of the mouth. Ground bark is used by tribal people as a remedy for toothache.

27.12.2 Culinary uses

The rusty-red berries contain bitter, black seeds that are usually removed before the spice is sold. The spice is used whole or ground and is an essential ingredient in most of the dishes of the Szechuan region of China as well as in other Chinese and Japanese cuisines, particularly for chicken and duck. It is one of the spices in the Chinese five-spice powder and is used in Japanese seven-spice seasoning mix. The leaves are dried and ground to make *sansho*, a Japanese spice. In Goa and in the Konkan region of India the dried immature fruit of *Z. rhesta* is used for flavouring fish and chicken preparations.

Szechuan pepper has an aromatic lemony, flavour and pungent, biting taste, and causes numbness of the tongue. Szechuan pepper leaves possess a flavour somewhat in between mint and lime. Due to the strong aroma and flavour, Szechuan pepper found use in a large number of dishes, especially meat and fish preparations in which the spice was employed to add flavour and taste, as well as to suppress undesirable taste and flavour. Szechuan pepper is also used as a condiment, for addition to food items before they are eaten. One such condiment is the widely popular Szechuan pepper-salt, which is invariably found in every household in the Szechuan province of China. For preparing this, Szechuan pepper and salt are roasted and ground; similarly in Japan, the condiment *sanshou* is a mixture of Szechuan pepper and salt. In Nepal, a close relative of Szechuan pepper, *Z. armatum*, is used in cooking. This is a strongly scented and more pungent spice than the Szechuan pepper. The

Indonesian lemon pepper used in the ethnic cookery of Indonesia is a species related to Szechuan pepper (*Z. avicennae*) (Katzer, 2012).

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28

Other herbs and spices: mango ginger to wasabi

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Abstract: In this second part of the chapter on underutilized spices, nine spices are discussed briefly. They are mango ginger, fragrant pandan, pink pepper, rue, sumac, savory and wasabi. Morphology, chemical composition, medicinal and culinary uses are given briefly in each of these spices. In addition short notes on 12 lesser known spices are provided; these are blue fenugreek, boldo leaves, chameleon plant, cicely, cresses, epazote, finger root, gale, lemon myrtle, Mexican pepper leaf, Tasmanian pepper and water pepper.

Key words: mango ginger, fragrant pandan, pink pepper, rue, sumac, savory, wasabi, less known spices.

28.1 Introduction

Brief reviews on eight herbs and spices are given in this chapter. These spices and herbs are not widely used, and many are restricted mostly to regional cuisines. Spices like sumac are used mainly in the Persian Gulf and Mediterranean regions, while fragrant pandan is prevalent in countries in the Far East and Pacific regions. Pink pepper is a specialty of South and Central American cuisines. Morphologically these spices vary from a rhizomatous perennial herb (mango ginger) to small herbs (rue) to fairly large trees (Californian pink pepper tree). In this chapter, these spices and their role in cooking are dealt with briefly. In addition brief notes are also provided on 12 other minor herbs and spices.

28.2 Mango ginger

Mango ginger (*Curcuma amada* L.) is a rhizomatous, aromatic herb of the ginger family, Zingiberaceae. It is cultivated throughout India, Sri Lanka, and Bangladesh and in many South East Asian countries for its rhizomes, which are used in both fresh and dried form for flavouring pickles and other vegetarian and meat dishes.

It is also valued for its medicinal properties. The fresh cut rhizome has the flavour and the colour of mango, hence the name mango ginger.

Mango ginger is a perennial, which is propagated through rhizomes. The plant reaches 60–90 cm in height; its leaves are long, petiolate and oblong–lanceolate; flowers are white or pale yellow in spikes produced at the centre of the tuft of leaves. The rhizomes have a bitter taste initially, turning sweeter and later becoming sour and aromatic. *Curcuma amada* is similar in taste and uses to the Indonesian species *Curcuma mangga* (Khare, 2007).

28.2.1 Chemical and functional properties

Earlier studies reported variation in the chemical composition of mango ginger rhizome depending upon the region of production. Major compounds identified include ocimene, linalool, linalyl acetate, safrole, curcumene, δ-3-carene, (*Z*)-β-β-ocimene, dihydrocimenes, myrcene and 1,8-cineole. (Chaudhuary *et al*., 1996; Gupta *et al*., 1999; Srivastava *et al*., 2001; Singh *et al*., 2002; Mustafa *et al*., 2005). Another study using advanced gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) led to the identification of 26 components comprising almost 98 % of the total volatiles (Mustafa *et al*., 2005). The oil contained ten monoterpenes, three hydrocarbons, four oxygenated hydrocarbons, two ketones and one ester. The predominant monoterpenes are camphor and thymol. The sequiterpene fraction consisted mainly of hydrocarbons such as (*Z*)-β-farnasene, guaia-6,9-diene, α-pinene and α-longipinene. The aromatic constituent contributing to the flavour is thymol. Jatoi *et al*. (2007) studied the chemical composition of mango ginger from Japan and reported over 130 compounds with biological activities, including antioxidant, antibacterial, antifungal and insecticidal properties. Nahar and Sarker (2007) provide an exhaustive list of the chemical constituents of various species of *Curcuma* including *C. amada.*

Mango ginger has many biological activities due to the presence of curcuminoids (curcumin, des-methoxy curcumin, demethoxy curcumin). Curcumin has been shown to be associated with a large number of physiological and medicinal properties (Aggarwal *et al*., 2007; Jatoi *et al*., 2007). It is known as a carminative, expectorant and antipyretic, and can stimulate the appetite. Studies have shown that curcumin (as well as its main source, turmeric) has a variety of biological activities, especially under *in vitro* cell culture and in animal systems.

Curcumin has a significant effect on different cell signaling pathways (such as, for example, its impact on the NFκB signalling pathway, inhibition on the AP-1 signaling pathway, modulation of the cytochrome *P*-450 pathway and inhibition the growth factor pathway). The anticancer properties of curcumin against some types of cancer cells have been tested and proved under *in vitro* systems. It was shown to inhibit the proliferation of breast cancer cells, colon cancer cells, prostate cancer cells and kidney cancer cells and to induce apoptosis in many cancer cells. It has also been found to enhance the chemosensitivity and radiosensitivity of cancer cells.

Curcumin is useful in cardiovascular-related ailments such as hypercholesterolemia and platelet aggregation: the rhizome extract was reported to exhibit a hypocholesterolemic effect in rabbits (Pachuri and Mukherjee, 1970). Majumdar *et al*. (2000) demonstrated the anti-inflammatory activity of mango ginger rhizome extract

in rats, and many other studies have also indicated that mango ginger extract exhibited strong inhibitory activity on bacteria and moulds (Sayyad and Chaudhari, 2010).

It has been found to be very effective in muscle regeneration and in wound healing, and is recommended for use in rheumatism, concussion and sprains (Khare, 2007). Curcumin is also effective in treating inflammation of the liver and in protecting the liver from alcohol- and drug-induced injury. A significant reduction in total lipids and serum triglycerides in the liver of adult female rats was observed when fed with 10 % mango ginger or 10 % curcumin in a normal diet or in a sucrose-based hyper-triglyceridemic diet (Khare, 2007). Lastly, one of the most noteworthy features of curcumin is its activity against Alzheimer's disease.

28.2.2 Culinary uses

The rhizome of mango ginger is a popular spice and vegetable due to its rich flavour, which is described as sweet with subtle earthy floral and pepper overtones and similar to that of raw mango. It is a delicious addition to salads and stir fries. It is used in South Asian and South East Asian as well as Far East Asian cuisines and, most commonly, in Thai cooking. In India, it is most widely used in chutneys and pickles. It is prepared for use in cooking like fresh ginger. Some of the dishes found on popular recipe websites in which mango ginger is used as a flavour and spice are: Kondaikadalai pachadi, mango ginger gravy, mango ginger pickle, grilled pan chicken with fiery mango ginger salsa, gingerbread cupcakes with mango ginger icing, hot grilled shrimp with mango ginger sauce, couscous cake with fresh mango ginger chutney, grilled Thai chicken salad with mango ginger, spicy mango ginger tofu, mango ginger sorbet.

