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Handbook of herbs and spices

Volume 3

Edited by K. V. Peter

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Introduction

Herbs and spices play a pivotal role in the day-to-day life of mankind as important flavouring agents in foods, beverages and pharmaceuticals and also as ingredients in perfumes and cosmetics. The manufacturers of foods, beverages, cosmetics and pharmaceuticals are responding to the growing wave of consumer resistance and legislative limitations set for products containing chemical additives. Spices as sources of natural colours and flavours present welcome opportunities in the international market. The nutritional, antioxidant, antimicrobial and medicinal properties of spices also have widespread applications.

I.1 Production of quality spices

Production of quality clean spices without any pesticide/chemical residues is important in this era of free international trade resulting from globalisation. Organic spices which fetch 20 to 50% higher prices than spices from conventional farms are devoid of pesticides and chemical residues and are superior in quality. Adoption of good agricultural practices helps to reduce the above contaminants. Quality assurance systems such as HACCP is of great relevance in the production of quality spices. Decontamination techniques and proper packaging and storage techniques play a major role in maintaining quality of spices.

I.1.1 Rational uses of pesticides and controlling the pesticide/chemicals residues in herbs and spices

All over the world, people are becoming more and more conscious of health problems due to consumption of foods contaminated with pesticide residues. It is estimated that a large number of people suffer from pesticide poisoning and suffer every year due to the toxic effects of chemicals. Promotion of a farming technique adopting ecologically sound plant protection measures, organic recycling and bio-waste management would go a long way in bringing back the health of soil and reducing the pesticide residues of farm produce. The role played by various beneficial microorganisms including mycorrhizae, biocontrol agents and plant-growth-promoting rhizobacteria are enormous in enhancing crop growth and disease control without leaving any chemical residues on plants. The effective bioagents for the control of major diseases of spice crops are listed in Table I.1.

Table I.1 Effective bio agents for the control of major diseases in spice crops

Crops	Major diseases	Causal organisms	Bio control agents
Cardamom (small)	Azhukal	*Phytophthora meadii, P. nicotianae* var. *nicotianae*	*Trichoderma viride* *T. harzianum* *Laetisaria arvalis*
	Rhizome rot	*Rhizoctonia solani, Pythium vexans*	*Gliocladium virens* *Arbiscular Mycorhizal Fungi (AMF)*
	Seed rot	*Fusarium oxysporum*	*Trichoderma* sp.
	Seedling rot	*R. solani, P. vexans*	*Pseudomonas fluorescens*
	Root rot	*F. oxysporum*	*Bacillus subtilis*
Black pepper	Foot rot (quick wilt)	*Phytophthora capsici*	*AMF* *T. viride, T. harzianum, Gliocladium virens* *Paecilomyces lilacinus*
	Slow decline (slow wilt)	*Rodophilus similies, Meloidogyne incognita*	*G. virens, T. viride* *T. harzianum, AMF* *Verticillum, Chlamydosporium* sp. *Pasteuria penetrans*
Vanilla	Root rot	*Fusarium oxysporum, Sclerotium rolfsi*	*T. viride, T. harzianum*
	Stem rot, stem blight, beans rot, beans yellowing and rotting shoot tip rot	*P. meadii, F. oxysporum* *Sclerotium rolfsii* *F. oxysporum, Colletotrichum gloeosporioides*	*B. subtilis* *P. fluorescens* *T. viride* *T. harzianum* *P. fluorescens*
Ginger	Soft rot (rhizome rot)	*Pythium aphanidermatum, P. myriotylum,*	*T. viride* *T. harzianum*
	Ginger yellows	*Fusarium* sp.	*Trichoderma* sp.
Turmeric	Rhizome rot Storage rots	*Rhizoctonia solani, Sclerotium rolfsii*	*Trichoderma* sp.
Chillies, Paprikas	Damping off in seedlings	*Pythium* sp., *Phytophthora* sp.	*T. viride* *T. harzianum* *P. fluorescens*
	Anthracnose (fruit rot)	*Colletotrichum lindemuthianum*	*B. subtilis* *P. fluorescens* *B. subtilis* *Trichoderma* sp.
Thyme	Wilt disease	*F. oxysporum*	*T. viride*
	Leaf rot	*F. oxysporum*	*T. harzianum*
Rosemary	Thread blight	*Rhizoctonia solani*	*T. harzianum*
Sage	Wilt	*R. solani*	*T. harzianum*
Mint	Wilt	*F. oxysporum*	*T. harzianum*
Horse-radish	Leaf blight	*Colletotrichum* sp.	*T. harzianum*
	Root rot, wilt	*Verticillium* sp.	*T. harzianum*
Burmese-coriander	Wilt	*Fusarium* sp.	*T. harzianum*
Marjoram	Leaf blight	*Colletotrichum* sp.	*T. harzianum*
	Leaf spot	*Phoma* sp.	*T. harzianum*
Oregano	Leaf spot	*Curvularia lunata*	*T. harzianum*

Guidelines for production of organic spices are developed for various producing countries. The Spices Board of India (2001) published the guidelines for production of organic spices in India. The nutrient composition of selected organic cakes and recommended quantity of organic manure for various spice crops are presented in Table I.2.

I.1.2 Radiation processing to decontaminate spices

Radiation processing offers good scope for increasing shelf life, enhancing quality and microbial safety without changing the natural flavour attributes of spices. This technique is widely practised in North America and Europe to decontaminate imported

Table I.2 (a) Nutrient composition of selected organic cakes and (b) recommended quantity of organic manure for various spice crops

(a)

Oil cakes	Nutrient contents (%)		
	Nitrogen	Phosphorus	Potash
Edible cakes			
Coconut cake	3.0	1.9	1.8
Niger cake	4.7	1.8	1.2
Sesamum cake	6.2	2.0	1.2
Sunflower cake	7.9	2.2	1.9
Groundnut cake	7.3	1.5	1.3
Non-edible cake			
Cotton seed cake (with shells)	6.4	2.9	2.2
Mahua cake	2.51	0.80	1.85
Neem cake	5.22	1.08	1.48

(b)

Spice crops	Organic manure	Quantity
Black pepper	Farmyard manure	4–10 kg/plant
Small cardamom	Neem cake/FYM/Vermicompost/Poultry manure	4–5 kg/plant
Large cardamom	Cattle manures/organic cakes	2 kg/plant
Vanilla	Farmyard manure/Vermicompost	4–5 kg/plant
Chilli	Farmyard manure/	4–5 t/ha
	Sheep manure/	3–5 q/ha
	Neem cake	3–4 q/ha
Ginger	Farmyard manure/	5–6 t/ha
	Neem cake	2 t/ha
Turmeric	Farmyard manure/	5–6 t/ha
	Neem cake	2 t/ha
Fennel	Farmyard manure	10–12 t/ha
Coriander	Farmyard manure	4 t/ha
Cumin	Farmyard manure	4–5 t/ha
Fenugreek	Farmyard manure	4–5 t/ha
Celery	Farmyard manure	10–12 t/ha
Clove	Farmyard manure	15–40 kg/plant
Nutmeg	Farmyard manure	15–40 kg/plant

Source: Spices Board of India (2001).

spices. The various producing countries also started installing facilities for radiation processing of spices. Radiation sterilisation along with good agricultural and manufacturing practices help to produce clean, high quality spices free from pesticide and chemical residues. Being a cold process, it does not affect the delicate aroma and flavour compounds in spices. The risk of post-treatment contamination can be eliminated by subjecting the pre-packed spices to irradiation. Table I.3 gives the list of countries that have approved irradiation processing of food products and spices items permitted for irradiation under the Indian Prevention of Food Adulteration Act (PFA) rules.

Low doses of irradiation (< 1 K.Gy) help to inhibit sprouting in onion, garlic, ginger, etc. A medium dose application (1–10 K.Gy) eliminates spoilage microbes and food pathogens and high dose application (>10 K.Gy) sterilises food for special requirements and for shelf-stable foods without refrigeration.

I.1.3 Packaging in spices for maintenance of quality

Spice products are hygroscopic in nature and being highly sensitive to moisture,

Table I.3 (a) Countries which have approved radiation processing of food products and (b) spice items permitted for irradiation under Indian Prevention of Food Adulteration Act (PFA) rules

(a)

S. no.	Country	S. no.	Country	S. no.	Country
1	Argentina	19	Ghana	37	Philippines
2	Australia	20	Greece	38	Poland
3	Austria	21	Hungary	39	Portugal
4	Bangladesh	22	India	40	Russian Federation
5	Belgium	23	Indonesia	41	South Africa
6	Brazil	24	Iran	42	Spain
7	Canada	25	Ireland	43	Sweden
8	Chile	26	Israel	44	Syria
9	China	27	Italy	45	Thailand
10	Costa Rica	28	Japan	46	Turkey
11	Croatia	29	Republic of Korea	47	Ukraine
12	Cuba	30	Libya	48	UK
13	Czech Republic	31	Luxemburg	49	Uruguay
14	Denmark	32	Mexico	50	USA
15	Egypt	33	Netherlands	51	Vietnam
16	Finland	34	New Zealand	52	Yugoslavia
17	France	35	Norway		
18	Germany	36	Pakistan		

(b)

Name of spice	Dose of irradiation		Purpose
	Minimum	Maximum	
Onion	0.03	0.09	Sprout inhibition
Shallots (small onion)	0.03	0.15	Sprout inhibition
Garlic	0.03	0.15	Sprout inhibition
Ginger	0.03	0.15	Sprout inhibition
Spices	6.0	14.0	Microbial decontamination

Source: Sharma *et al*. (2003).

absorption of moisture may result in caking, discolouration, hydrolytic rancidity, mould growth and insect infestation. As spices contain volatile aromatic principles, loss of these principles and the absorption of foreign odours as a result of inefficient packaging may pose serious problems. In addition, heat and light accelerate deterioration of aroma and flavour components.

Spices containing natural colouring pigments need protection from light (capsicum, cardamom, turmeric and saffron). Spice powders like onion and garlic contain highly volatile sulphur compounds and need rigorous protection from loss/absorption of flavour. The essential oil components naturally present in most of the spices are subject to oxidation by atmospheric oxygen, particularly at high storage temperature resulting in the development of off-flavours. Packing of spice oils and oleoresins is done in epoxy lined steel drums and high-density polythene containers. For certain oils and oleoresins, aluminium and stainless steel containers are used. Polyethylene terephthalate (PET) bottles, which possess very good odour barrier properties and food-grade high-molecular-weight high-density polyethylene (HMHDPE) containers are also used for storing essential oils and oleoresins. Most of the whole spices are protected by pericarp and the natural antioxidants present therein, and need less rigorous protection than ground spices. The packaging materials suitable for different spice products are listed in Table I.4.

Table I.4 Packaging in spices

Spice	Product	Type of packaging	Packing material
Black pepper	Whole pepper	Bulk	Gunny bags (burlap bags) polyethylene-lined double burlap bags.
	Whole pepper	Retail	HDPE pouches 200 gauge
	Ground pepper	Retail	Laminated heat stable aluminium foil (polyethylene coated) Moisture-proof cellulose film Double-lined polyethylene bags
Cardamom	Green cardamom	Bulk	Wooden boxes or tins lined with heavy gauge black polyethylene, metal foil or waterproof paper.
	Cardamom seed	Retail	Air-tight tin. Wooden chests lined with aluminium foil laminate
	Cardamom powder	Retail	Lacquered cans, PVDC and HDPE pouches
Ginger	Dry ginger	Bulk	Single/double gunny bags
Turmeric	Dry turmeric	Bulk	Double gunny bags
	Turmeric powder	Retail	Aluminium foil laminate
	Turmeric powder	Bulk	Fibreboard drums, multiwall bags and tin containers
Chilli	Dry chilli	Bulk	Wooden crate dunnage with a layer of matting
	Chilli powder	Retail	Plastic laminate and aluminium combination pouches with nitrogen gas. 3000 gauge low-density polyethylene pouches

Source: Pruthi (1993).

I.2 Herbs and spices as sources of natural colours and flavours

The food sector is now experiencing a trend back towards natural colourants due to changes in legislation and consumer preference as synthetic food colourants pose health hazards like cancer, asthma, allergy, hyperacidity and thyroidism. But low tinctorial power, poor stability (to changes in pH, oxygen, heat and light), low solubility, off-flavour and high cost limit the use of natural colours. These problems can be overcome by improving the traditional extraction methods using enzymes, microorganisms, super-critical CO_2, membrane processing and encapsulation techniques.

Before synthetic colours came into existence, spices like chilli, saffron, turmeric, etc., were used in Indian cuisines to add colour. The Central Food Technological Research Institute of India (CFTRI) has developed technology for the manufacture of certain natural food colours such as kokum (red) and chillies (red). Kokum contains 2–3% anthocyanin and is regarded as a natural colour source for acidic foods. Garcinol is the fat soluble yellow pigment isolated from rind of kokum fruit. Garcinol is added at 0.3% level to impart an acceptable yellow colour to butter. Colour components present in spices and natural shades available with spices are presented in Table I.5.

I.2.1 Sources of natural colours in spices

Paprika

The colour in paprika is due to carotenoids, namely capsanthin and capsorubin, comprising 60% of total carotenoids. Other pigments are cryptoxanthin, xeaxanthin, violaxanthin, neoxanthin and lutein. The outer pericarp of paprika is the main source of capsanthin and capsorubin. Indian paprika oleoresin is orange in colour which is less preferred in the international market. Oleoresin contains up to 50% capsorubin. Paprika oleoresin is insoluble in water whilst being readily soluble in vegetable oil and is made dispersible in water by the addition of polysorbate.

Applications are in sausages, cheese sauces, gravies, salad dressings, baked goods, snacks, icings, cereals and meat products.

Table I.5 Colour components in spices

Colour component	Tint	Spice
Carotenoid		
β-carotene	Reddish orange	Red pepper, mustard, paprika, saffron
Cryptoxantein	Red	Paprika, red pepper
Lutin	Dark red	Paprika, parsley
Zeaxanthin	Yellow	Paprika
Capsanthin	Dark red	Paprika, red pepper
Capsorubin	Purple red	Paprika, red pepper
Crocetin	Dark red	Saffron
Neoxanthin	Orange yellow	Parsley
Violaxanthin	Orange	Parsley, Sweet pepper
Crocin	Yellowish orange	Saffron
Flavonoids	Yellow	Ginger
Curcumin	Orange yellow	Turmeric
Chlorophylls	Green	Herbs

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

The ingredients of paprika colour are paprika oleoresin and refined vegetable oil. Stability is as follows:

Heat	good
pH (colour range)	pale pinkish
Light	good
Concentration	40000 IU

Turmeric

Curcumin is the golden-yellow pigment present in turmeric, regarded as the pure colouring principle with very little of flavour components. It is produced by crystallisation from the oleoresin and has a purity level of 95%. Pure curcumin is insoluble in water and hence is dissolved in food grade solvent and permitted emulsifier (Polysorbate 80). Curcumin gives a lemon-yellow colour in acidic pH. It is used at levels of 5–20 ppm. Curcumin is available in two basic forms, oleoresin and curcumin powder, both are used as food colourants.