28.3 Fragrant pandan

Fragrant pandan (also known as pandan wangi and fragrant screw pine) belongs to the screw pine family. It is an ancient cultigen which has never been found wild (Setyowati and Siemonsma, 1999). It might have originated from the Moluccas in Indonesia and is now grown in Sri Lanka, Thailand, India, Malaysia, Indonesia, the Philippines and probably in many other tropical and subtropical countries. Fragrant pandan is the only species of *Pandanus* with fragrant leaves, and it is non-flowering in all the cultivated regions except in the Moluccas. Throughout Southeast Asia, it is used in cooking to impart flavour and colour to rice, sweets, jellies, and in many other food products. It is widely used to flavour ordinary rice as a substitute for the expensive aromatic rice varieties (Setyowati and Siemonsma, 1999).

Fragrant pandan grows in two sizes, small and large. Both are perpetuated by suckers. In the small type, the stem is slender, 1–1.6 m tall, 2–5 cm in diameter, decumbent and ascending, producing many aerial roots. The large type produces an erect stem, 2–4.5 m tall, *c*.15 cm in diameter, sparsely branched, producing prominent prop roots. The taste and flavour quality are the same in both growth forms. The small form is usually cultivated, grown by suckers or by stem cuttings. It is usually grown mixed with various other crops in the home garden, and large plantations do not exist. Harvesting of leaves can start 6 months after planting. Individual

leaves are cut for use, leaving a tuft of four to five leaves at the top of the plant, and are marketed fresh. A mixed plantation of fragrant pandan may give approximately 60 kg fresh leaves per harvest or 6 tons/ha per year. It is also an ornamental plant and its leaves can be used for basket weaving.

Pandan leaves yield a very small amount of essential oil consisting of 6–42 % sesquiterpenes, hydrocarbons and 6 % linalool. The oil contains about 10 % of the aromatic compound, 2-acetayl-1-pyrroline, which is responsible for the aroma of pandan leaves (Buttery *et al.*, 1983). Pandan leaves also contain alkaloids such as pandamarine, and three pandamarilactones (pandamarilactone-1, 31 and 32) (Nonato *et al.*, 1993). More alkaloids and their derivatives were later identified in pandan leaves grown in Thailand and Jami (Indonesia). Pandan leaves are also rich in antioxidant carotenoids such as neoxanthin, violaxanthin, α-carotene, β-carotene, lutein, zeaxanthin and vitamin E analogues. Lutein is present in the highest concentration and is mainly responsible for the antioxidant property of the leaves. Pandanin is a lectin-type protein present in the leaves (Wongpornchai, 2006).

The fresh leaf has no aroma and flavour; flavour develops when the leaf is withered and when cut into pieces. This flavour development is due to a volatile product of the oxidative degradation of a yellow carotenoid pigment that forms only when the plant withers. The leaves have a pleasant aroma, similar to fresh hay, similar to the scent found in some aromatic rice varieties grown in South East Asia (such as Thai jasmine rice and Khao Dawak Mali 105). However, dried pandan leaves lose their fragrance quite quickly (Routray and Rayaguru, 2010).

28.3.1 Functional properties and medicinal uses

Pandan leaves are important in the traditional medicine of South East Asian and Far East Asian countries. The leaves are soaked in coconut oil for several days and the oil is then used in the treatment of rheumatic problems. An infusion of leaves is taken internally as a sedative in restlessness, and it is also indicated in curing internal inflammations. It is used in the treatment of weak nerves (neurasthenia), lack of appetite, hair loss and to darken hair and prevent dandruff. In Thailand, pandan leaves are used as a traditional medicine for treating diabetes. The mode of administration is usually as juice or as a concentrated infusion taken with or without the addition of sugar. Locally it is also used as a treatment for wounds, as an antipyretic, to relieve headache and earache, as a laxative for children, for relief from chest pains, in helping women to recuperate after delivery and in reducing stomach spasms (Wongpornchai, 2006; Anon., 2012a).

28.3.2 Culinary uses

Fragrant pandan, as mentioned earlier, is used extensively in South East Asian and Far East countries to impart aroma to ordinary rice. Juice expressed from the leaves is used to impart flavour and colour to foodstuffs. Pandan leaves release their aroma when sliced, and have a subtle grassy-nutty flavour, making them suitable for use in pot pourris alongside flower petals, especially rose. They are widely used in many Asian countries, such as Indonesia, Malaysia, Thailand, Philippines, Singapore, Sri

Lanka, India and even in Australia in a variety of dishes, including rice dishes, puddings, beverages and curries. *Nasi lemak*, *nasi kuning* and *nasi padang* are some of the pandan leaf-flavoured rice dishes widely eaten in Malaysia and Indonesia, and the leaf paste is used in green Thai and Malaysian curries; the flavour goes very well with coconut milk, glutinous rice, lemon grass, milk, brown sugar and turmeric. In India, pandan leaves, as well as pandan flowers from the related species *Pandanus fascicularis* (common screw pine, the *Ketaki* flower), are used to perfume biryani and other rice dishes and also used in spice blends. Pandan leaves are also used as wrappers in South East Asian cooking in order to provide a distinct flavour to foods. They are wrapped around chicken, pork, fish and desserts before grilling, roasting, barbecuing or steaming. The leaves also provide the green colour and flavour in Indonesian, Thai, Malaysian and Nonya-style rice baked desserts, candies, puddings, soups and coconut milk. In fact, all over South East Asia, the most important culinary application of pandan leaves is in desserts (Anon., 2012a). In Indonesia, pandan leaves are made into ice cream-like concoctions. Pandan leaves are also used in sweet puddings or custards based on sticky rice covered in thick coconut milk (Routray and Rayaguru, 2010). Indian desserts such as *rasagolla*, *gulab jamun* and *rasmalai* also often contain pandan leaf. Many dishes containing pandan leaf flavouring can be found on well-known recipe sites, including: coconut pandan rice custard, coconut pandan chiffon cake, pandan fudge cake, Thai pandan chicken, chicken wrapped in pandan leaves, Thai pandan custard, nasi kuning – festive yellow rice, Indonesian rice with pandan, lod-chong nam ka-ti (pandan noodles with coconut milk), buko pandan, pandan waffle, Thai pandan chicken, pandan coconut muffins.

28.4 Pink peppercorn

Pink pepper is derived from two species of the genus *Schinus*. Brazilian pink pepper is derived from *Schinus terebinthifolius*, which belongs to the cashew family. California pink pepper (also known as Peruvian pepper or American pepper tree) is derived from *S. molle*. Both are known as pink pepper and are used for similar purposes. The Brazilian pepper tree is native to Brazil, Argentina and adjoining regions. It was introduced into the USA as an ornamental tree; in areas such as Florida it has run wild, and has also became naturalized in many other tropical and subtropical countries (Langeland and Burks, 1998). The pink pepper tree is highly drought resistant and has become one of the most aggressive and widespread invasive weed species, extending over vast areas in the USA and displacing the native plants. This species invades aquatic as well as terrestrial habitats, greatly reducing the quality of native biotic communities (MacDonald *et al*., 2008).

The Brazilian pepper tree is a small evergreen tree, having alternate, compound leaves, with a turpentine-like aroma. This species is dioecious; male and female flowers are produced on separate trees and are borne on large panicles. The fruit is a fleshy drupe, which turns bright red when ripe, while the pulp is brown in colour and aromatic (Kramer, 1957; NIIR Board, 2002). The tree produces a large quantity of seeds that are dispersed by animals and birds.

The American pepper or Californian pepper (*S. molle*) is native to the arid zone of northern South America, Mexico and the Andean deserts of Peru, spreading as far as central Chile and central Argentina. It has become widely naturalized around the world where it has been planted both as an ornamental and for spice production. *S. molle* is a drought-tolerant, long-lived, hardy evergreen species that has become a serious invasive weed internationally (Iponga *et al*., 2008). This is a much larger tree than the Brazilian pink pepper, but has similar morphological characteristics and chemical composition.

28.4.1 Chemical and functional properties

Chemical analysis led to the identification of 57 and 62 compounds in the oils of *S. molle* and *S. terebenthifolius*, respectively. The main constituents of these oils are: α-phellandrene (46.52 % and 34.38 %, respectively), β-phellandrene (20.81 % and 10.61 %), α-terpineol (8.38 % and 5.60 %), α-pinene (4.34 % and 6.49 %), β-pinene (4.96 % and 3.09 %) and *p*-cymene (2.49 % and 7.34 %). A marked quantity of γ-cadinene (18.04 %) was also identified in the *S. terebinthifolius* essential oil, whereas only traces (0.07 %) were detected in the essential oil of *S. molle* (Bendaoud *et al*., 2010). The oil of *S. terebenthifolius* was reported to be more effective as an antioxidant and also against certain cancer cell lines tested (Bendaoud *et al*., 2010). Both pink peppers cause allergic reactions such as skin irritations and respiratory difficulties when the tree is in bloom, and allergic reactions can also result from excessive consumption. This allergic property seems to be due to the presence of urushiol-type allergens, but the spice grown in Réunion appears to be free of urushiols, and the less effective cardanoles (3-alkylphenoles) were found in lower concentration than in Florida-grown pink pepper (Katzer, 2002). Extract of this plant is a powerful bactericidal agent (Siddique *et al*., 1995).

Pink peppers are important medicinally and are used in traditional local cures. They are used in treating a variety of wounds and ulcers due to their antibacterial properties, and have been used as an antidepressant and diuretic. They are also used in treatments for toothache, rheumatism and menstrual disorders. Recent studies are providing some support for their use as an antidepressant, while other research is also focused on their potential for use as natural insecticides (Katzer, 2002).

28.4.2 Culinary uses

Pink pepper is a spice used in a variety of dishes in Brazil, Mexico, Argentina, Peru and other areas of Latin America. The flavour of the pink berries (also marketed as pepper rosé) is rather weak, and so these berries serve a predominantly ornamental purpose, although they can develop a subtle flavour in food that has little other flavouring added (Katzer, 2002). A number of recipes using pink pepper can be found on recipe sites, including the following: miso cod with deep fried cabbage and pink peppercorn dressed cabbage; peppercorn salmon; smoky lamb kebabs; ceviche with crab salad and ciabatta; Sardinian octopus; pan-fried sea bass; duck fillets; roasted duck breast with pink peppercorn sauce; peppered duck; salt marsh lamb with woodland mushroom and sorrel; spiced pork burgers.

28.5 Rue

Rue (*Ruta graveolens* L.; citrus family–Rutaceae) is a hardy, shrub-like evergreen plant, which is native to Southern Europe. Rue can grow in almost any conditions, but prefers a semi-sheltered dry environment. The lower part of the stem is woody, and the leaves are alternate, bluish-green and either bi- or tripinnate. They have a strong unpleasant odour and a very bitter disagreeable flavour. The plant blossoms from June–September, with greenish yellow flowers. Propagation can be carried out by direct seed planting, stem cuttings or root cuttings.

The whole herb is used as for medicinal purposes, the drug consisting of both the fresh and the dried herb, and may also be used in cooking in some regions. The shoots are gathered before the plant flowers, with the young shoot tops considered the most valuable. The volatile oil present in the herb is contained in glands distributed over the whole plant, and is distilled from the fresh herb, as are decoctions and infusions. The dried herb has a similar taste and odour, but is less powerful. Its powder is used for making tea.

28.5.1 Chemical properties

The main active principles of the plant are: glycosides, such as the flavonoid rutin; alkaloids, such as coquisagenine, skimmianine and graveoline; furocoumarins (psoralens), such as bergaptene (3-methoxypsoralen) and xantotoxine (8-methoxypsoralen); essential oils-containing compounds, such as methyl-nonyl-ketone, methyl-*n*-octyl-ketone and methyl-heptyl-ketone; alcohols, such as methyl-ethyl-carbinol, pinene and limonenes; and other compounds, such as dictamine, gammafagarine, pteleine and kokusaginine (Pronczuk, 1989). Soleimani *et al*. (2009) carried out a chemical analysis of the essential oil of rue plant and reported that the main classes of compounds are ketones (46.6 %), sesquiterpenoids (13.3 %) and monoterpenoids (4.1 %). The major constituents were 2-undecanone (33.9 %), 2-heptanol acetate (17.5 %), 1-dodecanol (11.0 %), geyrene (10.4 %) and 2-nonanone (8.8 %). The active principles of clinical importance are the psoralens, responsible for hepatotoxicity and photosensitization and methyl-nonyl-ketone, which accounts for the effects on the uterus. Rutin has the effect of supporting and strengthening the inner lining of blood vessels and reducing blood pressure.

28.5.2 Functional and medicinal properties

Rue is a traditionally used medicinal plant and is used in Indian traditional medicines (Ayurveda, Unani and Siddha) and in herbal medicine in many other countries, although its use in developed countries is now minimal. Khare (2007) provides the following list of its medicinal properties and uses, primarily in Indian traditional medicine:

- **Herb**: Stimulating, antispasmodic, stomachic, irritant, abortifacient; used as an emmenogogue and for the treatment of cough, colic and flatulence.
- **Leaf**: Used in amenorrhea, menorrhea, and colic; used externally for sciatica, headache, muscular chest pain, bronchitis, arthritis.
- **Oil**: Antispasmodic, anti-epileptic, emmenogogue, rubifacient.

The primary use of rue in Latin American traditional medicine is to stimulate menstrual flow by invigorating the muscles of the uterus: pregnant women should therefore avoid consumption of rue. It is also widely used in the treatment of eye problems; an infusion of the herb relieves tired and strained eyes and is believed to help improve vision. It has been used in the treatment of disorders of the nervous system such as multiple sclerosis and Bell's palsy, as well as in curing dizziness and vertigo. Rue is beneficial in treating gastrointestinal complaints such as colic and flatulence; in these cases, rue essential oil should be taken internally, mixed with water and sugar. If an overdose is taken, the herb acts as an acro-narcotic poison. It also tends to induce vomiting; any treatment should therefore not be taken directly after eating. Externally, it can be used as an ointment, while the leaves help to relieve pain caused by sciatica. Fresh leaves placed on the forehead are said to cure headaches, and a compressed and saturated decoction prepared from rue leaves applied to the chest helps in treating persistent bronchitis. Chewing rue leaves helps to eliminate bacteria on the gums (Grieve, 1931b).

28.5.3 Culinary uses

Katzer (2000a) provides a detailed discussion of the uses of rue in cooking:

> Apart from occasional use in Italy, rue's popularity is greatest in Ethiopia. Fresh rue leaves are sometimes used as a coffee flavourant (remember that coffee is probably native to Ethiopia), and rue is also sometimes mentioned as a components in the national spice mix, *berbere.* Ethiopian cuisine is unique in using not only rue leaves, but also dried fruits (rue berries) with their more intensive, slightly pungent flavour that is well preserved on drying.
>
> To cook with rue is usually considered old-fashioned, which is probably because half a century ago, rue was significantly more popular than today so that it is seen a leftover from past times; second, older people frequently develop a positive attitude towards bitter taste and tend to use bitter herbs and spices more liberally. And yet, rue is definitely worth a try; meat, eggs and cheese all can profit from this nearly unknown spice, provided care is taken not to overdose. The bitter taste is reduced by acids; thus, a leaf of rue may be used to flavour pickled vegetables, make a salad more interesting or add a very personal touch to home-made herbal vinegar.
>
> Because of its general affinity to acidic food, rue goes well with spicy Italian tomato sauces containing olives and capers (together with marjoram, basil and lovage). If a cook wants rue flavour without bitterness, he might make use of the fact that rue leaves excrete the essential oil much more quickly than the bitter rutin (very similar to tea leaves). Thus, the fresh leaves may be soaked in a slightly boiling sauce for a short time (typically, one minute) and discarded afterwards. By this a procedure, a maximum of flavour at a minimum of bitterness is achieved…
>
> Like many other bitter spices … rue is popular for flavouring liquors. Besides stimulating the appetite, bitter liquors have some tonic, stomachic and even bile-stimulating properties, all of which are advantageous after a rich feast. One of the most common liquors containing rue is grappa con ruta, an Italian draff brandy flavoured with a small branch of rue per bottle. For this, the related Fringed Rue (Aleppo rue, *R. chalepensis*) is usually preferred.

Below is a list of recipes found on popular recipe sites that use rue as a flavouring ingredient: peas with rue (rantas borbszho), cheese–rue casserole, scotch rues, potato soup, seafood gumbo, clam chowder, lentil soup, sauerkraut and bean soup, seafood

au gratin, linguini and white clam sauce, broccoli cheese soup, cheese soup, rue spice cake, ancient Roman-style garlic cheese with rue, endives wrapped in ham, etc.