The ingredients of turmeric colour (oil soluble) are curcumin and turmeric oleoresin Stability is as follows:

Heat	very good
pH (colour range)	greenish yellow to reddish yellow
Light	poor
Application	butter, margarine, cream desserts, fruit wine, bread, biscuit and cakes.

It is blended with other natural colours such as annatto and beetroot red for use in confectionary, ice cream, dairy products such as yoghurts.

Saffron

Saffron gives a wonderful golden colour to food but due to its powerful and distinctive flavour, it is prized in soups, stews, bread and rice dishes in many global cuisines. Saffron is perceived as luxurious and expensive and hence its use is restricted in foods. The intensive colour of saffron is caused by carotenoids, especially crocetine esters with gentobiose. Other carotenoids present are alpha and β carotene, lycopene and zeaxanthin.

I.2.2 Spices as sources of natural flavours

The increasing demand in developed countries for natural flavour offers tremendous potential for spice crops as sources of natural flavours. The main flavour compounds present in herbs and spices are presented in Table I.6. The recovery of essential oil and oleoresin from various spices and the major aromatic principles present in spices are illustrated in Table I.7. Extraction of oils and oleoresins is accomplished using a range of methods, including steam distillation, hydrocarbon extraction, chlorinated solvent extraction, enzymatic treatment and fermentation, and super-critical carbon dioxide extraction.

Carbon dioxide extraction from solid botanicals is now adopted on a commercial scale. The resulting essential oils have no solvent residue, fewer terpenes and enhanced black notes. Enzymatic treatment and fermentation of raw botanicals also result in greater yields and quality of essential oil. More recently, the use of genetic engineering

Table I.6 Important flavour compounds in spices

Spice	Important flavour compounds
Allspice	Eugenol, β-caryophyllene
Anise	(E)-anethole, methyl chavicol
Black pepper	Piperine, S-3 Carene, β-caryophyllene
Caraway	d-carvone, crone derivatives
Cardamom	α-terpinyl acetate, 1-80-cineole, linalool
Cinnamon, cassia	Cinnamaldehyde, eugenol
Chilli	Capsaicin, dihydro capsacin
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	Sabinene, α-pinene, myristicin
Parsley	Apiol
Saffron	Safranol
Turmeric	Turmerone, Zingeberene, 1,8-cineole
Vanilla	Vanillin, p-OH-benzyl-methyl ether
Basil, sweet	Methylchavicol, 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, Dalmation	Thujone, 1,8-cineole, camphor
Sage, Spanish	e- and t-sabinylacetate, 1,8-cineole, camphor
Savory	Carvacrol
Tarragon	Methyl chavicol, anethole
Thyme	Thymol, carvacrol
Peppermint	1-menthol, menthone, menthfuran
Spearmint	1-carvone, carvone derivatives

and recombinant DNA technology have resulted in *in vitro* production of natural esters, ketones and other flavouring materials. Cloning and single cell culture techniques are also of benefit to the flavourist.

I.2.3 Herbs and spices as medicinal plants

The medicinal properties of spices have been known to mankind from time immemorial. Spices were used extensively in the traditional systems of medicines such as Ayurveda, Sidha and Unani. In the recent past, there has been increasing interest in the biological effects of spices as they are safe and cause no side effects to humans. Extensive studies are going on in developed countries for the separation of medicinal components from spices and evaluation of their biological properties. A classic example for such study is the Piperine alkaloid separated from black pepper and marketed as Bioperine (98% pure piperine). This alkaloid could increase bioavailability of certain drugs and nutrients like beta carotene. The medicinal properties of spices are summarised in Table I.8.

Table I.7 Recovery of essential oil and oleoresin from spices and the major aromatic principle

Spice	Essential oil (%)	Aromatic principle	Oleoresin (%)
Black pepper	1–4.0	Terpin hydrate	10–13
Cardamom (small)	6–10	α–terpinyl acetate 1,8–cineole	10–12
Cardamom (large)	1–3	1,8–cineole	–
Ginger	1–2.5	Zingiberine	5–10
Turmeric	2–6	Turmerone	8–10
Nutmeg	7–16	Myristicine Elemicin	10–12
Clove	16–18	Eugenol	20–30
Cinnamon	1–3	Cinnamaldehyde (bark oil) Eugenol (leaf oil) Camphor (root bark oil)	10–12
Allspice	1–3 (leaf oil) 3–4.5 (berry oil)	Eugenol	–

Table I.8 Medicinal properties of spices

Spice	Medicinal property
Black pepper	Carminative, antipyretic, diuretic, anthelminthic, antiflammatory and antiepileptic
Cardamom	Antidepressive, carminative, appetizer, diuretic
Ginger	Carminative, anti nauseant, diuretic, antiflatulence, antihistaminic, aphrodisiac and cholesterol lowering
Turmeric	Carminative, antibiotic, antiflatulence, antiseptic and anti-inflammatory
Garlic	Antimicrobial, diuretic, diaphoretic, antiflatulence, cholesterol lowering and anti-inflammatory
Clove	Antiflatualnce, analgesic, stimulant, carminative and antinauseant
Nutmeg	Stimulant, carminative, astringent, aphrodisiac, anti-inflammatory
Cinnamon	Stiumlant, Carminative, astringent, aphrodisiac, anti-inflammatory
Chilli	Carminative and antirheumatic
Saffron	Stimulant, stomachic and anticarcinogenic
Allspice	Stimulant, digestive and carminative
Basil, sweet	Stomachic, anthelminitic, diaphoretic, expectorant, antipyretic carminative, stimulant, diuretic, demulcent
Bayleaves (laurel)	Stimulant, narcotic
Caraway	Stomachic, carminative, anthelminitic, lactagogue
Celery	Stimulant, tonic, diuretic, carminative, emmenagogue, anti-inflammatory
Chive	Stimulant, diuretic, expectorant, aphrodisiac, emmenegogue, anti-inflammatory
Coirander	Carminative, diuretic, tonic, stimulant, stomachic, refrigerent, aphrodisiac, analgesic, anti-inflammatory
Cumin	Stimulant, carminative, stomachic, astringent and antiseptic
Dill	Carminative, stomachic, antipyretic
Fennel	Stimulant, carminative, stomachic, emmenagogue
Fenugreek	Carminative, tonic, aphrodisiac
Leek	Stimulant, expectorant
Marjoram	Carminative, expectorant, tonic, astringent
Mint (peppermint)	Stimulant, stomachic, carminative, antiseptic
Mint (spearmint)	Stimulant, carminative and antispasmodic
Oregano	Stimulant, carminative, stomachic, diuretic, diaphoretic and emmenagogue
Parsley	Stimulant, diuretic, carminative, emmenagogue, antipyretic, anti-inflammatory
Rosemary	Mild irritant, carminative, stimulant, diaphoretic
Sage	Mild tonic, astringent, carminative
Tarrgon	Aperient, stomachic, stimulant, febrifuge
Thyme	Antispasmodic, carminative, emmenagogue, anthelmintic, spasmodic, laxative, stomachic, tonic, vermifuge

This volume is the third in the series *Handbook of herbs and spices* and has two parts. The first part deals with general aspects referred to the industry such as quality spice production, quality assurance systems, decontamination techniques, packaging, spices as sources of natural colours and flavours, effect of Agreement on Agriculture on spice production and export, etc. The second part deals with detailed information on individual spices. It is hoped that this book will form a good reference source for those who are involved in the study, cultivation, trade and use of spices and herbs.

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Contents

Part I

Improving the safety of herbs and spices

1

Detecting and controlling mycotoxin contamination of herbs and spices

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1.1 Introduction

Spices have been used in foods, mainly as flavouring and colouring agents as well as for their functional properties such as being antioxidant and antimicrobial. While some spices inhibit growth of microorganisms and some retard their growth others reduce mycotoxin production (Bullerman *et al.,* 1977; Akgül and Kıvanç, 1998; Yin and Cheng, 1998; Beuchat, 2001; Juglal *et al.,* 2002). They can be invaded by bacteria, yeast and moulds themselves immediately after harvesting till final consumption (Schwab *et al.,*1982; Garrido *et al.,*1992; McKee, 1995; Erdogrul, 2000; Garcia *et al.,* 2001). Mycotoxins are toxic metabolites produced by different genera of moulds under favourable conditions. Moulds can contaminate agricultural commodities during harvesting, drying, processing and storage and some of them are capable of producing secondary metabolites, causing acute or chronic diseases in human and animals. Mycotoxins can also be found in animals and animal products through the ingestion of mouldy feed.

There are approximately two secondary metabolites per fungal species which means that there are potentially 20,000 to 300,000 unique mycotoxins (CAST, 2003). Among these mycotoxins, the ones that have world-wide importance and are currently considered are aflatoxins, deoxynivalenol, fumonisins, ochratoxin A, T-2 toxin and zearalenone (WHO/FAO, 2001). This evaluation has been based on significant impact on human health and animal productivity (WHO/FAO, 2001), however, the emphasis can vary from country to country or among regions, altering the ranking. For example, fumonisin presence in corn is not considered to be an important hazard in Australia, because corn is not a frequent item in the diet (Pitt and Hocking, 2004). On the other hand, fumonisin in corn is considered to be among the primary important mycotoxins in the USA (Miller, 2002). Similarly, aflatoxin does not pose an important threat to consumers in Europe, since it does not appear in high concentrations due to inconvenient temperatures, and moreover the limits are extremely low and inspections are strict on imported products. It keeps its importance in countries where the temperature is convenient, such as in the USA (Bhatnagar *et al.,* 2004), Africa (Shephard, 2004; Njapau and Park, 2005), and Asia (Park *et al*., 2005).

Ochratoxin A (OTA) is a wide spread toxin found in crops in Europe and appears frequently in bread and flour (Jorgensen and Petersen, 2002). Actually, blood and milk analyses carried out in Europe show that consumers have been exposed to OTA (Skaug *et al.,* 2005). The kidney disease called Balkan Endemic Nefropati (BEN) particularly seen in the Balkans, has been proved to be a result of crop consumption containing ochratoxin A and citrinin (Vrabcheva *et al.,* 2000; Pfohl-Leszkowicz *et al.,* 2002). In the light of these evaluations, although it is difficult to arrange a general list according to their importance, covering every country and every product, researchers have agreed that aflatoxins, ochratoxin A, fumonisins, trichotecenes and zearalenone (ZEN) are important mycotoxins (Anklam and Stroka, 2002; Park, 2002a).

The most important element in defining the type of mycotoxin humans are exposed to, is the dietary habits of communities. For example, herbs and spices constitute an important part of the daily menu for some societies and are consumed in large quantities. It is customary to add red pepper to almost every dish in the southern and eastern regions of Turkey. Similarly, spices are more frequently used in countries in the Middle East, South Asia, some parts of Europe and South America. Hence contaminated spices and herbs will constitute a health hazard to consumers (Geeta and Kulkarni, 1987; Freire *et al.,* 2000; Thirumala-Devi *et al.,* 2000; Elshafie *et al.,* 2002). In essence aflatoxins are known hepatocarcinogens (Henry *et al.,* 2002); OTA is nephrotoxic and teratogenic (Walker, 2002); fumonisin has been associated with several fatal diseases in animals, including equine leukoencephalomalacia and esophegal cancer in humans (Yoshizava *et al.,* 1994; Bullerman *et al.,* 2002); trichothecenes inhibit proteins, causing dermal necrosis and gastroenteritis (Bullerman, 2000; CAST, 2003) and zearalenone has estrogenic activity (Ryu *et al.,* 2002); therefore their presence in foods and feeds should be eliminated.

1.2 Naturally occurring mycotoxins in herbs and spices

Spices can be obtained from fresh fruits after drying and grinding or they may be different parts of plants like the seed, the bark or the roots. Herbs are usually the leafy parts of the plant (Farkas, 2000) and are more commonly used for medicinal or therapeutic purposes. During harvesting and sun drying, spices and herbs can be contaminated with moulds. Many strains of moulds, while growing under favourable conditions, produce metabolites that are toxic to humans and animals. These toxic secondary metabolites are called mycotoxins.

The growth of mould and the production of mycotoxins are influenced by intrinsic and extrinsic factors as well as stress factors and physical damage of kernels. Intrinsic factors are related to the properties of the products such as moisture content or water activity (aw), pH, redox potential (Eh), nutrient content (substrate), inhibitors and osmotic pressure. The extrinsic factors are related to environmental conditions such as temperature, relative humidity (ERH) and gases in the environment. Intrinsic and extrinsic factors promoting mycotoxin production can differ from mould to mould. For example *P. verrucosum* is an important ochratoxin-producing mould in temperate climates like Central and Northern Europe. The temperature range for its growth is 0–31 °C. The same range for ochratoxin production is 4–31 °C (FAO, 2001). It has already been shown in Denmark that ochratoxin A production in cereal depends strongly on climatic conditions (Jorgensen and Petersen, 2002). *A. carbonarius* is another OTA-producing mould which grows at high temperatures and produces

ochratoxin in tropical climates (Heenan *et al.,* 1998; Pitt, 2002). The maximum temperature for the growth of *A. carbonarius* is approximately 40 °C, whereas the optimum temperature 32–35 °C (WHO/FAO, 2001). Deoxynivalenol is produced under conditions of low oxygen tension. In growing crops, DON is not found (Miller *et al.,* 1983). In contrast, zearalenone requires oxygen saturation for optimal production, a condition seen after field crop senescence (Miller, 2002).

The optimum temperature for aflatoxin production is 25–30 °C and the maximum is 48 °C. The higher temperatures and drought conditions also may favour *A. flavus* over other fungi because of its ability to grow on substrates with low water activity (CAST; 2003). These conditions should be present simultaneously; the presence of only one is not sufficient (Payne, 1998). Researchers found that peanuts grown with adequate moisture did not contain aflatoxin. Similarly, peanuts grown under prolonged drought with temperatures less than 25 °C or greater than 32 °C were free of aflatoxin. Colonisation by *A. flavus* and aflatoxin contamination maximised at 30.5 °C (CAST, 2003). In addition to the production of aflatoxin before harvesting, the adverse conditions during drying, transporting and storing cause accumulation of higher amounts of aflatoxin. Aflatoxin synthesis starts after 24 hours depending on the conditions and reaches its maximum level between 36–60 hours (Cary *et al.,* 2000).

Mycotoxins found in spices and herbs and the analysis method used are presented in Table 1.1. As seen from the table, among spices and herbs the most frequently studied spice is red pepper and the most frequently encountered mycotoxins are aflatoxin and ochratoxin. Several mycotoxins were detected in spices and herbs such as aflatoxin, fumonisin, ochratoxin A, mycophenolic acid, penitrem A, zearalenone and trichothecenes.