28.6 Sumac

Sumac (*Rhus coriaria* and related species; cashew family–Anacardiaceae) is a very popular spice in countries of the Middle East, where it is widely used in rice, vegetable and meat dishes and in desserts. Sumac is used in most Arab countries, and has spread from there to a number of regions across the world. The term sumac is derived from the Arabic root, *summaq*, meaning red, referring to the colour of sumac fruit. Sumac belongs to the genus *Rhus*, found in subtropical and temperate regions throughout the world. Many species are used as a spice, the most important being Sicilian sumac, *Rhus coriaria.* Other species used include Chinese sumac (*Rhus chinensis*), smooth sumac (*R. glabra*), Staghorn sumac (*R. typhina*), fragrant sumac (*R. aromatica*), lemonade sumac (*R. integrifolia*), sugar sumac (*R. ovata*) and Muller's sumac (*R. mulleria*) among others. Sumac plants are large shrubs or small trees, reaching a height of 3–10 m, with pinnately compound leaves. They bear greenish white flowers in dense panicles and red drupaceous fruits (also called sumac bobs), from which the spice is derived. Sumac is an invasive species that has spread over large stretches of land in many areas of the USA, where it has proved difficult to eradicate.

28.6.1 Chemical and functional properties

Sumac fruits, leaves and bark contain many components (Brunke *et al.*, 1993). Sixty constituents have been identified in the essential oil extracted through hydrodistillation of *R. coriaria* leaf (principally β-caryophyllene (0.33–16.95 %) and a sesquiterpene hydrocarbon, patchoulane (3.08–23.87 %)); 63 in the essential oil from the bark/branch (mainly β-caryophyllene (12.35–21.91 %) and cembrene (10.71–26.50 %)); and 85 in the essential oil from the fruit pericarp (principally limonene (0.17–9.49 %), nonanal (10.77–13.09 %) and (*Z*)-2-decenal (9.90–42.35 %)). The composition of oils from two different phytogeographic regions showed variations (Rayne and Mazza, 2007; Kossah *et al.*, 2009). Brunke *et al.* (1993) studied the chemical composition of Syrian and Chinese sumac and reported a large number of compounds in their oils. They found the main constituents to be terpene hydrocarbons (i.e. α-pinene, β-caryophyllene and cembrene), oxygenated terpenes (i.e. α-terpineol, carvacrol and β-caryophyllene alcohol) as well as farnesyl acetone, hexa-hydro-farnesyl acetone and aliphatic aldehydes.

The flavour characteristics of ground sumac are described as 'oil and acid aroma, dried lemon balm, cellulose/ woody, spicy, earthy and astringent' (Bahar and Altun, 2009). The astringent–acidic flavour of sumac spice is mostly caused by two different types of constituent: tannins (gallotannins, with a total content of 4 %) and organic acids (malic, citric and tartaric acid, along with smaller amounts of succinic, maleic, fumaric and ascorbic acids). The sensory and flavour profile analysis carried out by Bahar and Altung (2009) indicated that the malic acid present in sumac fruit is mainly responsible for its sour taste. β-caryophyllene contributes both the spicy and

woody flavour; cembrine, the woody flavour and caryophyllene oxide, the spicy flavour. The fruit wall (pericarp) is dark red and contains anthocyanin pigments, including chrysanthemin, myrtillin and delphinidin.

Sumac fruits (as well as all other plant parts) contain the group of chemicals known as urushiols (3-alkyl pyrocatechol derivatives), which are powerful allergens. Sumac can cause painful dermatitis in sensitive people, and the toxins are effective in sub-microgram amounts. Lethal poisonings have been reported, particularly on ingestion or inhalation, which allows the urushiols to attack the mucous membranes of the mouth, nose and intestines (Katzer, 1998b).

Sumac is used medicinally in Arab countries. Studies on sumac extracts to date have indicated that the plant may be a source of bioproducts with the following bioactivities: antifibrogenic, antifungal, anti-inflammatory, antimalarial, antimicrobial, antimutagenic, antioxidant, antithrombin, antitumorigenic, antiviral, cytotoxic, hypoglycaemic and leukopenic (Rayne and Mazza, 2007).

Sumac extracts, as well as the extracts of other species of *Rhus*, are most notable for their antimicrobial activities. In one study, crude methanolic extracts of *R. glabra* branches exhibited both the widest zones of inhibition in a disc assay, and the broadest spectrum of inhibition (active against the species of bacteria tested: *Bacillus subtilis*, *Enterobacter aerogenes*, *Escherichia coli* DC2, *Klebsiella pneumoniae*, *Mycobacterium phlei*, *Pseudomonas aeruginosa* H187, *Serratia marcescens*, *Staphylococcus aureus* and *Salmonella typhimurium* TA98) (Rayne and Mazza, 2007).

28.6.2 Culinary uses

Sumac is widely used as a spice throughout the Middle East, and its use in cooking has also spread to the Iberian peninsula. Often it is simply provided as a condiment to be sprinkled on food at the table. In Turkey and Iran, sumac is often put on the table in shakers or bowls, especially in kebab houses, and is used like salt and pepper are used in the west, to improve the flavour of meat dishes, curries, fish, vegetables, rice, salads, stews and sauces and, combined with onions and salt, as a seasoning for roast meat. In other Arab countries, particularly in the Eastern Mediterranean, sumac is mixed with sesame seeds, salt and thyme or hyssop in the popular spice mix called *za'atar* (also spelled *za'htar*), used as a seasoning for fried and barbecued meat, or combined with olive oil for use as a dip for breads (Katzer, 1998b). In Egypt, it sometimes appears in another spice mix called *dukkah* (spelling varies). In addition to being used as a condiment, sumac is also commonly rubbed on meats, chicken or fish, added to marinades and used to increase the acidity in yogurt sauces or vinaigrettes. It is used for the enhancing taste and flavour of egg dishes and salads and, because it provides an attractive red colour, it is used as a decorative garnish on dishes such as hummus and other dips.

Native Americans also use the fruits of smooth sumac and staghorn sumac (*R. glabra* and *R. typhina*) to make a beverage known as sumac-ade, Indian lemonade or rhus juice. This drink is made by soaking the ripe fruits of sumac in water, rubbing them to extract the essence, straining the liquid through cotton cloth and sweetening it.

Examples of dishes using sumac, taken from various sources, are as follows:

Azerbaijanis herbed potato slices, za'atar spice blend, devilled eggs with tahini, shawerma, mousakhan (Palestinian chicken), Middle Eastern-style lamb pizzas, fattoush (Middle Eastern salad), parsley and sumac salad, spiced kumara (sweet potato) dip with crisp flatbread, Middle Eastern thyme breads, sumac onions, roasted red capsicum soup, tofu sour cream, piyaz (Turkish black-eyed pea salad), Turkish potato salad, chicken thighs in yogurt and onions, African spice mix, gavurdagi.

28.7 Summer savory and winter savory

The genus *Satureja* (*Satureja* spp. L.; sage family–Lamiaceae) comprises about 14 species of highly aromatic, hardy annual or perennial herbs or under-shrubs. Two important species of this genus are *S. hortensis* (summer savory) and *S. montana* (winter savory), with the former more widely used; hence this discussion is mainly on summer savory.

Summer savory is a hairy aromatic annual, grown as a popular garden herb, while winter savory is a semi-evergreen bushy and woody perennial shrub, with a stronger flavour. Savory is indigenous to southern Europe and the Mediterranean area, and is now distributed across the warmer regions of both hemispheres, growing wild in dry, light soils and on rocky hillsides on chalk. It is locally cultivated for commercial use, with France, Albania and countries of the former Yugoslavia the major producers (NIIR Board, 2002). Savory is also cultivated in Spain, Germany, and other parts of continental Europe, England, Canada the USA and in India, in Kashmir, although the variety grown in the former Yugoslavia is recognized as the premier grade.

Summer savory plants grow to 30–60 cm tall, and have slender green leaves with lilac tubular flowers, while winter savory can reach over 200 cm tall, has a woody stem and pale pink to white flowers. Both types can be propagated clonally, from cuttings or divisions of the root, or preferably through seeds. They are cold-sensitive, preferring a cool climate, full sun and rich and light soil. Harvesting of leaves can start 75–120 days after sowing; the harvest is dried in shade or at 35 °C and stored in closed containers. The dried leaves are brownish green in colour and are fragrantly aromatic, with a warm, slightly sharp taste. The flavour of winter savory tends to be more bitter than that of summer savory. In commerce, the plant is marketed in a number of forms: the plant as harvested at flowering time and dried; freshly harvested leaves and flowering tops, collected during the flowering season; the leaves harvested before flowering; or the whole ground dried leaves and flowering tops.