1.2.1 Red pepper

Mycotoxins and their maximum levels detected in red pepper were 969 μg/kg AFB_1 (Reddy *et al*., 2001), 50.4 μg/kg OTA, 15.4 μg/kg ZEN, and 81 μg/kg trichothecenes (Patel *et al.,* 1996). Aflatoxin can be produced before and after harvest in red pepper. Taydaş and Aşkın (1995) determined AFB_1 with maximum concentration 1.45 μg/kg in three of 33 red pepper pod samples, collected from fields before harvest. Reddy *et al.,* (2001) studied 124 samples of three different qualities of chili pods and found that aflatoxin contamination could be correlated with sample grades such as 50% in grade 1, 66% in grade 2, 93% in grade 3. The highest concentration of 969 μg/kg AFB_1 was found in one sample representing grade 3 (low quality).

Heperkan and Ermiş (2004) studied 36 ground (flakes) red pepper samples obtained from different producers from four regions in Turkey. Aflatoxin B_1 was detected in five samples (14%) at levels between 10.5–31.2 μg/kg. The amount of toxin was higher but the incidence was lower than that noted by other researchers (Taydaş and Aşkın, 1995) who studied similar areas in Turkey. AFB_2 (El-Dessouki, 1992) and AFG_1 (El-Dessouki, 1992; Dokuzlu, 2001) were also detected in addition to AFB_1, in red pepper.

The amount of aflatoxin in red pepper listed in Table 1.1 was higher than the limits of EC standards (2 μg/kg) except for one study. Low levels of aflatoxin B_1 (0.8 μg/kg) were found in one of two red pepper samples (Taguchi *et al.,* 1995). In addition to aflatoxins and ochratoxin A, other mycotoxins such as fumonisin, zearalenone and trichothecenes (Patel *et al.,* 1996) were also determined in red pepper.

Table 1.1 Mycotoxins in spices and herbs

Spice	Property/country	Methods	Incidence	Mycotoxins (µg/kg) range or amount of toxin/ type of toxin/incidence	References
Red pepper-pod (*Capsicum annuum*)					
	Turkey	TLC + fluorescence spectrofotometer	3/33	1.45 µg/kg Aflatoxin B_1 (max)	Taydaş and Aşkın, 1995
	High quality (grade 1) India	ELISA	21/42	<10 Aflatoxin B_1 16/42 11–30 3/42 >31 2/42	Reddy *et al.*, 2001
	(grade 2) India	ELISA	25/38	<10 Aflatoxin B_1 10/38 11–31 6/38 >31 9/38	Reddy *et al.*, 2001
	Low quality) (grade 3) India	ELISA	41/44	<10 Aflatoxin B_1 21/44 11–32 4/44 >31 16/44	Reddy *et al.,* 2001
Red pepper-ground (*Capsicum annuum*)					
Chillies/cayenne	Germany	TLC	11/22	< 5 Aflatoxin B_1 7/22 8.4–24 Aflatoxin B_1 4/22	Majerus *et al.,* 1985
Paprika[(a)]	Turkey	TLC + fluorescence spectrofotometer	30/30	1.2–15.9 Aflatoxin B_1	Taydaş and Aşkın, 1995
Red pepper	Turkey	HPLC	5/36	10.5–31.2 Aflatoxin B_1	Heperkan and Ermiş, 2004
Red pepper-powder (*Capsicum annuum*)					
Chilli	Imported samples/USA		9/12	10 AFs (total) avarage 30 Aflatoxin max	Wood, 1989
Paprika[(a)]	Sweet and hot/ Germany	TLC	7/15	2.8–14.5 Aflatoxin B_1 10.1–1.7 Aflatoxin B_2 2.9–15.3 AFs (total)	El-Dessouki, 1992
Chillies	Germany	TLC	13/24	9.6–211 Aflatoxin B_1 30.3–7.1 Aflatoxin B_2 0.2–18.3 Aflatoxin G_1 10.2–218.4 AFs (total)	El-Dessouki, 1992

Table 1.1 Continued

Spice	Property /country	Methods	Incidence	Mycotoxins (μg/kg) range or amount of toxin/ type of toxin/incidence	References
Paprika[(a)]	Turkey	TLC + fluorescence spectrofotometer	28/31 % 90.3	Max 28.5 Aflatoxin B_1	Taydaş and Aşkın, 1995
Chilli	121 samples of ethnic foods UK			1.1–5.4 Aflatoxin B_1 1.6–50.4 Ochratoxin A 4.5–15.4 Zearalenone 8–81 Trichothecenes	Patel *et al*., 1996
Red pepper	Imported foods/ Japan	TLC + fluorescence TLC scanner	1/2	0.8 Aflatoxin B_1	Taguchi *et al.,* 1995
Red pepper	Samples collected from Egypt, analysed in USA	LC	1/2	10 Aflatoxin B_1	Selim *et al.,* 1996
Red pepper	Turkey	TLC	14/30	5–25 μg/kg 13/30 Aflatoxin B_1 5–15 μg/kg 1/30 Aflatoxin B_1, G_1	Dokuzlu, 2001
Paprika[(a)]	Prepackaged samples/Portugal	HPLC + IAC	8/12	1–20 Aflatoxin B_1	Martins *et al.,* 2001
Cayenne pepper	Prepackaged samples/Portugal	HPLC + IAC	5/5	2–32 Aflatoxin B_1	Martins *et al.,* 2001
Chilli	Prepackaged samples/Portugal	HPLC + IAC	3/8	1–5 Aflatoxin B_1	Martins *et al.,* 2001
Chilli	Capsicum annuum/ Indıa	ELISA	17/43	<10 Aflatoxin B_1 12/43 11–33 Aflatoxin B_1 1/43 >30 Aflatoxin B_1 and Ochratoxin A 4/43	Reddy *et al.,* 2001
Chilli		HPLC + IAC	4/6 2/6	5.60–69.28 Aflatoxin 2.34–4.91 Ochratoxin A	Abdulkadar *et al.,* 2004
Bay leaf	The Netherlands	TLC		Aflatoxin B_1 5.1 max.	Beljaars, 1975

Table 1.1 Continued

Spice	Property /country	Methods	Incidence	Mycotoxins (μg/kg) range or amount of toxin/ type of toxin/incidence	References
Black pepper	Samples collected from Egypt, analysed in USA	LC	1/2	33	Selim *et al.,* 1996
Black pepper	Brazil	TLC		Chaetocin, penitrem A, xanthocillin	Freire *et al.,* 2000
Black pepper	Seed	ELISA	14/26	15–69 Ochratoxin A	Thirumala-Devi *et al.,* 2001
Black pepper	Grain/imported from India/ Portugal	HPLC with fluorescence detection	3/4	0.2–4.2 Aflatoxin B_1 0.08–0.3 Aflatoxin B_2 0.08–21 Aflatoxin G_1 0.08 Aflatoxin G_2	Ferreira *et al*., 2004
Cinnamon	Samples collected from Egypt, analysed in USA	LC	2/2	10 and 42 Aflatoxin B_1	Selim *et al.,* 1996
Coriander	Germany	TLC	2/12	< 5.2 Aflatoxin B_1	Majerus *et al.*, 1985
Coriander	Seed	ELISA	20/50	10–51 Ochratoxin A	Thirumala-Devi *et al.,* 2001
Cumin	Prepackaged samples/Portugal	HPLC+IAC	3/7	1–5 Aflatoxin B_1	Martins *et al.,* 2001
Curry powder				0.4–61.2 Aflatoxin 1.8–23.9 Ochratoxin A 1.2–10.8 Zearalenone 15–230 Fumonisins 7–281 Trichothecenes	Patel *et al.,* 1996
Curry powder	Prepackaged samples/Portugal	HPLC+IAC	2/5	1–5 Aflatoxin B_1	Martins *et al.,* 2001
Ginger				4.2–13.5 Aflatoxin 2.1–7.5 Ochratoxin A trace levels of trichothecenes	Patel *et al.,* 1996
Ginger	Imported samples/ USA		1/3	2 AFs (total) max	Wood, 1989
Ginger	Powder/India	ELISA	2/25	23/80 Ochratoxin A	Thirumala-Devi *et al.*, 2001

Table 1.1 Continued

Spice	Property /country	Methods	Incidence	Mycotoxins (μg/kg) range or amount of toxin/ type of toxin/incidence	References
Ginger	Mouldy ginger/Denmark	HPLC	17/20	Mycophenolic acid	Overy and Frisvad, 2005
Mustard seed	India		44/100	Aflatoxin B_1	Sahay and Prasad, 1990
Mustard seed	Field experiment			106*; 35** Aflatoxin B_1 first planting date 110*; 56** Aflatoxin B_1 second planting date 272*; 279** Aflatoxin B_1 third planting date	Bilgrami *et. al*., 1991
Nutmeg	The Netherlands	TLC	30/32	23.2 Aflatoxin B_1 max	Beljaars *et al.,* 1975
Nutmeg	Germany	TLC	11/28	< 5 Aflatoxin B_1 (8/28) 5.4–7.7Aflatoxin B_1 (3/28)	Majerus *et al.*, 1985
Nutmeg	Japan	TLC + fluorescence TLC scanner	2/3	0.4–0.6Aflatoxin B_1	Taguchi *et al.,* 1995
Nutmeg	Prepackaged samples/Portugal	HPLC + IAC	8/10	1–5 Aflatoxin B_1 3/10 6–20 Aflatoxin B_1 3/10 21–60 Aflatoxin B_1 2/10	Martins *et al.,* 2001
Saffron	Prepackaged samples/Portugal	HPLC + IAC	2/5	1–5 Aflatoxin B_1	Martins *et al.,* 2001
Turmeric	Powder/India	ELISA	9/25	11–102 Ochratoxin A	Thirumala-Devi *et al.,* 2001
White pepper	Japan	TLC + fluorescence TLC scanner	1/13	0.6 Aflatoxin B_1	Taguchi *et. al*., 1995
White pepper	Brazil	TLC		Tenuazonic acid	Freire *et al.,* 2000
White pepper	Prepackaged samples/Portugal	HPLC + IAC	3/7	1–5 Aflatoxin B_1	Martins *et. al*., 2001

Table 1.1 Continued

Spice	Property /country	Methods	Incidence	Mycotoxins (μg/kg) range or amount of toxin/ type of toxin/incidence	References
White pepper	Grain/imported from India/Portugal	HPLC with fluorescence detection	7/10 5/10 7/10 7/10	0.09–1.1 Aflatoxin B_1 0.036–2.1 Aflatoxin B_2 0.07–5.3 Aflatoxin G_1 0.07–0.45 Aflatoxin G_2	Ferreira *et al.,* 2004
White pepper	powdered/imported from India/Portugal	HPLC with fluorescence detection	4/8 2/8 4/8 3/8	0.07–5.1 Aflatoxin B_1 0.6–6.3 Aflatoxin B_2 0.09–7.2 Aflatoxin G_1 0.09–4.5 Aflatoxin G_2	Ferreira *et al.,* 2004
Mixed spices powder		HPLC + IAC	5/6 1/6	0.16–5.12 Aflatoxin 0.86 Ochratoxin A	Abdulkadar *et al.,* 2004

a: author's preferences.
*: first year.
**: second year.

1.2.2 Black pepper and white pepper

Black pepper was contaminated with AFs (Selim *et al.*, 1996; Freire *et al.*, 2000), OTA (Thirumala-Devi *et al.*, 2001), penitrem A, chaetocin and xanthocillin (Freire *et al.,* 2000). White pepper was contaminated with AFs (Martins *et al.,* 2001; Ferreira *et al.*, 2004) and tenuazonic acid (Freire *et al.*, 2000). However, other scientists did not detect any aflatoxins in black pepper (0/4 samples) (Taguchi *et al*., 1995) (Elshafie *et al.,* 2002). In white pepper, the incidence (1/13) and the amount of aflatoxin B_1 (0.6 μg/kg) were low (Taguchi *et al*., 1995).

Freire *et al.,* (2000) studied mycoflora and mycotoxins in Brazilian black and white pepper. Twenty metabolites were observed from black pepper, and seven from white pepper which were also detected in black pepper. Tenuazonic acid was identified in the acid fraction of white and black pepper. Chaetocin and penitrem A were identified from the neutral fraction and xanthocillin from the acid fraction of black pepper. The toxicities of the metabolites were also studied. Chaetocin was cytostatic, xanthocillin was not known, tenuazonic acid inhibited plant growth, and penitrem A was tremorgenic (Freire *et al.,* 2000).

Madhyastha and Bhat, (1984) studied the growth of *A. parasiticus* and production of aflatoxin on black and white pepper and found that black pepper supported fungal growth and aflatoxin production better than white pepper, the values being 62.5 μg/kg and 44 μg/kg respectively under laboratory conditions. In spite of these high aflatoxin values, researchers claim that both black and white pepper could be considered as poor substrates for fungal growth and aflatoxin production because they found that piperine and pepper oil inhibited *A. parasiticus* growth and aflatoxin production.

Ferreira *et al.,* (2004) studied 18 samples of white and four samples of black pepper imported from India. They used silica and C18 columns together which provided good clean up of pepper extracts for HPLC analysis, with sensitivity at the low $\mu g/kg^{-1}$ level. Only one white pepper sample was found to be heavily contaminated with aflatoxins (total aflatoxins > 20 μg/kg). Most of the analysed samples contained two or four aflatoxins, however, they were below the limit of 20 μg/kg fixed by the European Union. No aflatoxin was detected in one black pepper and seven white pepper samples.

Aziz and Youssef (1991) examined 130 spice samples used in meat products for aflatoxins and aflatoxigenic moulds in a study conducted in Egypt. Spice samples used in the investigation were collected from local meat-processing companies. Aflatoxin B_1 was detected in four samples of black pepper (35 μg/kg) and four of white pepper (22 μg/kg). The most commonly isolated moulds were *Aspergillus flavus* (24 isolates) and *A. parasiticus* (16 isolates). Aflatoxin contamination of processed meat was found to be correlated with the addition of spices to fresh meat ingredients.

1.2.3 Other spices and herbs

Cinnamon oils were found to suppress the growth of *A. parasiticus* (Juglal *et al.*, 2002) completely. On the other hand, cinnamon samples collected from Egypt and analysed in the USA were contaminated with aflatoxin B_1 with high levels (Selim *et al.,* 1996). Coriander was contaminated with two types of mycotoxins namely, AFB_1 (Majerus *et al.*, 1985) and OTA (Thirumala-Devi *et al.,* 2001). However no aflatoxin was found in the coriander sample in another research (Selim *et al.,* 1996).

Cumin was contaminated with AFB_1 at levels above the tolerance level set by the World Health Organization (Roy and Chourasia, 1990; Martins *et al.,* 2001). Curry

powder is rich in mycotoxins, contaminated with AFB_1 (Patel *et al.*, 1996; Martins *et al.*, 2001), OTA, ZEN, FUM, and trichothecenes (Patel *et al.*, 1996).