28.7.1 Chemical and functional properties

The chemical properties of the fresh leaves differ from those of the dried commercial product. Fresh summer savory leaves contain moisture (72 %), protein (4.2 %), fat (1.65 %), sugar (4.45 %), fibre (8.60 %) and ash (2.11 %). The commercial product should have the following specifications: about 10 % total ash, 2 % acid insoluble ash, 10 % moisture, 25 ml volatile oil per 100 g and granulation 95 % (95 % of the ground product should pass through a US standard sieve No. 40).

The leaves on a dry weight basis contain 11.95 % pentosans and also labiatic acid, ursolic acid, β-sitosterol and volatile oil. There are many reports on the composition of essential oil of the aerial parts and leaves of savory from different parts of the world (Opdyke, 1976; Gora *et al*., 1996; Hajhashemi *et al*., 2000). The essential oil distilled from the full flowering spice is between 0.1 and 0.15 %. Savory oil is described as light yellow to dark brown liquid, and it comprises carvacrol, *p*-cymene, pinene, dipentane, ursolic acid, etheral oil, phenolic substances, resins, tannins and mucilage (Prakash, 1990; Karnick, 1994). Lawrence (1981) compared the chemical composition of savory oils from Europe, Canada and North Africa. The oil exhibited differences in *p*-cymene, myrcene and γ-terpinene contents. Prakash (1990) carried out a comprehensive literature survey on the chemical composition of savory oil.

Seed oil of summer savory is also a commercial product. Seeds contain fixed oil (45 %) and protein (24 %) on a dry basis. Ghannadi (2002) analysed the seed oil of savory collected from Iran using GC and GC–MS. The seeds yielded 0.3 % of pale yellowish oil with a pleasant spicy odour. Forty-two components were characterized, representing 96.7 % of the total oil. The major components were carvacrol (59.7 %), γ-terpene (12.8 %), *p*-cymene (9.3 %) and α-terpinine (2.1 %). Gora *et al*. (1996) analysed a sample from Poland and reported the following compounds: γ-terpinene (40.9 %), carvacrol (39.3 %), *p*-cymene (6.2 %), α-terpinene (4.0 %), myrcene (2.5 %), α-thujene (1.9 %), α-pinene (1.5 %) and smaller quantities of β-caryophyllene, β-pinene, β-bisbolene and limonene. Many of these compounds are also common in the oil from the vegetative parts. Pfefferkorn *et al*. (2008) reported variations in the chemical composition of the oil depending upon the developmental stages of the plant.

The properties of savory as summarized by Khare (2007) include: carminative, digestive, laxative, stomachic, diuretic, sudorific and vermifuge. It is used as a treatment for colic and flatulence, and to regulate or suppress menstrual bleeding. An infusion (savory tea) is given as a carminative and expectorant (Karnick, 1994). Essential oil distilled from the aerial part has antibacterial, antifungal and spasmolytic properties, while the labiatic acid present in the plant is an antioxidant. *In vitro* studies provided some evidence for the antibacterial, antifungal, antispasmodic, antidiarrheal and anti-inflammatory activities of savory essential oil (Adiguzel *et al*., 2007). The main active ingredients seem to be carvacrol and thymol. Finally, the flowering stalks are also used as a moth repellant for clothes.

28.7.2 Culinary uses

Savory has a distinctive taste, somewhat similar to that of marjoram, and has been used in cooking since Roman times. It is now used extensively in western Asia, Europe, the Middle East, the USA and Canada, but is less common in the cuisines of tropical Asia and in South and Far East Asian countries. The leaves are used as a seasoning in meat dishes and stuffings, and sprigs of the plant are boiled with peas, beans and cabbage to improve their digestibility (Verghese, 2003; Katzer, 2007). It has also been used as a garnish, as a substitute for parsley and chervil and in liqueurs.

Popular websites provide an extensive list of dishes in which savory is used; a small selection of these is provided below:

- Meat dishes, such as crock pot chicken with blue cheese and mustard sauce, veal stew à la hongroise, herbed lemon spareribs, summer savory perfumed roast pork loin, capitolade of chicken, Newfoundland fishcakes and Quebec meat pie.
- Vegetable dishes, such as vegetable paté, pan-fried peppers with fancy grits, mushroom bruschetta, spinach and artichoke casserole, blue cheese and spinach puffs, skillet squash and onions, black-eyed pea and corn chowder, and mushroom lentil barley stew.
- Seasonings and condiments, such as herbs de provence seasoning, savory cranberry stuffing, turkey stuffing and Italian-style seasoning.
- Soups, such as Michigan asparagus soup, tomato rice soup and Cape Verde vegetable soup and green string bean soup.

28.8 Wasabi

Wasabi (*Wasabia japonica* (Miq.) Matsum; mustard family – Brassicaceae), also known as Japanese horseradish, is a native aromatic herbal spice crop of Japan. The natural distribution of wasabi ranges from Russia's Sakhalin Island, north of Hokkaido (the most northern Japanese island) to Kyushu (the southernmost major Japanese island). However, the Shimane region is the largest area of wasabi production and breeding research in Japan at present. Wasabi is now being grown in many countries in the world, including New Zealand, Taiwan, Korea, Israel, Brazil, Thailand, Columbia and the Vancouver region of Canada and in Oregon, USA. However, Japanese wasabi is still considered as the best quality variety. Horseradish (*Armoracia rusticana*) is a widely used adulterant and substitute for wasabi.

The genus *Wasabia* consists of two species, the cultivated wasabi (*W. japonica*) and the wild, uncultivated wasabi (*W. tenuis*). *W. japonica* is a glabrous, perennial aromatic herb that grows about 45 cm high, producing leaves on long petioles from the crown of the plant. Wasabi crop matures after 18 months when the plants have thickened rhizome. According to some Japanese farmers wasabi has nine well-known cultivars: Mazuma, Daruma, Takai, Shimane, Midori, Sanpoo, Izawa Daruma, Medeka and Hangen (Oates, 2008). Daruma is the most popular variety, known to grow well under marginal environmental conditions, such as warmer temperatures. For poor quality locations, researchers developed cultivars like Fuji Daruma, Izawa Daruma, Ozawa Daruma and Sanpoo. Wasabi is grown in two ways: in water and in soil. The water-grown type is considered superior and this is known as *sawa wasabi*; the lower quality land grown wasabi is known as *oka wasabi* (Oates, 2008; Sultana and Savage, 2008).

28.8.1 Chemical composition

The characteristic flavour of wasabi, similar to that of mustard leaves, is due to the presence of a group of compounds known as isothiocyanates. Wasabi (along with European horseradish and other members of the Brassicaceae family) contains the precursor of the isothiocynates, namely glucosinolates, which are glucosides present in the vacuoles of the wasabi plant. When the tissue gets damaged or cut or ground, the glucosinolates are acted upon by the enzyme myrosinase. Isothiocynates are the

final product of this enzymatic reaction, which is aided by neutral and alkaline conditions. Wasabi contains several types of isothiocynates, and the flavour quality of wasabi varieties differ depending upon the relative concentration of these compounds. So far, about 116 isothiocynates have been reported in wasabi (Masuda *et al*., 1996; Anon., 2011).

28.8.2 Medicinal properties

Wasabi has been grown and eaten in Japan for centuries. It is believed that the daily consumption of wasabi improves the health and helps fighting a large number of ailments. Many studies on the actions of the ingredients of the wasabi plant confirmed the plant's medicinal properties and its usefulness as a nutraceutical. The active ingredients in wasabi are thought to be able to destroy a number of different types of cancer cells, to reduce the possibility of getting blood clots and to have antimicrobial properties. A great deal of research has been carried out into the medicinal properties of wasabi and its active ingredient, isothiocynate derivatives, especially on 6-methylisothiocynate, which is found in a relatively high concentration in wasabi. Such studies, combined with traditional knowledge, are providing some insight into the uses and the actions of wasabi in the human body. The following are the major biological effects of wasabi and the isothicynates (ITCs) contained in it (Depree *et al*., 1998; Nagel and Oates, 2007; Anon., 2011). The medicinal properties of wasabi are excellently summarized by Nagel and Oates (2007); details are also available in the website of Wasabi world. The major properties are given below.

Anti-inflammatory effects

Wasabi can control seasonal allergies, asthma and eczema (Nagel and Oates, 2007). ITCs present in wasabi are effective agents for suppressing inflammation based on their rapid action and the low levels needed. 6-MITC (6-methyl isothiocynate) can inhibit lipoxygenase, cyclooxygenase and cyclic AMP phosphodiesterases that are involved in inflammation. Studies have shown that wasabi isothiocynates (along with other members of the mustard family like horseradish), have strong anti-inflammatory and anti-asthmatic effects. Natural health practitioners suggest that Wasabi can be an effective treatment for seasonal allergies as well as asthma and eczema.