Ginger was contaminated with AFs (Patel *et al.*, 1996; Wood, 1989), OTA (Patel *et al.*, 1996; Thirumala-Devi *et al.*, 2001) and mycophenolic acid (Overy and Frisvad, 2005). Mycophenolic acid produced by *Penicillium brevicompactum* may cause secondary mycotoxicosis by affecting the immune system of humans, thus making them more susceptible to bacterial infections and foodborne diseases (Overy and Frisvad, 2005).

Mustard is a susceptible substrate for aflatoxin contamination (Sahay and Prasad, 1990; Bilgrami *et al.*, 1991). Bilgrami *et al.*, (1991) found mustard seeds of pre-harvested crops to be contaminated with various levels of aflatoxin. Delayed planting resulted in a high incidence of aflatoxin. The amount of aflatoxin detected in the samples of the third planting date was 272 and 279 μg/kg during the first and second years respectively. These values were significantly higher than the amounts detected in the samples of the first (106; 35 μg/kg) and second (110; 56 μg/kg) planting dates of the respective years. Differences between the two varieties with respect to aflatoxin contamination can be attributed to the variation in their maturity period as well as their ability to resist aflatoxin elaboration. However, aflatoxins were not detected in mustard (0/3 samples) (Taguchi *et al.*, 1995).

Nutmeg and saffron were also found contaminated with AFs (Beljaars *et al.*, 1975; Martins *et al.*, 2001). High levels of OTA (110 μg/kg) were detected in turmeric, which is one of the most widely used spices in Indian cooking (Thirumala-Devi *et al.*, 2001) Elshafie *et al.*, (2002) screened fifteen samples of spices (ginger, cumin, cinnamon, clove, black pepper, cardamom and coriander) that were heavily contaminated by *A. flavus*, for the presence of aflatoxins using HPLC. No aflatoxins were detected on the samples.

Abou-Arab *et al.*, (1999) collected medicinal plant samples such as peppermint, chamomile, anise, caraway and tilio, randomly from the Egyptian market and analysed for aflatoxins. *A. flavus* was predominant in most samples with the highest level in peppermint. Aflatoxin contamination was not detected in any of the samples. In another study it was found that spices such as coriander, cardamon, pippali, and emblic are contaminated with aflatoxin B_1 at levels above the tolerance level set by the World Health Organization (Roy and Chourasia, 1990).

Herbs and medicinal plants commonly used in Egyptian foods were collected from Egypt and analysed in the USA by reversed phase liquid chromatography with UV detection. Aflatoxin B_1 was found in Karkadia (24 μg/kg), Halfa bar (camel's hay) (64 μg/kg), rawind (48 μg/kg), khashab keena (cinchona bark) (49 μg/kg), misht ballot (26 μg/kg), kesher romman (pomegranate peel) (105 μg/kg), somowa (cleme) (26 μg/kg) and salamakka (senna pods) (48 μg/kg) (Selim *et al.*, 1996).

The results of a survey by Majerus *et al.*, (1985) on 185 spices yielded aflatoxins in 16 cases less than 5 μg/kg (eight nutmeg, one coriander and seven chilies/cayenne) and in eight cases more than 5 μg/kg (three nutmeg: 5.4–7.7 μg/kg; one coriander: 5.2 μg/kg; four chilies: 8.4–24 μg/kg). Ochratoxin A and sterigmatocystin could not be detected. However Reddy *et al.*, (2001) have detected aflatoxin above 30 μg/kg in chili powder and at the same time ochratoxin A above 30 μg/kg.

1.3 Mycobiota of spices and herbs and possible mycotoxin production

Fungi can infect spices and herbs in the field, during harvesting, drying, sorting, grinding, processing, packaging and storage. Pre-harvest mycotoxin production occurs when environmental conditions are suitable for mould growth. Most of the time, these conditions are beyond the control of man (Park and Troxell, 2002). Whereas post-harvest contamination can be controlled through several factors such as agricultural practices, handling during harvesting, methods and time spent for drying, conditions during storage and the quality of the seed and minimisation of physical damage.

Aflatoxins are a group of mycotoxins produced by different species such as *Aspergillus flavus, A. parasiticus*, *A. nomius* (Samson *et al.,* 2002), *A. ochraceoroseus, Emerciella venezuelensis* (Frisvad *et al.,* 1999), *A. argentinicus* and *A. bohemicus* (Ostry *et al.,* 1999). *Aspergillus flavus* and *A. parasiticus* are the most commonly encountered species in food. Moulds isolated from spices and herbs and their possible mycotoxin contamination (from literature) are shown in Tables 1.2 to 1.8.

Spices and herbs were seen to be contaminated by a number of fungi including potentially mycotoxigenic species. Among the spices red, black and white peppers, caraway, cardamom, cinnamon, coriander, cumin, ginger, mustard, peppermint, rosemary, tilio and turmeric were found to be contaminated with *A. flavus* and/or *A. parasiticus*. On the contrary, bay leaves and oregano did not contain aflatoxin-producing moulds. However, there is only one study in the literature (Beljaars *et al.*, 1975) that reports that bay leaf contained aflatoxin.

There are three types of pepper used as spice. The difference between them is not only their colour, their botanic names and properties are also different. Red pepper is a member of the *Capsicum* genus; the sweet red peppers belong to the *Capsicum annuum* species whereas the hot peppers to *Capsicum frutescens* (Bosland, 1994). Black and white peppers belong to the *Piper nigrum* L. Both are the grape-like fruit of the plant. Black pepper is obtained from immature corn if it is directly ground after drying; if the thin skin is removed from mature corn before grinding white pepper is produced. Unlike other peppers and spices, red pepper can be consumed fresh, in ground or powdered form. For this reason studies are included covering all three types, separately, in Table 1.1. However, there are various names related to red peppers in literature such as paprika in the USA, paprika and chili in Europe for sweet red pepper, cayenne and chili for hot pepper (Heperkan and Ermiş, 2004). Therefore under the common title red pepper in Table 1.1, the original names have also been kept.

Mycobiota, mycotoxigenic species and possible mycotoxin production (from literature) from these toxic species in red pepper, black pepper and white pepper are shown in Tables 1.2, 1.3 and 1.4 respectively. As seen in Table 1.2, nine different species of *Aspergillus* were found in red pepper. Most of them are able to produce different types of mycotoxins. Mycotoxigenic *Aspergillus* species in red peppers are *Aspergillus flavus*, *A. parasiticus*, *A. niger*, *A. ochraceus*, *A. oryzae*, *A. terreus*, and *A. versicolor*. In addition to *Aspergillus* species other mycotoxigenic species isolated from red peppers include *Emerciella nidulans*, *Penicillium brevicompactum*, *P. chrysogenum*, *P. crustosum*, *P. griseofulvu*m and *P. viridicatum*. *Trichoderma* sp. was also isolated but not in species level. In the literature, *T. virens* and *T. viride* produce mycotoxins (Frisvad and Thrane, 2002).

When the data is compared with the literature, it can be observed that in addition

Table 1.2 Mycobiota, mycotoxigenic species and possible mycotoxin production in red pepper

Mycobiota	Incidence (%)	Mycotoxins produced by moulds according to the literature[a]	References
Absidia sp.			Flannigan and Hui, 1976
Chaetomium jodhpurense	80*a		Abdel-Hafez and El Said, 1997
Aspergillus alutaceus	30		Abdel-Hafez and El Said, 1997
A. flavus	43–100	Aflatoxin B_1 Cyclopiazonic acid 3-nitropropionic acid	Flannigan and Hui, 1976; Bhat *et al.,* 1987; Martinez-Magana *et al.,* 1989;Abdel-Hafez and El Said, 1997; Heperkan and Ermiş, 2004
A. niger	12.5–100	Ochratoxin A	Flannigan and Hui, 1976; Martinez-Magana *et al.*, 1989 Abdel-Hafez and El Said, 1997 Heperkan and Ermiş, 2004
A. ochraceus	12.5	Penicillic acid Ochratoxin A Xanthomegnin Viomellein Vioxanthin	Martinez-Magana *et al.,* 1989
A. oryzae	10	Cyclopiazonic acid 3-nitropropionic acid	Heperkan and Ermiş, 2004
A. parasiticus	60	Aflatoxin B_1, B_2, G_1, G_2	Abdel-Hafez and El Said, 1997 Bhat *et al.,* 1987
A. sclerotia	33		Heperkan and Ermiş, 2004
A. terreus	7	Citrinin Citreoviridin Patulin	Heperkan and Ermiş, 2004
A. versicolor	3	Sterigmatocystin Nidulotoxin	Flannigan and Hui, 1976 Heperkan and Ermiş, 2004
Emerciella nidulans (A.nidulans)	10–30	Sterigmatocystin Nidulotoxin	Flannigan and Hui, 1976 Abdel-Hafez and El Said, 1997 Heperkan and Ermiş, 2004
Eurotium amstelodami	33–70**		Abdel-Hafez and El Said, 1997 Heperkan and Ermiş, 2004
E. chevalieri	10–50**		Abdel-Hafez and El Said, 1997 Heperkan and Ermiş, 2004
E. rubrum	3–50**		Abdel-Hafez and El Said, 1997 Heperkan and Ermiş, 2004
Gibberella (Fusarium)*	50		Abdel-Hafez and El Said, 1997
Monascus ruber	3		Heperkan and Ermiş, 2004
Mucor sp.	90		Abdel-Hafez and El Said, 1997
P. brevicompactum	7	Mycopenolic acid Botryodipliodin	Heperkan and Ermiş, 2004
*P. chrysogenum**	30	Roquefortine C	Abdel-Hafez and El Said, 1997
P. crustosum	3	Roquefortine C Penitrem A	Heperkan and Ermiş, 2004
*P. corylophilum**	40		Abdel-Hafez and El Said, 1997
P. griseofulvum	3	Roquefortine C, Cyclopiazonic acid Patulin Griseofulvin	Heperkan and Ermiş, 2004

Table 1.2 Continued

Mycobiota	Incidence (%)	Mycotoxins produced by moulds according to the literature[a]	References
P. viridicatum	30	Xanthomegnin Viomellein Vioxanthin Viridic acid Penicillic acid	Heperkan and Ermiş, 2004
Rhizopus sp.	40		Abdel-Hafez and El Said, 1997
Scopulariopsis brevicaulis	3		Heperkan and Ermis, 2004
*Stachybotrys*sp. (Melanopsamma)*	70		Abdel-Hafez and El Said, 1997
*Trichoderma*sp.*	40	(*T.virens, T. viride*) Gliotoxin Emodin Trichodermin	Abdel-Hafez and El Said, 1997

a: adopted from Frisvad and Thrane, (2002).
*Cellulose agar.
**50% sucrose agar.

to aflatoxin, ochratoxin A, trichothecenes and zearalenone, secondary important mycotoxin production such as; citrinin, cyclopiazonic acid, patulin, sterigmatocystin is also possible in red pepper. The presence of moulds in food does not necessarily mean that mycotoxins are also present; environmental conditions such as temperature and relative humidity should also be favourable as well as the type and structure of the food (Heperkan and Ermiş, 2004).

The mycobiota of the red pepper flakes collected from four different regions in Turkey consisted mainly of *Aspergillus* (56%), *Eurotium* (17%) and *Penicillium* (16%) species, while *Monascus* and *Scopuloriopsis* were detected only once in two different samples. Among the *Aspergillus* species, *A. niger* and *A. flavus-A. parasiticus* mould counts were higher 17% and 16% of the mycobiota, respectively, followed by *A. sclerotia* (12%). *E. amstelodami* (12%) and *P. viridicatum* (11%) (Heperkan and Ermiş, 2004). Red pepper flakes are produced by drying fresh pepper followed by coarse grinding. Bhat and co-workers (1987) studied the microbial profile on chilli powder (red pepper) in the USA. Aflatoxin producing *A. flavus* and *A. parasiticus* were detected in 88% of the chilli samples. Heperkan and Ermiş, (2004) found that 36% of red pepper flake samples were contaminated with aflatoxigenic fungi such as *A. flavus* and *A. parasiticus*.

As seen in Table 1.3 and Table 1.4 mycobiota were similar in black pepper and white pepper. Important mycotoxins and their potential producers isolated from black pepper and white pepper were as follows; aflatoxin producers such as *A. flavus*; ochratoxin producers such as *A. ochraceus* and *A. niger*; trichothecenes producers such as *F. equiseti* and zearalenone producers such as *F. equiseti* and *F. semitectum*. Other mycotoxin-producing moulds such as *A. fumigatus*, *A. tamari*, *A. terreus*, *A. versicolor*, *Emerciella nidulans*, *P. brevicompactum* and *P. glabrum* were also isolated. The only exception was *A. parasiticus* which was isolated from black pepper but not from white pepper.

Martinez-Magana *et al*., (1989) studied the mycobiota of pepper and found that

Table 1.3 Mycobiota, mycotoxigenic species and possible mycotoxin production in black pepper

Mycobiota	Incidence (%)	Mycotoxins produced by moulds according to the literature[a]	References
Aspergillus aureolus			Garcia *et al.,* 2001
A. candidus			Flannigan and Hui, 1976 Garcia *et al.,* 2001
A. flavus	15–43.8	Aflatoxin B_1, B_2 Cyclopiazonic acid 3-nitropropionic acid	Flannigan and Hui, 1976 Geeta and Kulkarni, 1987 Martinez-Magana *et al.,* 1989 Freire *et al.*, 2000
A. fumigatus	13	Gliotoxin Verrucologen Fumitoxins Fumigaclavines	Martinez-Magana *et al.,* 1989 Garcia *et al.,* 2001
A. niger	16–48	Ochratoxin A	Martinez-Magana *et al.,* 1989 Garcia *et al.,* 2001 Geeta and Kulkarni, 1987 Freire *et al.,* 2000 Elshafie *et al.,* 2002
A. ochraceus	3.8–26	Penicillic acid Ochratoxin A Xanthomegnin Viomellein Vioxanthin	Martinez-Magana *et al.,* 1989 Freire *et al.,* 2000 Garcia *et al.,* 2001
A. parasiticus *A. restrictus*	15	Aflatoxin B_1, B_2, G_1, G_2	Geeta and Kulkarni, 1987 Moreno-Martinez and Christensen, 1972 Rami *et al.*, 1995 Chourasia, 1995 Geeta and Reddy, 1990
A. sydowii	4		Martinez-Magana *et al.,* 1989
A. tamarii	7.2	Cyclopiazonic acid	Moreno-Martinez and Christensen, 1972 Rami *et al.*, 1995 Chourasia, 1995 Geeta and Reddy, 1990 Freire *et al.*, 2000
A. terreus		Citrinin Citreoviridin Patulin	Elshafie *et al.*, 2002
A. versicolor	4–5.8	Sterigmatocystin Nidulotoxin	Martinez-Magana *et al.*, 1989 Freire *et al.*, 2000
Alternaria alternata			Moreno-Martinez and Christensen 1972 Rami *et al.*, 1995 Chourasia, 1995 Geeta and Reddy, 1990
Chaetomium sp.	15.3	(*C.globosum*) Chaetoglobosins Chetomin	Moreno-Martinez and Christensen, 1972 Rami *et al.*, 1995 Chourasia, 1995 Geeta and Reddy, 1990
Circinella			Garcia *et al.*, 2001
Cladosporium sp.		(*C.herbarum*) Cladosporic acid	Freire *et al.*, 2000
Cunninghamella			Garcia, *et al.*, 2001
Curvularia			Garcia, *et al.*, 2001
Emerciella nidulans (*A. nidulans*)	13	Sterigmatocystin Nidulotoxin	Freire *et al.*, 2000 Elshafie *et al.*, 2002

Table 1.3 Continued

Mycobiota	Incidence (%)	Mycotoxins produced by moulds according to the literature[a]	References
Eurotium chevalieri	6.5		Freire *et al.*, 2000
E.rubrum	4		Freire *et al.*, 2000
Fusarium oxysporum			Freire *et al.*, 2000
F. sacchari			Freire *et al.*, 2000
F. solani			Freire *et al.*, 2000
Geotrichum candidum 5			Freire *et al.*, 2000
Mycelia sterilata			Elsafie *et al.*, 2002
P. brevicompactum	15	Mycopenolic acid Botryodipliodin	Freire *et al.*, 2000
P. glabrum	8.3	ctromycetin	Freire *et al.*, 2000
R. oryzae	10		Freire *et al.*, 2000
Syncephalastrum racemosum			Elshafie *et al.*, 2002
Trichoderma sp T. artroviride	3.5		Garcia *et al.*, 2001

a: adopted from Frisvad and Thrane, (2002).

Aspergillus and *Penicillium* were the main components of the flora. The most common aspergilli were *A. flavus* (group) (46%) and *A. niger* (20%). They also found that 28% of 72 strains of *A. flavus* isolated from spices were toxigenic. Freire *et al.,* (2000) studied mycoflora and mycotoxins in Brazilian black and white pepper and found that *A. flavus* and *A. niger* were isolated more frequently from black than from white pepper. A total of 42 species was isolated from surface sterilised corns of the two pepper types. *A. flavus* was most frequently isolated and was more prevalent on black pepper than white pepper (43.8 and 3.4%). *A. niger* was the second dominant species on both peppers (16.2 and 4.5%). Other potential mycotoxigenic species isolated were: *A. ochraceus* (3.8% black pepper), *A. tamarii* (7.2 and 4.0%), *A. versicolor* (5.8 and 2.5%), *E. nidulans* (13.0% black pepper), *Chaethomium* (15.3 and 3.7%), *P. brevicompactum* (15 and 12.5%), *P. citrinum* (7.4%), *P. islandicum* (2.4%), *P. glabrum* (2.4% black pepper). The high fungal contamination of black pepper and white pepper and the high incidence of potential producers of mycotoxins show that these peppers can be a means of contamination of food.

Cinnamon, coriander and ginger are suitable substrates for mould growth and mycotoxin production. Mycobiota and possible mycotoxin production are shown in Tables 1.5, 1.6 and 1.7 respectively. Mycotoxigenic fungi isolated from cinnamon samples were *A. flavus*, *A. niger*, *A. fumigatus, A. ochraceus, Chaethomium globosum*, *E. nidulans*, *P. chrysogenum*, *P. citrinum,* and *P. oxalicum* (Table 1.5). As seen in Tables 1.6 and 1.7, *A. flavus*, *A. niger*, *A. terreus, E. nidulans, F. equiseti and F. semitectum* were isolated from coriander whereas *A. flavus A. fumigatus*, *A. parasiticus, A. niger, E. nidulans* and several mycotoxin producer *Penicillium species* were isolated from ginger.

Mycobiota and mycotoxigenic species in anise, bay leaves, caraway, cardamom, cumin mustard, oregano, peppermint, rosemary, tilio and turmeric are shown in Table 1.8. *Alternaria* and *Fusarium* species dominated over other fungi of mustard seed from the mixed cropping treatment in India. *A. flavus*, however had the highest incidence among mono-cropping samples (Bilgrami *et al.,* 1991).

Table 1.4 Mycobiota, mycotoxigenic species and possible mycotoxin production in white pepper

Mycobiota	Incidence (%)	Mycotoxins produced by moulds according to the literature[a]	References
A. candidus	2		Flannigan and Hui, 1976 Freire *et al.*, 2000
A. flavus	3.4–47	Aflatoxin B_1, B_2 Cyclopiazonic acid 3-nitropropionic acid	Martinez-Magana *et al.*, 1989 Flannigan and Hui, 1976 Freire *et al.*, 2000
A. fumigatus	12	Gliotoxin Verrucologen Fumitoxins Fumigaclavines	Martinez-Magana *et al.*, 1989 Flannigan and Hui, 1976
Emerciella nidulans (*A. nidulans*)	6	Sterigmatocystin Nidulotoxin	Martinez-Magana *et al.*, 1989 Flannigan and Hui, 1976
A. niger	4.5–24	Ochratoxin A (few isolates)	Martinez-Magana *et al.*, 1989 Flannigan and Hui, 1976 Freire *et al.*, 2000
A. ochraceus	6	Penicillic acid Ochratoxin A Xanthomegnin Viomellein Vioxanthin	Martinez-Magana *et al.*, 1989
A. tamarii	4	Cyclopiazonic acid	Freire *et al.*, 2000 Flannigan and Hui, 1976
A. terreus	6	Citrinin, Citreoviridin Patulin	Flannigan and Hui, 1976 Martinez-Magana *et al.*, 1989
A. versicolor	2.5	Sterigmatocystin Nidulotoxin	Flannigan and Hui, 1976 Freire *et al.*, 2000
Chaetomium globosum	3.7	Chaetoglobosins Chetomin	Freire *et al.*, 2000
Cunninghamella elegans	1.5		Freire *et al.*, 2000
Curvularia lunata	2.2		Freire *et al.*, 2000
Eurotium (*A.glaucus*)	18		Martinez-Magana *et al.*, 1989 Flannigan and Hui, 1976
E. chevalieri	2		Freire *et al.*, 2000
Fusarium oxysporum	1.5		Freire *et al.*, 2000
Microascus cinereus	2.5		Freire *et al.*, 2000
P. brevicompactum	12.5	Mycopenolic acid Botryodipliodin	Freire *et al.*, 2000
P. citrinum	7.4	Citrinin	Freire *et al.*, 2000
P. islandicum	2.4	Rugulosin Luteoskyrin Islanditoxin Cyclochlorotine Erythroskyrin Emodin	Freire *et al.*, 2000
Rhizopus oryzae	5.5		Freire *et al.*, 2000
Sporendonema sp.	1		Freire *et al.*, 2000
Spormiella minima	2.3		Freire *et al.*, 2000
Trichoderma artroviride	1.5		Freire *et al.*, 2000

a: adopted from Frisvad and Thrane, (2002).

Elshafie *et al.,* (2002) detected mycobiota of seven different spices from a group consisting of one hundred and five samples. Coriander was found to be the most heavily fungal contaminated among the spices (18 out of 20) followed by black pepper, ginger, cinnamon, cumin, and cardamom. Clove was the least contaminated spice due to its microbial inhibitory effect. Cinnamon was found to be contaminated by a number of fungi (11 out of 20) including potentially mycotoxin producing fungi. Fifteen samples of spices (ginger, cumin, cinnamon, clove, black pepper, cardamom, ginger and coriander) that were heavily contaminated by *A. flavus* were screened for the presence of aflatoxins using HPLC. No aflatoxins were detected on the samples. Of the seven spices studied, clove was found to be the least contaminated, while cumin was the most heavily contaminated.

Medicinal plants such as peppermint, chamomile, anise, caraway and tilio were analysed for moulds and aflatoxins (Abou-Arab *et al.,* 1999). Samples were collected randomly from the Egyptian market. *Aspergillus* and *Penicillium* genera were more frequently detected and in greater abundance in the samples than other genera of fungi. For the *A. flavus* infection, the results showed that all tested medicinal plants were infected with the exception of packed tilio. The highest percentage of infection was in peppermint (15.8%) followed by non-packed tilio (15.4%) as well as non-packed caraway (13.5%). The other tested medicinal plants showed a low percentage of *A. flavus*. However, natural aflatoxin contamination was not detected.

Rizzo *et al.,* (2004) studied toxigenic fungi on 56 species of medicinal and aromatic herbs, which were used as raw material for drugs in Argentina. *A. flavus* and *A. parasiticus* were the predominant species isolated, 50% out of 40 isolates were toxigenic, 26% of isolates produced OTA in low concentrations, 27% of the isolates were *F. verticilloides* and *F. proliferatum*, which produced fumonisin B_1 and fumonisin B_2. Other *Fusarium* species were able to produce neither group A and B trichothecenes nor zearalenone.

Martins *et al.,* (2001) studied microbiological quality of seven species (chamomile, leaves of orange tree, flower soft linden, corn silk, marine alga, pennyroyal mint and garden sage) of 62 medicinal plants in Lisbon, Portugal. Corn silk samples were the most contaminated. *Fusarium* spp., *Penicillium* spp., *A. flavus* and *A. niger* were predominant in all samples with the exception of garden sage.

1.4 Detecting mycotoxins in herbs and spices

Various methods have been published to determine the mycotoxin content of foodstuffs by international organisations such as the Association of Official Analytical Chemists (AOAC), the International Union of Pure and Applied Chemistry (IUPAC), the European Standardization Committee (CEN). However, an official method related to the determination of mycotoxin in herbs and spices does not exist. The aim of this section is not to repeat a specific method developed by the official bodies mentioned above, but to present information regarding issues to be considered during method selection and application for herbs and spices together with alternative methods that can be used in mycotoxin analyses of herbs and spices and the recent development in the field.

1.4.1 Mycotoxin determination methods in spices and herbs

All mycotoxin analyses consist of three steps; sampling, sample preparation and analytical procedure. Sampling should be performed such as to collect a representative amount of the lot. The sample size in the EU is 30 kg. Sample preparation is the second step, where the sample is ground to particle sizes as small as possible and

Table 1.5 Mycobiota, mycotoxigenic species and possible mycotoxin production in cinnamon

Mycobiota	Incidence (%)	Mycotoxins produced by moulds according to the literature[a]	References
Absidia spp.			Flannigan and Hui, 1976
*Aspergillus alutaceus**	50		Abdel-Hafez and El Said, 1997
A. flavus	100	Aflatoxin B_1 Cyclopiazonic acid 3-nitropropionic acid	Abdel-Hafez and El Said, 1997, Elshafie *et al.*, 2002
A. niger	70	Ochratoxin A	Flannigan and Hui, 1976 Abdel-Hafez and El Said, 1997 Elshafie *et al.*, 2002
A. fumigatus	60	Gliotoxin Verrucologen Fumitoxins Fumigaclavines	Abdel-Hafez and El Said, 1997
A. ochraceus		Penicillic acid Ochratoxin A Xanthomegnin Viomellein Vioxanthin	Elshafie *et al.*, 2002
*Chaetomium globosum**	100	Chaetoglobosins Chetomin	Abdel-Hafez and El Said, 1997
*Emerciella nidulans**	30	Sterigmatocystin Nidulotoxin	Abdel-Hafez and El Said, 1997
*E. amstelodami***	100		Abdel-Hafez and El Said, 1997
*E. chevalieri***	70		
*E. rubrum***	40		
Mucor sp.	60		Abdel-Hafez and El Said, 1997 Elshafie *et al.*, 2002
Mycelia sterilata			Elshafie *et al.*, 2002
Mycosphaerella tassiana	30		Abdel-Hafez and El Said, 1997
*Myrothecium*sp.*	30		Abdel-Hafez and El Said, 1997
*Nectria*sp.*	40		Abdel-Hafez and El Said, 1997
P. chrysogenum	60	Roquefortine C	Abdel-Hafez and El Said, 1997
*P. citrinum***	30	Citrinin	Abdel-Hafez and El Said, 1997
P. corylophilum	30		Abdel-Hafez and El Said, 1997
*P. oxalicum**	40	Secalonic acid F	Abdel-Hafez and El Said, 1997 Elshafie *et al.*, 2002
Rhizopus nigricans			
R. stolonifer	50		Abdel-Hafez and El Said, 1997
*Stachybotrys*sp.*	30		Abdel-Hafez and El Said, 1997
Syncephalastrum racemosum			Elshafie *et al.*, 2002
*Trichoderma*sp.*	40		Abdel-Hafez and El Said, 1997

a: adapted from Frisvad and Thrane, (2002).
*Cellulose agar.
**50% sucrose agar.

Table 1.6 Mycobiota, mycotoxigenic species and possible mycotoxin production in coriander

Mycobiota	Incıdence (%)	Mycotoxins produced by moulds according to the literature[a]	References
A. flavus		Aflatoxin B_1, B_2 Cyclopiazonic acid 3-nitropropionic acid	Rami *et al*., 1995 Elshafie *et al*., 2002
A.niger		Ochratoxin A	Rami *et al.*, 1995 Elshafie *et al*., 2002
A.terreus		Citrinin Cireoviridin Patulin	Elshafie *et al.*, 2002
Alternaria alternate			Hashmi and Ghaffar, 1991
A. longissima			Hashmi and Ghaffar, 1991
A. porri			Hashmi and Ghaffar, 1991
Ascochyta spp.			Hashmi and Ghaffar, 1991
Botryodiplodia			Hashmi and Ghaffar, 1991
Botrytis cinerea			Hashmi and Ghaffar, 1991
Cephalasporium acremonium			Hashmi and Ghaffar, 1991
Colletotrichum			Hashmi and Ghaffar, 1991
capsici			Hashmi and Ghaffar, 1991
Curvularia lunata			Rami *et al.*, 1995
Drechslera bicolor			Hashmi and Ghaffar, 1991
D. rostrata,			Hashmi and Ghaffar, 1991
D. tetramera			Hashmi and Ghaffar, 1991
Emerciella nidulans		Sterigmatocystin Nidulotoxin	Rami *et al.*, 1995 Elshafie *et al.*, 2002
Fusarium equiseti		Fusarochromanone Trichothecenes type A & B Zearalenone	Hashmi and Ghaffar, 1991
F. oxysporum			Hashmi and Ghaffar, 1991
F. semitectum		Zearalenone	Hashmi and Ghaffar, 1991
F. solani,			Hashmi and Ghaffar, 1991
Macrophomina phaseolina			Hashmi and Ghaffar, 1991
Myrothecium roridum			Hashmi and Ghaffar, 1991
M. verrucaria			Hashmi and Ghaffar, 1991
Paecilomyces,	53		Elshafie *et al.*, 2002
Penicillium spp.			Elshafie *et al.*, 2002
Phoma spp.			Hashmi and Ghaffar, 1991
Protomyces macrosporus			Hashmi and Ghaffar, 1991
Pythium spinosum			Hashmi and Ghaffar, 1991
Rhizopus nigricans			Elshafie *et al.*, 2002
Syncephalastrum racemosum			Elshafie *et al.*, 2002
Verticillium alboatrum			Hashmi and Ghaffar, 1991
Absidia sp.			Garcia, *et al.*, 2001

a: adapted from Frisvad and Thrane, (2002).