Antimicrobial effects

As reported (Nagel and Oates, 2007), the isothiocynates present in wasabi have an inhibitory effect on many bacteria, yeasts and fungi. The compound 6-MITC from *wasabi* extracts have potent antibacterial properties against *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus mutans*, which is responsible for dental cavities. *Wasabi* extracts inhibit various strains of *Helicobacter pylori,* the bacteria implicated in stomach ulcers and cancers.

Antiplatelet effects

Nagel and Oates (2007) cite studies to show that 6-MITC from *Wasabi* has been found to inhibit platelet aggregation in the elderly, where preventing excessive clotting is vital, by a number of different mechanisms, including the inhibition of lipoxygenase, cyclooxygenase and cGMP phosphodiesterase.

Anticancer effects

Wasabi ITCs inhibit the phase I enzymes, responsible for the conversion of precarcinogenic compounds to turn into carcinogenic compounds. These isothicynates can also induce detoxifying phase II enzymes like glutathione-S-transferase. 6-MITC has also been shown to block the cell cycle of cancerous cells and to affect protein production in cancerous cells.

Metastasis, a critical stage in spreading cancer beyond the local site, can be blocked by ITCs and, in particular, 6-MITC. Fuke and her co-workers (2000, 2006) and Manesh and Kuttan (2003) have shown that 6-MITC from *Wasabi* suppressed dissemination or metastasis of certain tumour cells. ITCs from *Wasabi* have been shown to cause cancerous cells to undergo apoptosis or cell death. This has been shown in leukaemia cells (Fimognari *et al*., 2005), breast cancer cells (Nomura *et al*., 2005), lung cancer (Kuang and Chen, 2004), colorectal cancer (Lund *et al*., 2001) and cancerous cells of other types (Watanabe *et al*., 2003; Fimognari *et al*., 2005). *Wasabi* ITCs inhibit only cancerous cells and not healthy cells, and there are no reported side-effects (Nagel and Oates, 2007).

Other health effects

Wasabi and its isothiocynates also have the following medical effects (Nagel and Oates (2007):

- reduce diarrhea;
- protect nephrons in diabetes patients;
- act as antioxidants;
- provide immune modulation;
- protect cardiovascular function.

28.8.3 Preparation of wasabi paste

In traditional Japanese cuisine, wasabi is prepared by grating the fresh rhizome against a rough surface, such as a ginger grater. From ancient times, the most preferred grating surface had been shark skin ('oroshi'), and this is still regarded as the preferred method of obtaining the best flavour, texture and consistency in freshly ground wasabi. In order to minimize the loss of volatiles, the rhizome is kept at a 90° angle to the grating surface; in this way, the volatile compounds are allowed to develop with minimal dissipation. Commercially wasabi paste is prepared using mincers to finely grind the rhizomes and then it is mixed with other ingredients depending on the end use of the paste (Savage, 2012).

28.8.4 Culinary uses

Sultana and Savage (2008) provide a useful overview of the use of Wasabi in cooking:

> Wasabi adds a unique flavour, heat and greenish color to foods and, thus, it is a highly valued plant in Japanese cuisine. Wasabi is described as having 'a sharp hot taste with pungent smell', but the heat component in wasabi is different from chillies, and the

hotness quickly dissipates in the mouth, leaving an extremely pleasant mild vegetable taste, with no burning sensation at all. Wasabi adds aesthetic and culinary appeal to many foods and is considered a staple condiment in the Japanese diet. Recently, it has found widespread appeal in western cuisine due to its ability to change an ordinary dish to an extra special one by improving the taste (with addition of a spicy flavour). As a result, it is fast becoming a new flavour for the rest of the world. All the plant parts of wasabi possess same flavour but vary in the sharpness they deliver' and are, therefore, used for different purposes. Basically, wasabi can be served in three ways. These are as a condiment on the side of a dish, as a spice or herb in a dish and as wasabi flavour in processed foods. Rhizomes are the most popular tissues used to prepare fresh paste to be placed in a mound on a dish next to sliced raw fish (sashimi), spread on the raw fish in sushi preparations, or served on a small dish to accompany a bowl of cooked noodles. Sometimes grated wasabi is mixed with other ingredients like soya sauce and vinegar to prepare a dip for use with raw fish or other dishes, according to individual's choice. Tofu (soybean curd) is often decorated with grated wasabi. Wasabi petioles and leaves are pickled in sake brine or soya sauce and are popular accompaniments for white rice. Sometimes fresh leaves are used in salads and dried leaves are used to flavour cheese, salad dressings or crackers. A wasabi wine is sold (mainly as a novelty) in some Japanese specialty stores, as well as a high alcohol content wasabi liqueur.

28.8.5 Wasabi dishes

Wasabi is used in a variety of recipes in Japan and in the west (Anon., 2011d). A selection of dishes in which wasabi is used as a spices or condiment is provided below. The World of Wasabi website is a particularly useful source of wasabi recipes.

- **Dips, condiments and dressings**: Soy wasabi mayonnaise, wasabi soy sauce, avocado wasabi cream, wasabi maple dip, wasabi oil, wasabi ginger dressing, wasabi sesame dip, wasabi coconut dip, wasabi sandwich spread, cucumber wasabi sauce, wasabi butter, wasabi lime dressing.
- **Meat recipes**: Wasabi flank steak with mizo glazed potatoes, lamb shanks with wasabi, corned silverside with wasabi sauce, wasabi chicken wings, rabbit fillets with wasabi, coconut crusted chicken with wasabi sauce, wasabi lime steak, wasabi beef salad, wasabi meatballs, duck with orange wasabi glaze, meat loaves with wasabi garlic mash.
- **Fish recipes**: Tuna and ginger burgers, wasabi salmon and rice salad, sesame crusted tuna steaks, marinated sea bass, mini salmon wasabi rounds, wasabi goat cheese stuffed salmon, wasabi fish cakes, peppercorn crusted salmon with wasabi soy drizzle, crispy halibut with wasabi panzanella, tempura trout roll with caramelized onions and spinach, grilled Pacific salmon with soy-wasabi sauce and pickled ginger, tuna with wasabi cream.
- **Seafood recipes (excluding sushi)**: Crab cake salad with wasabi vinaigrette, prawn mousse filled zucchini flowers tempura, tempura king prawns, crabmeat and wasabi vol au vents, wasabi and shrimp cheese rolls, barbecue oysters with wasabi mayonnaise, wasabi prawns, wasabi crab waffles, warm wasabi seafood salad, shrimp with wasabi ponzu butter sauce.
- **Sushi recipes**: Sushi is the most famous Japanese dish outside Japan, and one of the most popular dishes among the Japanese themselves. In Japan, sushi is usually enjoyed on special occasions. The basic ingredients of sushi are sushi rice, sushi

vinegar and various types of sea food items. The fish used in sushi is raw and safety guidelines have been evolved in order to avoid toxicity issues. The original use for wasabi was with Sashimi only. Sushi recipes include: smoked salmon and cucumber sushi rolls, California nori roll, avocado and crab meat sushi roll, kappa maki (cucumber roll sushi), tuna sushi, pickled daikon sushi, sushi shrimp, sorba noodle sushi, shiitake tofu rolls, sweet brown rice sushi, lamb sushi roll, California maki, cucumber and avocado sushi, egg and pesto sushi, inside out sushi roll, boat sushi (Gunkan–maki).

- **Vegetarian wasabi recipes**: Cold avocado wasabi-soup, hot potato salad, wasabi pickled cucumber, wasabi tomato salad, rice salad with wasabi dressing, wasabi tofu soup, tempura wasabi crisps, wasabi mango rice, wasabi stuffed potatoes, Japanese wasabi snack bars, wasabi crusted tofu, wasabi ginger popcorn, wasabi flavoured spinach, wasabi roasted asparagus, wasabi pea soup, wasabi almonds, wasabi potato fritters.
- **Desserts, cakes and cookies**: Wasabi cookies, wasabi muffins, wasabi cheesecake, pumpkin and wasabi pie, wasabi chocolate shortbread, wasabi fresh fruit compote, wasabi apple crumble, white chocolate wasabi praline, hot wasabi chocolate drink, frozen wasabi icecream pudding.
- **Drinks**: Namida gimlet, namida and tonic, namida samurai, namida martini, namida vesper, namida mary, namida Caesar, angry red planet, wasabi margarita, bloody samurai, bloody samurai's revenge.

28.8.6 Adulteration issues

Wasabi is one of the rarest spices in common use, and thus the availability of genuine wasabi is very limited. Horseradish is used in most of the products currently on the market, and up to 99 % of 'wasabi' being sold around the world is only coloured European horseradish (*Armoracia rusticana*) (Anon., 2009). This means that the valuable medicinal properties of wasabi do not reach consumers. This adulteration and substitution of genuine wasabi with horseradish has been going on for over six decades and seems likely to continue unless stringent measures are employed to prevent it (Anon., 2009).

In 2009, an organization known as the 'World Wasabi Council' (WWC) was formed by wasabi growers and manufacturers with the purpose of addressing this problem. Any genuine products should carry an 'Authentic Wasabi' logo prominently displayed for easy recognition by consumers. The WWC carry out independent scientific tests to verify that products carrying this logo do not contain any European horseradish and/or artificial colourings.

28.9 Less well-known spices and herbs

In addition to the herbs and spices discussed briefly in this chapter as well in Chapter 27, there are many more with restricted distribution and use. Such herbs and spices are used by certain communities in certain regions only and they may be of little commercial importance at the current time. However, these spices and herbs are very useful in the context of the innovations being attempted by the chefs around

the world, who are always in search of novel flavours and tastes to create new dishes having exotic appeal and taste. Some such herbs and spices are listed below.

28.9.1 Blue fenugreek

Blue fenugreek (*Trigonella coerulea* [Desr.ex Lam] Ser.) is also known as blue trigonella, blue–white clover, curd herb and blue melilot. This is a rare culinary herb found in the Alpine region, Western Europe and in the Europe–Asia border regions, and is seldom used outside these areas. The whole plant or dried seeds (sometimes the whole pods) are widely used as flavouring agents in breads, cheeses (such as sapsago and schabziger), stews and in other recipes in these regions. Blue fenugreek can also intensify other flavours. It forms part of the national herb–salt mixture of Georgia (called *khmeli suneli*), used as a seasoning for many meat and vegetable dishes. It is the most commonly used spice in rye bread made in the alpine regions, to which it imparts a unique flavour. For this purpose, the herb is harvested and subjected to a fermentation process as a result of which it acquires a very strong unique aroma (Katzer, 2008).

28.9.2 Boldo leaves

Boldo leaves *(Peumus boldus* Molina; Syn. *Boldu boldus*, *Boldea fragrans*; Monimiaceae) are known and used only in South America. Boldo leaves are strongly aromatic, similar to cinnamon or bay leaves and, when used, impart a very agreeable aroma to dishes. They are most useful in fish dishes, and can also enrich the taste and flavour of sauces, mushroom dishes, pickled vegetables and gravies. Boldo leaves contain about 2 % essential oil, which contributes to the flavour and aroma of this leaf. The main component of the essential oil is ascaridol, a monoterpene peroxide with a disagreeable smell and found only very rarely in plants. The flavour-contributing compounds are *p*-cymene, 1,8-cineol and linalool. The leaves also contain alkaloids such as boldine, isoboldine and *N*-methyllaurotetanine. They are used in herbal teas, and were part of traditional Mayan medicine. Research has verified the various indigenous uses of boldo leaves: in animal studies, they have been shown to stimulate digestion and to have anti-inflammatory, antioxidant and bile-producing and diuretic properties which are attributed largely to the plant chemical boldine. A US monograph reports that boldo can increase urine output by 50 %, which again validates the plant's traditional use as a diuretic (Taylor, 2004; Katzer, 1998a). An ethanol extract of the leaf administered to mice was shown to have a liver protective effect, preventing damage from chemical exposure. The extract also relaxes smooth muscle tissue and prolongs intestinal transit.

28.9.3 Chameleon plant

Chameleon plant (*Houttuynia cordata* Thunb.; Chinese lizard plant, fish wort, heart leaf; Saururaceae) is a native of the South East Asian region. There are two distinct types differing in flavour: the Chinese variety, native to China and Vietnam, has a strong coriander-like aroma and resembles Vietnamese coriander (*Eryngium foetidum*); the Japanese variety has a lemony and ginger-like aroma, and is distributed

in an area from Nepal to the Korea region, extending to Japan. In these regions, the leaves of this plant are used in a variety of local dishes, especially in salads and fish recipes. In Japan, it is also used as a fresh leaf garnish for soups, salads and sushi dishes. In China, its roots are used as a vegetable. The major flavour-contributing components are myrcene and 2-undecanone. It is a medical plant with a proven anti-inflammatory effect and has been found to be useful in combating severe acute respiratory syndrome (SARS) (Lau *et al.*, 2008; Katzer, 2012b).

28.9.4 Cicely

Cicely (*Myrrhis odorata* [L.] Scop.; sweet cicely, anise cicely, garden myrrh, sweet scented myrrh; Apiaceae) is one of the very few aromatic culinary herbs found in the very cold climates of the Scandinavian region, and is distributed over an area extending as far north as Iceland. Cicely has a sweet aromatic taste; its fruits are a good substitute for anise or fennel, while the leaves impart an aroma and flavour similar to that of chervil, but significantly stronger. The plant contains volatile oil, the components of which are *trans*-anethole, germacrene-*D*, β-caryophyllene, limonene, chavicol methyl ether, α-pinene, α-farnesene and myrcene. It also contains flavonoids such as luteolin and apigenin glucosides. Cicely leaves contain an essential oil with anethol as the predominant component. Cicely is used principally in fish recipes. The fresh leaves can be used in salads and chopped leaves are used in dishes along with rhubarb, gooseberries and other fruits. Cicely leaves are also used in fruit salads and drinks. The roots can be eaten as a vegetable, and can also be candied. The seeds can be used in cakes and candy, while the dried leaves are used in herbal tea (Katzer, 2000b; Hyde, 2011).

28.9.5 Cresses

There are many herbaceous plants known by the common name cress; however, the most common are garden cress *(Lepidium sativum* L., Brassicaceae), water cress (*Nasturtium officinale* L., Brassicaceae) and nasturtium (*Tropaeolum majus* L. Tropeolaceae). Garden cress and water cress belong to the mustard family (Brassicaceae), and nasturtium belongs to the Tropeolaceae family. All three types of cress have similar flavour qualities and are used in similar ways. Cresses have a spicy aroma and a refreshing peppery, pungent taste. The unique taste and flavour are due to the presence of isothiocynate derivatives, which are characteristic of plants in the mustard family. The main component of water and garden cresses is gluconasturin, which yields 2-phenylethyl-isothiocyanate. Nasturtium leaves contain glucotropeolin, which on hydrolysis gives benzyl-isothiocyanate. Cresses must be used fresh: they cannot be dried because drying removes the flavour almost completely. Cresses are used in local traditional medicine as a remedy for cough, cold, asthma, diabetes, anaemia, constipation and also as a body deodorizer. They are also a good source of iodine (Shipard, 2003). All three cresses are very commonly used in European and American cuisines. In America, they are used for spreads (especially based on cheese) and salads and often served along with bread and butter. Chopped cress leaves are used as toppings on warm dishes like vegetable soups, scrambled eggs and fish dishes. In Europe, cress leaves are used in flavouring

vinegar, sauces and soups, particularly in the form of a herb mixture known as mustard cress. This is a mixture of mustard and garden cress seedlings grown together for use (Katzer, 2012c). The BBC cook book lists 107 recipes using cresses. (Anon., 2011c).

28.9.6 Epazote

Epazote (*Chenopodium ambrosiodes* L.; wormseed, Mexican tea; Chenopodiaceae) is a Mexican aromatic herb with a strong aroma and is characteristically used in the Mayan cuisines of Mexico and Guatemala, being relatively unknown outside Central America. All aerial plant parts of this herb contain essential oil (0.7 % in the leaves, 2.5 % in the unripe fruits), which is composed of various monoterpenoids (α-pinene, α-phellandrene, thymol, myrcene, *p*-cymene, terpinene, campher, *trans*-isocarveol) and ascaridole, a monoterpenoid peroxide (Katzer, 2000c; Stuart, 2003). Its essential oil and its infusion are anthelmintic; the oil is active against intestinal nematodes, and aqueous extract is active against plant nematodes. Methanol extracts are anti-inflammatory and analgesic and ethanol extracts are used to reduce tumour growth, most likely due to an immunomodulatory effect. Tea made from its dried leaves is patented as a treatment for uterine fibroids (Diroff, 2008). Diroff (2008) has provided a detailed review on all aspects of epazote, including its medicinal properties and, biological actions. The herb is used fresh in soups, salads, meat dishes, sauces and, most commonly, in bean dishes (such as the famous Mexican refried beans). It is also used in Mexico as herbal tea. The www.food.com site lists 79 recipes using epazote (Anon., 2011d).