Table 1.7 Mycobiota, mycotoxigenic species and possible mycotoxin production in ginger

Mycobiota	Incidence (%)	Mycotoxins produced by moulds according to the literature[a]	References
Absidia sp.			Aziz *et al.*, 1998
A. flavus		Aflatoxin B_1, B_2 Cyclopiazonic acid 3-nitropropionic acid	Aziz *et al.*, 1998 Elshafie *et al.*, 2002
A. fumigatus		Gliotoxin Verrucologen Fumitoxins Fumigaclavines	Elshafie *et al.*, 2002
A. parasiticus (dominant)		Aflatoxin B_1, B_2, G_1, G_2	Aziz *et al.*, 1998
A. niger		Ochratoxin A	Elshafie *et al.*, 2002
Alternaria alternate	96.7		Elshafie *et al.*, 2002
Cladosporium herbarum			Aziz *et al.*, 1998
Emerciella nidulans		Sterigmatocystin Nidulotoxin	Elshafie *et al.*, 2002
Eurotium amstelodami			Elshafie *et al.*, 2002
Fusarium spp.			Aziz *et al.*, 1998
Mucor sp.			Aziz *et al.*, 1998 Elshafie *et al.*, 2002
Paecilomyces variotii			Aziz *et al.*, 1998
Penicillium brevicompactum	85	Mycopenolic acid Botryodipliodin	Overy and Frisvad, 2005
P. crustosum	55	Roquefortine C Penitrem A	Overy and Frisvad, 2005
P. polonicum	35	Penicillic acid Verrucosidin	Overy and Frisvad, 2005
P. cyclopium	25	Penicillic acid Xanthomegnin Viomellein	Overy and Frisvad, 2005
P. aurantiogriseum	10	Penicillic acid Verrucosidin	Overy and Frisvad, 2005
P. steckii,	10		Overy and Frisvad,
P. bialowiezense	10		2005
P. freii	10	Xanthomegnin Viomellein Vioxanthin Penicillic acid	Overy and Frisvad, 2005
P. allii	5		Overy and Frisvad, 2005
P. commune	5	Cyclopiazonic acid	Overy and Frisvad, 2005
P. viridicatum	5	Xanthomegnin Viomellein Vioxanthin Viridic acid Penicillic acid	Overy and Frisvad, 2005
P. expansum	5	Roquefortine C Patulin Citrinin	Overy and Frisvad, 2005
P. discolor	5	Chaetoglobosin C	Overy and Frisvad, 2005
Rhizopus nigrificans			Elshafie *et al.*, 2002
Syncephalastrum racemosum			Elshafie *et al.*, 2002

a: adapted from Frisvad and Thrane, (2002).

Table 1.8 Mycobiota, mycotoxigenic species and possible mycotoxin production in other spices and herbs

Spices and herbs	Mycobiota	Incidence (%)	Mycotoxins produced by moulds according to the literature[a]	References
Anise	*Alternaria sp.*	100		Abou-Arab *et al.*, 1999
	Aspergillus flavus	50	Aflatoxin B_1, B_2 Cyclopiazonic acid 3-nitropropionic acid	Abou-Arab *et al.*, 1999
	Fusarium spp.	50		Abou-Arab *et al.*, 1999
	Penicillium spp,	100		Abou-Arab *et al.*, 1999
	Trichoderma sp.	50		Abou-Arab *et al.*, 1999
Bay leaves	*Aspergillus fumigatus*		Gliotoxin Verrucologen Fumitoxins Fumigaclavines	Garcia, *et al.*, 2001
	A. niger		Ochratoxin A	Garcia *et al.*, 2001
	Alternaria sp.			Garcia *et al.*, 2001
	Cunninghamella sp.			Garcia *et al.*, 2001
	Cladosporium sp.			Garcia *et al.*, 2001
	Monilia sp.			Garcia *et al.*, 2001
	Penicillium spp.			Garcia *et al.*, 2001
	Paecilomyces sp.			Garcia *et al.*, 2001
	Trichodermasp.			Garcia *et al.*, 2001
Caraway	*Aspergillus crothecium*			Abou-Arab *et al.*, 1999 Abou-Arab *et al.*, 1999
	Penicillium sp.	56		Abou-Arab *et al.*, 1999
	Rhizoctonia sp.	33		Abou-Arab *et al.*, 1999
	Fusarium sp.			Abou-Arab *et al.*, 1999
	A. flavus	13	Aflatoxin B_1, B_2 Cyclopiazonic acid 3-nitropropionic acid	Abou-Arab *et al.*, 1999
	A. niger	12.5	Ochratoxin A	Abou-Arab *et al.*, 1999
	A. terreus	12.5	Citrinin Cireoviridin Patulin	Abou-Arab *et al.*, 1999
Cardamom	*Alternaria alternate*			Elshafie *et al.*, 2002
	Emerciella nidulans (Aspergillus nidulans)		Sterigmatocystin Nidulotoxin	Elshafie *et al.*, 2002
	A. niger		Ochratoxin A	Elshafie *et al.*, 2002
Cardamom	*Fusarium spp.*			Elshafie *et al.*, 2002
	Rhizopus nigricans			Elshafie *et al.*, 2002
	Syncephalastrum racemosum			Elshafie *et al.*, 2002
Cumin	*A. fumigatus*		Gliotoxin Verrucologen Fumitoxins Fumigaclavines	Geeta and Reddy, 1990
	A. niger (dominant)		Ochratoxin A	Garcia, *et al.*, 2001
	A. aureolus			Garcia, *et al.*, 2001
	Alternaria alternate			Geeta and Reddy, 1990
	Curvularia lunata			Geeta and Reddy, 1990
	Cunninghamella sp.			Garcia *et al.*, 2001
	Circinella sp.			Garcia *et al.*, 2001

Table 1.8 Continued

Spices and herbs	Mycobiota	Incidence (%)	Mycotoxins produced by moulds according to the literature[a]	References
	Emerciella nidulans (A. nidulans)		Sterigmatocystin Nidulotoxin	Geeta and Reddy, 1990
	Fusarium spp.			Geeta and Reddy, 1990
	Helminthosporium sativum			Geeta and Reddy, 1990
Cumin	*Mucor sp.*			Garcia, *et al.*, 2001
	Rhizopus sp.			Garcia, *et al.*, 2001
	Scopuloriopsis sp.			Garcia, *et al.*, 2001
	Trichoderma spp.			Garcia, *et al.*, 2001
	Penicillium spp.			Garcia, *et al.*, 2001
Mustard	*A. flavus*		Aflatoxin B_1, B_2 Cyclopiazonic acid 3-nitropropionic acid	Bilgrami *et al.*, 1991
	Alternaria spp.			Bilgrami *et al.*, 1991
	Eurotium spp. (A.glaucus gr)			Flannigan and Hui, 1976
	Fusarium spp.			Bilgrami *et al.*, 1991
Oregano	*Cunninghamella sp.*			Garcia *et al.*, 2001
	Trichoderma sp.			Garcia *et al.*, 2001
	A. niger		Ochratoxin A	Garcia *et al.*, 2001
	A. versicolor		Sterigmatocystin Nidulotoxin	Garcia *et al.*, 2001
	Mucor spp.			Garcia *et al.*, 2001
	Nigrospora sp.			Garcia *et al.*, 2001
	Chaetomium sp.			Garcia *et al.*, 2001
	Phoma sp.			Garcia *et al.*, 2001
	Rhizopus sp.			Garcia *et al.*, 2001
	Penicillium spp.			Garcia *et al.*, 2001
Pepper-mint	*Alternaria sp*			Abou-Arab *et al.*, 1999
	A. condius			Abou-Arab *et al.*, 1999
	A. flavus	15.8	Aflatoxin B_1, B_2 Cyclopiazonic acid 3-nitropropionic acid	Abou-Arab *et al.*, 1999
	A. niger	15.8	Ochratoxin A	Abou-Arab *et al.*, 1999
	A. ochraceus		Penicillic acid Ochratoxin A Xanthomegnin Viomellein Vioxanthin	Abou-Arab *et al.*, 1999
	A. terreus		Citrinin Citreoviridin Patulin	Abou-Arab *et al.*, 1999
	Fusarium spp.			Abou-Arab *et al.*, 1999
	Penicillium sp.			Abou-Arab *et al.*, 1999
	Trichoderma sp.			Abou-Arab *et al.*, 1999
Rosemary	*Alternaria*			Abdel-Hafez and Said, 1997
	A. flavus		Aflatoxin B_1, B_2 Cyclopiazonic acid 3-nitropropionic acid	Abdel-Hafez and Said, 1997

Table 1.8 Continued

Spices and herbs	Mycobiota	Incidence (%)	Mycotoxins produced by moulds according to the literature[a]	References
	A. niger		Ochratoxin A	Abdel-Hafez and El Said, 1997
	A. sydowii			Abdel-Hafez and El Said, 1997
	Cladosporium sp.			Abdel-Hafez and El Said, 1997
	Mycospharella sp.			Abdel-Hafez and El Said, 1997
	E. amstelodami			Abdel-Hafez and El Said, 1997
	E. chevalieri			Abdel-Hafez and El Said, 1997
	E. rubrum			Abdel-Hafez and El Said, 1997
	P. chrysogenum		Roquefortine C	Abdel-Hafez and El Said, 1997
Tilio	*Alternaria sp.*			Abou-Arab *et al.*, 1999
	A. crothecium			Abou-Arab *et al.*, 1999
	A. flavus		Aflatoxin B_1, B_2 Cyclopiazonic acid 3-nitropropionic acid	Abou-Arab *et al.*, 1999
	A. niger		Ochratoxin A	Abou-Arab *et al.*, 1999
	Penicillium spp.			Abou-Arab *et al.*, 1999
	Rhizoctonia sp.			Abou-Arab *et al.*, 1999
Turmeric	*A. flavus*		Aflatoxin B_1, B_2 Cyclopiazonic acid 3-nitropropionic acid	Geeta and Kulkarni, 1987
	A. parasiticus		Aflatoxin B_1, B_2, G_1, G_2	Geeta and Kulkarni, 1987
	A. niger		Ochratoxin A	Geeta and Kulkarni, 1987
	Eurotium spp. (A. glaucus gr)			Flannigan and Hui, 1976
	Mucor sp.	15		Geeta and Kulkarni, 1987

a: adapted from Frisvad and Thrane, (2002).

homogenised, from which a sub-sample is taken (approximately 100 g). In checking all the factors associated with the variability of mycotoxin results, it was found that the contribution of sampling is the greatest single source of error (Ahmed, 2000). Specially, for products like peanuts where the aflatoxin distribution is not homogeneous, the variability associated to the test procedures (sampling, sample preparation and analysis) can cause misclassification (Adams and Whitaker, 2004). On the other hand, in a study on sampling wheat for deoxynivalenol, even with the use of a small sample size (0.454 kg), the sampling variation was not the largest source of error as found in other mycotoxin test procedures (Whitaker *et al.,* 2002). Therefore, whether the particle size or the mycotoxin distribution in the particle is homogeneous or not does not affect the variability in the sampling stage in wheat (Whitaker *et al.,* 2002).

The levels of mycotoxin are regulated in several countries by set maximum

permissible levels therefore the method selected to be used must have appropriate sensitivity. In addition, having short analysis time, ease-of-use, being a reliable, less interfering, and inexpensive substance are other important factors (Ahmed, 2000; Lombaert, 2002).

The technical infrastructure of the laboratory is also very important for the selection of the method. For example, thin layer chromatography (TLC) can be considered as an appropriate method due to its low initial investment. However, the application and evaluation of the method is very difficult. Evaluation should be made with a densitometer. Thus the visual errors in the determination of the equivalent mycotoxin to the standard should be eliminated. During an interlab study with 28 participating laboratories, on DON analysis of agricultural products, it was reported that the results from TLC were considerably lower than the average value ($p = 0.01$) (Josephs *et al.*, 2001). In fact, thin layer chromatography is suitable for confirmation of positive samples (De Nijs and Notermans, 2000).

Almost all analytical procedures consist of the similar basic steps which include:

- extraction
- purification and clean-up
- separation, detection and determination
- confirmation.

Not all methods for mycotoxins in foodstuffs incorporate a cleanup step. In particular, ELISA methods may not require any cleanup (Scott, 2002). However, Yu *et al.,* (1998) developed and used immunoaffinity columns (IAC) for the cleanup of CPA extracts prior to ELISA analysis of corn, peanuts, and mixed feed (Dorner, 2002). ELISA is a useful tool for screening purposes before LC or GC.

The analytical methods for mycotoxins include thin layer chromatography (TLC), high performance liquid chromatography (HPLC) or reversed phased liquid chromatography (LC), enzyme-linked immunosorbent assay (ELISA), and, more recently, by tandem mass spectrometry (MS) (Scott, 2002; Trucksess, 2000; Ventura *et al.,* 2004). Aflatoxins, ochratoxin A, deoxynivalenol and zearalenone can be determined by fluorescence detection after chromatographic separation (De Nijs and Notermans, 2000). Gas chromatography (GC) has also been used for many mycotoxin analysis especially for the identification and quantification of multiple trichothecenes in foods (Lombaert, 2002).

Capillary electrophoresis (CE) may be used as an alternative technique to analyse mycotoxins (Cancalon, 1995; Martin *et al.,* 2005). A particular type of CE, micellar electrokinetic capillary electrophoresis or micellar electrokinetic capillary chromatography (MECC) has been used for aflatoxin, cyclopiazonic acid, citrinin, griseofulvin, mycophenolic acid, ochratoxin A, patulin, penicillic acid, and sterigmatocystin analyses (Cancalon, 1995; Martin *et al.,* 2005). The method has several advantages such as more rapid analysis, reduced amount of organic solvents, smaller sample volume, and increased efficiency and resolution (Martin *et al.,* 2005). According to a survey of the literature, HPLC was the leading approach, followed by TLC and ELISA in mycotoxin analyses in herbs and spices (Table 1.1). In addition, a single study was found on liquid chromatography-tandem mass spectrometry in medicinal herbs in the literature (Ventura *et al.,* 2004).

Ventura *et al.,* (2004), extracted aflatoxins in *Rhammus purshiana* which is a medicinal herb, with methanol:water and tested by liquid chromatography and detected by mass spectrometry single quadruple using an electrospray ionisation source (LC-

MS) in order to avoid derivatisation. The detection limit was 10 ng and the quantification limit 25 ng. The advantages of the method can be stated as follows (Ventura *et al.,* 2004). Using a short column (C18) for chromatographic separation allows rapid determination obtaining sharp chromatographic peaks and minimising consumption of the mobile phase. Using low quantities of methanol for the extraction steps avoids the use of chlorate solvents that are harmful. The polymeric sorbent is as easy to apply as immunoaffinity columns but is cheaper. Mass spectrofotometric detection is employed in order to avoid derivatisation which presents several disadvantages.