28.9.7 Finger root

Finger root (*Boesenbergia pandurata* (Roxb.) Schltr.; Chinese ginger; ginger family–Zingiberaceae) is a commonly used spice in Thai cuisine. It is less popular in China, Vietnam, Cambodia and Indonesia. It is a rhizomatous herb; the rhizomes develop like the fingers of a hand, have a strong flavour and are best used fresh. The rhizome contains 1–3 % essential oil, the important components of which are 1,8-cineol, camphor, *d*-borneol and methylcinnamate. Li Ching (2008) reported the presence of eucalyptol, camphor, α-citral, β-citral, β-linalool and methyl cinnamate. Geraniol was also reported in the hydrodistilled oil. The solvent extract of finger root was reported to have antibacterial properties. Cell line screening using HL-60 cancer cell line indicated that the extract also has an anticancer effect, with a compound called bossenbergin A, reported to have the strongest cytotoxic effect. Finger root is used mainly in fish curries, vegetable stews and sea food soups (Katzer, 2003a). Grated rhizomes of finger root are also used in recipes for pork fried rice, roast pork, dishes containing broccoli, cheese and chicken, and in dishes like almond crusted chicken tender salad, apricot ginger teriyaki, garlic chilli pepper wings, roasted soy Dijon lamb racks, among others.

28.9.8 Gale

Gale leaves (*Myrica gale* L.; sweet gale, candleberry, bog myrtle; Myricaceae) are used for flavouring dishes in Europe and America. The plant is found in northern

Europe, West Asia and the USA. Gale leaves are aromatic, and can be used either fresh or dried, the aroma intensifying after drying. The leaf contains essential oil, the main components of which are α-pinene, 1,8-cineol, myrcene and limonene. Gale leaves are used like bay and cinnamon leaves: the whole leaf should be steeped in the dish during preparation, and the leaves are removed before serving. Gale leaves impart a very agreeable and pleasant flavour to boiled vegetable stews and legume dishes, but are less commonly used in meat dishes. Historically, the most important use of gale leaf has been for flavouring beer. In the Scandinavian countries, gale leaves are also used to flavour schnapps (a type of distilled alcoholic beverage) (Katzer, 2003b ; Anon., 2011e)

28.9.9 Lemon myrtle

The original home of lemon myrtle (*Backhousia citriodora*; sweet verbena tree, lemon scented myrtle, lemon ironwood; myrtle family–Myrtaceae) is Queensland and the Australian mainland. The leaf of this small tree has an intense, refreshing, lemon-like aroma and warm pleasant taste.

The leaves contain about 4–5 % essential oil, and two chemotypes can be identified based on the predominant component in the essential oil. In one, the predominant component is citral (citral type) and in the other, the main component is citronellal (citronellal type). The former is more common and is the one used for culinary purposes. It is often described as the queen of lemon herbs, because its essential oil contains around 95–97 % citrals, in contrast to lemon peel, which contains only up to 10 % citrals. The oil of lemon myrtle is antifungal, antiseptic, antiviral, calmative and sedative. Its fragrance, together with its medicinal properties, makes it ideal for use in cosmetics, toiletries, incense burners and massage oils. Citral is used extensively as a food flavouring, but a leaf or two of lemon myrtle is a good substitute. Lemon myrtle is used in cooking and it blends well with macadamia nut oil. The leaves are used fresh, dry, as powder or as encapsulated extracted flavour. It is used widely in a variety of food products from bread to pasta, in fish preparations, as a herbal tea, in milk-based products, creams, cheesecakes; and also in native beer. Lemon myrtle is also a widely grown ornamental and avenue tree (Katzer,1999).

28.9.10 Mexican pepper leaf

Mexican pepper (*Piper auritum* Kunth; hoja santa, yerba santa, anisello, rootbeer plant; sacred pepper leaf; black pepper family–Piperaceae) is a member of the black pepper family with large fleshy leaves. It is known as *hoja santa* in Mexico, which means sacred leaf. Mexican pepper leaf and its young stems are widely used to flavour various dishes in Mexican cuisine; outside Mexico it is seldom used mainly due to a lack of availability. Both fresh and dried leaves are used; they have a pleasant aroma, somewhat akin to anise, nutmeg and black pepper. The young stems have a stronger flavour, which is also warm and mildly pungent. Safrole is the major component of the Mexican pepper leaf; minor components include α-pinene, camphene, sabinene, β-pinene, myrcene and α-phellandrene (Gupta *et al.*, 1985). Mexican pepper leaf is also used medicinally by the local people in Mexico. It is said to help relieve nervous anxiety, stress and restlessness. It can be eaten or taken as a tea;

however, it is very powerful and should be used on a limited basis. Long-term use may cause liver problems.

Mexican pepper leaf is widely used for tamales, being wrapped around fish, meat steaks and chicken before steaming, baking or grilling. The famous Mexican dish *pescado en hoja santa* is fish wrapped in Mexican pepper leaves baked and served with a spicy tomato sauce. Unlike other wrappers, the Mexican pepper leaf wrapper is eaten along with the fish or meat. In central Mexico, pepper leaves are used for flavouring chocolate drinks (the famous Aztec chocolate). It is an important ingredient in *mole verde*, the famous Mexican sauce. An American cheese brand, hoja santa cheese, is goat's milk cheese wrapped in Mexican pepper leaves. The leaves are often gently fried in oil before being used to flavour salads, sauces and various other dishes (Katzer, 2012a).

28.9.11 Tasmanian pepper

Mountain pepper or Tasmanian pepper (*Tasmania lanceolata* L.; Mountain pepper, Australian pepper tree; Winteraceae) is native to Australia (New South Wales), and plays an important role in Australian cuisine. For cooking, the dried berries and dried powdered leaf are used; both have a strong woody fragrance, with a black pepper-like taste with a cinnamon-like notes. Tasmanian pepper is initially intensely hot and pungent, so caution is required in its use. It then produces a strange sensation of numbness similar to the effect of Szechuan pepper. The component responsible for this pungency is polygodial, which is a dialdehyde with a bicyclic sesquiterpenoid skeleton. Tasmanian pepper goes well with other aromatic herbs and can be mixed with other spices like coriander, lemon myrtle, wattle seed and salt. In Australia, it is essential for preparing bush food dishes, like emu burgers, kangaroo steaks and so on. The berries are crushed and mixed with oil, and the resulting product is used for marinating meat. The berries are used in flavoured breads, pastas and patés, mustards and cheeses. In stews or sauces this spice imparts a vibrant red colour which can be very attractive. Tasmanian pepper is now being marketed internationally and is available in many western supermarkets. Dishes using Tasmanian pepper include: salmon with acacia seed and Tasmanian pepper berries, Australasian roast pork, Australasian roast chicken, balmian bugs and whiting, and Tasmanian pepper beef stew (Katzer, 2001a).

28.9.12 Water pepper

Water pepper (*Polygonum hydropiper* L.; marsh pepper; Polygonaceae) grows in wet environments in temperate to tropical Eurasia, North Africa and North America. Young shoots form the useful part. This plant has no smell but a very pungent taste and, like Tasmanian pepper, produces numbness in the tongue and mouth. The leaf contains essential oil, the main components of which are α-pinene, β-pinene, 1,4-cineol, fenchone, α-humulene, β-caryophyllene and *trans*-β-bergamotene. As with Tasmanian pepper, the bicyclic sesquiterpenoid, polygodial, has been found to be responsible for the pungent taste. The pungency imparted by the water pepper is such that it is difficult to substitute it with any other spice. However, the use of water pepper is restricted to Japanese cooking and to the cuisine of some areas of South

East Asia such as Vietnam. In Japanese cookery, water pepper leaf is widely used in soups, salads, and also for garnishing sushi. Since water pepper does not have a taste or flavour of its own except pungency, it is used in Japanese cuisine wherever pungency is required without masking the original flavour and taste of a dish and its ingredients, such as in fish recipes and sushi. Certain types of water pepper have very low pungency, and these are used in Japan as a vegetable. Water pepper seeds are also very pungent, and were used once as a substitute for black pepper, but are rarely used today (Katzer, 2000d).

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