Mycotoxins are extracted from the food matrix using a suitable solvent mixture. Mycotoxins such as aflatoxins, ochratoxin A and penicillic acid dissolve in chloroform better than in a hydrophilic solvent. However, since chloroform is carcinogenic, it should be replaced by solvents such as methanol:water, acetonitrile:phosphoric acid or acetic acid, toluene:acetic acid (Ahmed, 2000; Scott, 2002).

For the purification and cleanup steps, commercially available and disposable SPE columns or cartridges, of which those incorporating silica and immobilised antibodies for immunoaffinity chromatography are the most widely used (Scott, 2002). A recently introduced technique is the use of molecularly imprinted polymers, polymers with cavities or imprints complementary in shape to an analyte of interest (Scott, 2002).

1.4.2 Points to be borne in mind in mycotoxin research

Moulds can cause allergic reactions and some of them are pathogenic. As mould spores are easily spread in the air, care must be taken while working with them, using a separate laboratory with restricted entry. As mycotoxins are toxic chemical substances, care must be taken to avoid exposure to mycotoxins by direct contact and inhalation. Work must be carried out in a separate laboratory, equipped with a fume hold. Protective goggles, gloves and lab-coats must be worn. Disposable laboratory wastes must only be disposed of after they have been soaked in a 10% solution of household bleach for 30 minutes. In order to remove aflatoxin remnants on glass surfaces, the articles must be rinsed with methanol, soaked in a 1% solution of household bleach for two hours, and acetone added to 5% of total volume. They should be left for 30 minutes to react and then washed thoroughly (Trucksess, 2000). As mycotoxin standards are sensitive to external influences such as light, oxygen and temperature, a suitable laboratory environment must be ensured.

1.5 Preventing and controlling mycotoxin contamination

Mycotoxins can lead to various diseases in human beings. They also lead to loss of products and product quality, diseases in animals, low yield generally and reduction in the number and size of eggs produced. Mycotoxins also seriously threaten the health of future generations. Aflatoxin B_1 is a human carcinogen (IARC, 1993). As an etiological agent, it is associated with several human diseases encountered particularly in Africa, Asia and South America, e.g., primary hepatic carcinoma, hepatic cirrhosis in children, chronic gastritis, Kwashiorkor and Reye's syndrome (Ostry *et al.,* 1999).

The products on which most work is being done to bring the mycotoxin hazard under control are ground nuts, cotton seed and maize. The factors influential in formation of mycotoxins have been determined with the work carried out so far and

attempts have been made to create control strategies in this respect. However, research indicates that mycotoxin has still not been brought entirely under control. The most important factor in arriving at this conclusion is the fact that mycotoxin formation does not take place in agricultural products such as ground nuts, cotton seed and maize, in spices such as red pepper and mustard and in dried fruits such as figs only after they are harvested, it also occurs before they are harvested. The critical stages after harvesting are drying and storage, but actual contamination takes place before harvesting, while the product is still ripening. As has been stressed by many researchers, one of the major factors in aflatoxin formation is the stress period caused by drought at the end of the season (Dorner *et al*., 1992; Park, 2002b). Taking this finding as a starting point, a 'biocontrol method based on biological competition' has been developed (Dorner *et al.,* 1992).

1.5.1 Preharvest controlling

Many types of mould produce mycotoxins which are toxic for human beings, warm-blooded animals and birds under suitable conditions (Moss, 1998). Although the presence of mould does not always indicate the presence of mycotoxins, it signals a mycotoxin hazard. From time to time the presence of mycotoxins in the form of aflatoxin and ochratoxin is encountered in spices and herbs as well. Incidence of infection with mycotoxygenic mould is high in physically damaged products which have been in contact with the soil. Formation of mycotoxin, with certain exceptions, usually commences at the drying stage following harvesting in red pepper and mustard (mycotoxin formation has also been observed in these products before harvesting) – and continues throughout the storage and transportation periods as well. For this reason it is vital to prevent contact of the product with the soil during harvesting and drying to avoid mycotoxin formation. Prevention of damage by vermin, insects and other similar harmful agents, adherence to the rules of hygiene, rapid and effective drying must also be ensured. Although, following an effective drying process, stability related to the reduction in water activity is achieved in microbiological terms in spices and herbs, transportation and storage are other stages which need to be borne in mind.

Storage conditions, particularly if the product is stored in heaps, encourage the development of mould. The product can become completely contaminated with mould and thus be rendered totally unsuitable for consumption. In certain situations, although development of mould is not observed, mycotoxins may be present in large quantities. It is for this reason that the practice of storing herbs and spices in heaps should be abandoned. In addition, it should not be forgotten that mycotoxin control can only be achieved by means of systematic work among different disciplines. Good agricultural practices, (GAP), good manufacturing practices (GMP), good hygiene practices (GHP) and hazard analyses critical control points (HACCP) systems must be implemented.

1.5.2 Technological methods

Work done to bring mycotoxins under control and the latest information on the methods developed are explained below.

Controlling mycotoxins by microorganisms

Two different strategies can be applied to control mycotoxins in the substrate by

microorganisms. First of all specific microorganisms which possess the ability to eliminate mycotoxins from contaminated substrates can be added. Second, atoxigenic mould species inoculated to the soil prevent mycotoxin production by toxigenic species before harvest. Removing mycotoxins by microorganisms from contaminated foods or feeds is one promising approach to be considered. Several bacteria (Ciegler *et al.,* 1966; Line *et al.,* 1994; El-Nezami *et al.,* 1998; Oatley *et al.,* 2000; Haskard *et al.,* 2001), yeast (Yiannikouris *et al.,* 2004a,b), mould (Varga *et al.,* 2000) and even protozoa (Kiessling *et al.,* 1984) have been used to remove various type of mycotoxins from different substrates. However, the mechanisms by which mycotoxins are eliminated, which vary according to the type and the number of the organisms (El-Nezami *et al.,* 2002a) involved, and the pH of the substrate (Haskard *et al.,* 2001) are still being investigated.

The first bacteria reported to remove aflatoxin from solution was *Flavobacterium aurantiacum* (Ciegler *et al.,* 1966). *F. aurantiacum* NRRL B-184 degrades aflatoxin B_1 in liquid medium as well as in several types of food (corn, peanuts, corn oil, milk, soybeans, peanut milk, and peanut butter) (Hao and Brackett, 1988; Line and Brackett, 1995). The bacterium actually metabolises the toxin to water-soluble and chloroform-soluble degradation products and CO_2 (Line and Brackett, 1995). Line *et al.,* (1994) reported that dead *F. aurantiacum* cells bind some aflatoxin but are unable to further degrade their water-soluble compounds or carbon dioxide. They also reported that a high population of cells (ca. 1×10^{10} CFU/ml) was necessary to effect degradation (Line *et al.,* 1994). Smiley and Draughon, (2000) studied the mechanism of degradation of AFB_1 by *F. aurantiogriseum* and reported the crude protein extract of the bacterium to bind AFB_1, suggesting the mechanism to be enzymatic.

Specific lactic acid bacterial strains remove toxins from liquid media by physical binding (Haskard *et al.,* 2001). *Lactobacillus rhamnosus* strain GG (LGG) removed AFB_1 (Haskard *et al.,* 2001) and ZEN (El-Nezami *et al.,* 2004) from solution most effectively. Surface components of these bacteria are involved in binding (Haskard *et al.,* 2001). Haskard *et al*. (2001) suggested that binding of aflatoxin B_1 appears to be predominantly extracellular for viable and heat-treated bacteria. Acid treatment may permit intracellular binding. Lahtinen *et al.,* (2004) also investigated the AFB_1 binding properties of viable *L. rhamnosus* and suggested that cell wall peptidoglycan, or components bound covalently to peptidoglycan, are important for AFB_1 binding. It was found that other carbohydrates such as teichoic acid (Knox and Wicken, 1973) and exopolysaccarides existing in the cell wall have no positive role for binding aflatoxin as well as cell wall proteins, Ca^{+2} or Mg^{+2} (Lahtinen *et al.,* 2004). The researchers suggested that the use of lactic acid bacteria had been recommended as a method for removing aflatoxins from food and feed (El-Nezami *et al.,* 2002a,b; Pierides *et al.,* 2000, Haskard *et al.,* 2001).

Aflatoxin was not the only mycotoxin removed from substrates by lactic acid bacteria, but also common *Fusarium* toxins such as trichothecenes were also removed by *Lactobacillus* and *Propionibacterium* (El-Nezami *et al.,* 2002a). The researchers indicated that significant differences exist in the ability of the bacteria to bind tricothecenes *in vitro* (El-Nezami *et al.,* 2002a). Several reports describe the OTA degrading activities of the microbial flora of the mammalian gastrointestinal tract, including rumen microorganisms of the cow and sheep, and microbes living mainly in the caecum and large intestine of rats. The human intestinal flora can also partially degrade OTA (Varga *et al.,* 2000).

The cell wall fraction of *Saccharomyces cerevisiae* represented 13.3–25.0% of the

dry weight of the total cell and was composed of various glucan, mannan and chitin contents (Yiannikouris *et al.,* 2004a). Among the cell wall components β-D-glucans were the main molecules responsible for ZEN adsorption. Weak noncovalent bonds (hydrogen bonding reactions) are involved in the complex-forming mechanisms associated with ZEN. The chemical reactions between β-D-glucans and zearalenone are therefore more of an adsorption type than a binding type (Yiannikouris *et al.,* 2004a).

An atoxigenic *A. niger* strain was found to decompose OTA in both liquid and solid media, and the degradation product, ochratoxin α was also decomposed. (Xiao *et al.,* 1996; Varga *et al.,* 2000). *A. niger* secreted carboxypeptidase which could decompose OTA to ochratoxin α and phenylalanine (Varga *et al.,* 2000). This method might allow the elimination of OTA from solid substrates such as green coffee beans and cereals (Varga *et al.,* 2000).

Control of mycotoxins by means of biocontrol based on biological competition is implemented before harvesting for products such as ground nuts, cotton seed and maize in particular. Implementation was described as follows (Dorner *et al.,* 1992); an *A. parasiticus* strain, which does not produce toxin but has extremely competitive features, is added to the soil. The mould becomes dominant in the soil microflora and replaces the *A. flavus/parasiticus* strain, which is a natural producer of aflatoxin, thus preventing its development. Thus, groundnuts exposed to the stress of end-of-season drought are also exposed to the attack of the dominant competitive mould. However, due to the fact that the mould does not form a toxin, no aflatoxin is formed in the product, or is formed in smaller quantities. In research carried out in the three-year period between 1987 and 1989, it was observed that while in groundnuts grown in soil in which no implementation had taken place, aflatoxin quantities were 531, 96 and 241 ppb; in products raised in soil injected with non-toxin-producing mould the quantities were low, being 11, 1 and 40 ppb respectively (Dorner *et al.,* 1992). The research indicated that the biological control method was applicable in pre-harvesting control of aflatoxin contamination and that it possessed a potential which could be of assistance in obtaining a product free of aflatoxin or containing a smaller quantity of it.

Various binding agents were added to the feed, thus binding the aflatoxin, and reducing the amount of aflatoxin absorbed by the gastrointestinal tract, decreasing aflatoxin intake and bioavailability. Phillips *et al.,* (2002) stated that processed calcium montmorillonite clay (HSCAS) was a powerful agent binding the AFB_1 and that addition of 0.5% w/w or lower-quantity HSCAS to poultry-feed would cause no adverse effects.

The effect of thermal processing on mycotoxins

The effects of thermal degradation at high temperatures vary according to the type of mycotoxin. While the heat applied during cooking processes commonly applied at home (roasting, frying, boiling) results in thermal degradation of some mycotoxins, it has no effect on aflotoxins, neither does it degrade AFB_1 and AFG_1 (Park, 2002b). The temperature required for partial degradation and thus thermal inactivation of the aflatoxin must be over 150 °C (237–306 °C). Other factors contributing to the degree of thermal inactivation of mycotoxins by means of roasting are the initial contamination level, moisture content of the product, temperature and duration of roasting. The type of food and the type of aflatoxin also affect the level of degradation and inactivation (Rustom, 1997). While the presence of water in the environment aids the inactivation

of aflatoxin, the presence of salt delays inactivation. While water leads to the opening up of the lacton ring of AFB_1, it also leads to the formation of carbolic acid; however, the ionic salts lengthen the duration of the inactivation process (Rustom 1997). Roasting is a good method for reducing aflatoxin levels in certain commodities, i.e., oil and dry-roasted peanuts, microwave-roasted peanuts (Park, 2002b). In the study made of samples of red pepper flake obtained from different regions as well, no mould or aflatoxin was encountered in the samples of red pepper flake which are roasted in oil and known as 'isot' (Heperkan and Ermiş, 2004).

When the effect of thermal processing on other mycotoxins apart from aflatoxin is studied, it is observed that DON, FUM and ZEN are resistant to thermal processing. DON is known to be stable up to 170 °C at neutral to acidic pHs (Wolf-Hall and Bullerman, 1998). Baking has been shown to cause little or no effect on DON levels in flour and dough (Trigo-Stockli, 2002). Seitz *et al.,* (1986) stated that, with cooking, the DON concentration in dough was reduced by 20–40%. (DON concentration in dough 0.2–0.9 mg/kg flour). On the other hand, Scott *et al.,* (1984) stated that little or no reduction in DON concentration took place in the DON concentration of bread made from flour with a DON concentration of 1–7 mg/kg. Roasting of wheat contaminated with 30 mg/kg DON using a commercial gas-fired roaster was shown (Stahr *et al.,* 1987) to reduce DON levels by 50% (Trigo-Stockli, 2002).

Bullerman *et al.,* (2002) reported that although generally heat stable, fumonisin concentrations appear to decline as processing temperatures increase. At processing temperatures of 125 °C or lower, losses of fumonisin are low (25–30%), whereas at temperatures of 175 °C and higher, losses are greater (90% or more). Processes such as frying and extrusion cooking, where temperatures can exceed 175 °C, result in greater loss (Bullerman *et al.,* 2002).

ZEN is known for its marked heat stability. In general, thermal processing was not effective in reducing ZEN. However, use of heat in combination with pressure during processing (extrusion cooking) resulting in substantial losses of ZEN in corn (Ryu *et al.,* 2002). Ryu *et al.* (1999) reported that the amount of reduction in ZEN in spiked corn grits ranged from 66–83% at temperatures of 120–160 °C. The moisture content of the grits (18–26%) had no significant effect on reduction of ZEN during extrusion. Flame roasting of naturally contaminated corn (0.02–0.06 μ/g) at temperatures of 110–140 °C reduced the concentration of ZEN by 50% (Hamilton and Thompson, 1992).

Citrinin is more sensitive to heat in comparison with other mycotoxins. At the same time, it has been observed that exposure to UV light resulted in a certain reduction of citrinin activity (Frank, 1992). Therefore thermal processing can be an effective method in citrinin detoxification (Kitabatake *et al.,* 1991). Decomposition and detoxification of citrinin can be realised under dry conditions with heat processing at 175 °C. Under moist conditions temperature of detoxification can be reduced to 35 °C, but when citrinin is thermally treated under these conditions additional toxic compounds are formed. One of these is citrinin H_1, which is more toxic then citrinin (Fouler *et al.,* 1994).

In recent years studies have been made of the effects of cooking in microwave ovens; it has been established that the power of the microwave, duration of thermal processing and the presence of water in the environment results in a decline in mycotoxin quantities. It is considered that thermal effects play the most important role in the inhibition of microorganisms, that in the absence of thermal effect microwave

energy does not render microorganisms inactive, but at the same time enhances and complements the thermal effects (Mertens and Knorr, 1992).

The effects of irradiation on mycotoxins

As the irradiation process depends on the dose applied, the type of the product, of the moulds and their number, it has a preventative action on the development of moulds. Doses of 1–3.5 Gy irradiation delayed the growth of moulds such as *Penicillium expansum* on some fresh fruits (Tiryaki *et al.,* 1994). Wolf-Hall and Schwartz (2002) reported that *Fusarium* survival decreased on malting barley by approximately 78% at 10 kGy using electron beam irradiation. However, researchers drew attention to the following subject; in the course of prevention of mould development, sub-lethal or inhibitory concentrations of chemicals may prevent fungal growth, but actually stimulate mycotoxin production (Wolf-Hall and Schwartz, 2002). In the same way, it was established in research where the effects of irradiation on *Aspergillus flavus* and *A. parasiticus* were studied, that the aflatoxin-producing characteristics of surviving isolates in irradiated cereals were enhanced (Moss and Frank, 1987). Gamma irradiation (2.5 Mrad) did not significantly degrade aflatoxin in contaminated peanut meal (Feuell, 1996). Ochratoxin A is also stable to gamma ray irradiation at a dose of 7.5 mrad (75 kGy) (Paster *et al.,* 1985). The high cost of equipment, limited positive results and lack of consumer acceptance of the irradiation process, are disadvantages of this method as a commercial application (Park, 2002b).

How chemicals affect mycotoxins

A great deal of work has been done on the effects of such chemicals as ammonia (Park *et al.,* 1992), hydrogen peroxide (Clavero *et al.,* 1993), calcium hydroxide (Charmly and Prelusky, 1994), sodium bisulphite (Accerbi *et al.,* 1999) and ozone (McKenzie *et al.,* 1997) on mycotoxins, but although positive results have been obtained, it has been observed that these substances would lead to loss of certain characteristics in agricultural products and thus render them unfit for consumption; at the same time, it has been established that certain chemicals form more toxic reaction products than the existing mycotoxin and thus their use was limited. It has been reported that certain food compounds and additives are effective against mycotoxins and that they do not lead to any changes in the structure and nutritive qualities of the foodstuff. The effect of ammonium peroxidedisulphatine on aflatoxins has been cited as an example (Tabata *et al.,* 1994).

Mycotoxins such as Aflatoxin B_1, Fumonisin B_1, T2 toxin and Ochratoxin A enhance lipid peroxidation and result in membrane damage in living organisms. Selenium, vitamins A, C and E, act as superoxide anion scavengers due to their antioxidising effects and protect the organism from the harmful effects of mycotoxins (Rustom, 1997).

Biotechnological approaches

Increased interest has been observed in the use of biotechnological methods in the development of plant defence against mycotoxin-forming (and at the same time) pathogenic moulds, together with plant-improvement work. Many new techniques in transgenic approaches in particular, and in marking of molecules have been developed and are in use; thus, the numbers, locations and effects of resistant or target genes can be assumed. The effects of mycotoxins can also be neutralised by means of anti-fungal proteins, binding and carrying of molecules are also prevented. For example,

in the fight against head blight in wheat, caused by *Fusarium* species, selection of resistant genes can be realised with the aid of marked molecules at very early stages such as the seed-sowing stage (Miedaner, 2004). Similar research has been concentrated on fungal pathogenity and host defence mechanisms, and antifungal protein originating in plants and microorganisms has been transferred to wheat. Work was done on plants such as rice and barley and on microorganisms for the chitinase enzyme and glucanase genes which degrade the cell walls of the fungus in particular (Miedaner, 2004). Work is continuing on genes that code antifungal proteins such as osmotin, which prevents the pathogenic fungus from affecting the plant (Miedaner, 2004). In a similar manner, marked DNAs are used in order to establish plant resistance emerging at a later stage under the influence of environmental factors. Use is made of different sources instead of one single donor to obtain resistant genes (Paul *et al.,* 2002; Buerstmayr *et al.,* 2003; Miedaner, 2004). Other methods which can be used in mycotoxin control are neutralisation of effect, acceleration of flow of carrier proteins, destruction of the mycotoxin molecule and making changes in the target (Miedaner, 2004).

1.5.3 Regulatory aspects for herbs and spices

As it is not always possible to prevent formation of mycotoxins, which have been proved to be a health hazard, the aim is to ensure that products with the lowest possible mycotoxin contents reach consumers. These considerations have resulted in a further lowering of maximum permissible mycotoxin values for agricultural products in the European Union (Adams and Whitaker, 2004). Approximately 90 countries have regulations that establish maximum aflatoxin limits in food and feed products. Regulations and limits vary from country to country (Adams and Whitaker, 2004). Mycotoxin limit values in the United States are approximately five times in excess of EU limits, but it has been reported that work is in progress to reduce these values. Maximum permitted mycotoxin values in spices in the EU are 5 ppb for aflatoxin B_1 and 10 ppb for total aflatoxin (OJEC, 2002).

1.6 Future trends

A great deal of intensive research has been done during the 46 years that have passed since the presence of mycotoxins was first established in poultry in Britain in 1960. Specific reliable new methods and techniques have been developed which enable results to be obtained at low detection levels and short periods of time in detection of mycotoxin in plant and animal products, body fluids such as milk and blood. Much progress has been achieved in the field of biotechnology as well, the location and characteristics of mycotoxin-producing genes having been established and transgenic products partially resistant to mycotoxin formation generated. Another extremely important development is the positive results obtained from work on the addition of various biological and non-biological (clay-based) binding agents to food or feed, thus binding the mycotoxins and reducing their absorption and bio-utilisation by the body. Thus, it will be possible to reduce the amount of mycotoxin to which the body is exposed. However, in spite of all these favourable developments mycotoxin continues to be a serious hazard in certain products.

There is a need for more research into biomarkers, which enable constant monitoring of mycotoxin. Thus, effective techniques enabling toxin-contaminated products to be separated at source could also be developed. Another important matter, particularly in developing countries, is to do something about the present lack of effective organisations bringing information to farmers and to ensure inter-disciplinary collaboration in this respect. The setting up of international working groups would be extremely useful in terms of achieving a regular exchange of information concerning mycotoxins.

1.7 Sources of further information and advice

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2

Controlling pesticide and other residues in herbs and spices

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2.1 Introduction

Spices have occupied the centre stage of world trade since time immemorial. Many Europeans travelled to such distant countries as India and China in search of spice commodities. During the period from the 15th to the 17th centuries, the Spanish, the English, the Portuguese, and the Dutch traders competed for prominence in the spice trade in the Far East, and by the 19th century, America had also entered the spice trade. It was a tradition for many families in the colonies of those times to have their own herb and spice gardens. Herbs were consumed for medicinal and culinary purposes, apart from their use as preservatives.

Quality standards in spice trade had taken a definite shape by the 1800s and many improved processing techniques were put in place. During the early 1900s, spice brands such as Golden Rule, Watkins, Raleigh, and McNess were well known among the trade community and consumers in the west. Over a period of time, the international market witnessed sweeping changes with regard to the quality of spices and herbs. New food safety systems and good manufacturing practices (GMP) based on hazard analysis and critical control points (HACCP) influenced the traceability and safety of ingredients used. Before the era of globalisation and liberalisation, exporters had to comply with the pre-shipment inspection and quality specifications prescribed by various governmental agencies. Post liberalisation, as trade barriers started to ease, pre-shipment inspection and quality control were withdrawn and the exporters became free to export products according to the specifications prescribed by the importing countries.

The most popular standard for whole spices and herbs is the *ASTA-USDA cleanliness specifications for spices, seeds and herbs*. Since the beginning of 1990, this has been an international standard for cleanliness, and major producing countries have aligned their supplies to meet the requirements of this standard. The European Spice Association (ESA), comprising the members of the European Union, has brought out a *Quality Minima for Herbs and Spices*, which serves as a standard for individual member countries of the European Union. Apart from this, individual member countries like

the UK, Germany and the Netherlands have laid down their own cleanliness specifications for spices. In addition to the cleanliness specification, importing countries insist on meeting specified limits for chemical parameters like pesticide residues, aflatoxin, heavy metals as well as microbial contamination. While the USA, Japan and the individual member countries of the EU have prescribed MRLs (maximum residue limits) in spices, the European Union has not prescribed specific limits for pesticide residues in spices and spice products.

For any exporting country in this international business, there has to be an overall strategy to cope with such demanding quality standards. The strategy has to be comprehensive, with sufficient attention given to such factors as insulation from commodity price fluctuations, improvements in productivity, reduction in costs of production, investment in state-of-the-art processing facilities, control of chemical residues by means of corporate/contract farming, and diversifying and expanding products into value-added areas. Despite the rising quality standards of importing countries, developing countries like China and Vietnam have exhibited exponential growth in their spice exports. Traditional spice exporters like Indonesia and Mexico have also shown positive growth rates in the past few years. The challenge ahead is in exploring the potential of value-added spice products like retail packs, seasoning blends, marinades, dressings, relishes, dips, dehydrates, etc., in addition to exploring new developments in natural colours and flavours, nutraceutical and other new applications.

Before finding their way to the product shelf in the market, new applications need to go through the hurdle of regulatory approval. This is tough indeed, more so because the laws and regulations of importing countries are varied. The United States, the largest consumer of nutraceutical food and beverages, has been evolving regulatory strategies to counter false claims, at the same time minimising the infrastructural delays. The FDA regulates food products depending on how they are classified. There are two categories under the FDA directive: conventional foods that are consumed for aroma, taste and nutrition, and the dietary supplements which are consumed for health benefits. The FDA does not have a regulatory category for functional foods, so these foods have to be marketed as either of the above. Further, the FDA has categorised new products into two groups: having qualified or unqualified health claims. The qualified new products should have more studies supporting the health claim, which are not likely to be reversed by future studies. Examples being folate, folic acid, omega 3 fatty acids, phosphatidylserine, antioxidant vitamins, etc. The unqualified products are those that have potential health benefits based on significant scientific agreement (e.g. calcium for osteoporosis, dietary fat and cancer, fibre-containing vegetable and cancer, plant sterols – plant stanol and heart disease). The labelling should ensure that the classified claims are depicted correctly.

2.2 The regulation of pesticide residues

Pesticides are a group of chemicals designed to control weeds, diseases, insects or other pests on crops, landscape plants or animals. The most commonly used pesticides are insecticides (for controlling insects), fungicides (for controlling fungi) and herbicides (to control weeds). Prudent use of pesticides has played a vital role in feeding the world's growing population by dramatically increasing crop yields. However, their safety and effects on the environment have been a serious concern. National regulations

have tried to standardise permitted residue levels by product category. As the range of herbal products continues to grow, this has become an increasingly difficult task. At the same time, new cultivation techniques are evolving to increase productivity of high-quality raw materials with a higher content of active ingredients, resulting in increasing use of chemicals to boost yields and control pests. The high-yielding hybrid varieties are often more susceptible to pest attack, and hence require greater use of pesticides.

Regulations covering the use of pesticides are based on data generated by environmental impact assessment (EIA) systems, which compare the characteristics and effects of different pest control systems and generate an index or ranking of pest control options. These types of assessment tools are also called pesticide risk indicators. There are three categories of assessment system:

1. Those that aid farmers/growers and other land managers.
2. Research and policy tools for use by governments, industry or academia.
3. Eco-labelling systems designed to influence consumer opinion and market behaviour.

The methodologies employed by the EIA include simulation of environmental effects (e.g. by computer modelling), sampling, monitoring and tracking changes in biophysical indicators (such as species diversity, soil respiration rate, and chemical levels in the environment), surveys and qualitative research methods, and indexing or ranking the extent and severity of pesticide (both chemical and non-chemical pest controls) impacts on one or more environmental indicators.

One of the primary objectives of assessing the environmental impacts of agriculture is to choose those pest control practices that have the least negative impacts on the environment, and on human health and safety. Policy makers then need to make broad-brush appraisals of the impacts of such choices. Today, the challenge before them is more complex as the number of chemical classes of pesticide has quintupled from approximately 25 in the 1970s to about 130 in 1990s, and the modes of pesticide activity affecting the environment have also diversified. In the USA, approval for the use of pesticides is given by the U.S. Environmental Protection Agency (EPA). The EPA is authorised by law to regulate the development, distribution, use and disposal of pesticides. Before approving or registering a pesticide for use in agriculture, the EPA normally requires close to 120 different tests – depending on the uses of the pesticide – to determine its safety. The agency registers only those pesticides that meet their standards for human health, the environment and wildlife. If new research shows that any registered pesticide does not meet their standards, the EPA can cancel or modify its use. While approving a pesticide, the EPA specifies instructions for its use on the label, which must be followed by law. The agency also establishes a tolerance (maximum residue level of a pesticide legally permitted in or on a food) for each pesticide it approves. The tolerance ensures that, when pesticides are used according to label directions, the residues will not pose an unacceptable health risk to anyone, including infants, who consumes the food. Tolerances are considered an enforcement tool and are used by the FDA in its monitoring program to ensure a safe food supply. If any pesticide residue is found to exceed its tolerance on a food, then the food is not permitted to be sold.

The Food Quality Protection Act, signed into law in 1996, sets an even tougher standard for pesticide use in food. The EPA will consider the public's overall exposure to pesticides (through food, water and in home environments) when making decisions

to set standards for pesticide use in food. Such new standards are intended to protect infants and children who may be more vulnerable to pesticide exposure. To determine pesticide safety for humans, the EPA establishes a reference dose (RfD) for each pesticide that is approved for use. The RfD is the amount of a chemical that, if ingested over a lifetime, is not expected to cause any adverse health effects in any population subgroups. Using food consumption and other data, the EPA estimates how much pesticide residue is likely to be consumed, and if the RfD is exceeded, the agency takes steps to limit the use of the pesticide. To monitor the food supply for pesticide residues, the FDA enforces pesticide tolerances for all foods (except for meat, poultry and some egg products, which are monitored by the USDA). Laboratory equipment used by these agencies usually can detect residues at one part per billion or lower. Over the years, the FDA and other monitoring agencies have concluded that pesticide residues in the food supply are well below established safety standards. Many independent health experts who have examined studies on the effects of pesticides in the diet have also concluded that the benefits of a diet rich in fruits and vegetables far outweigh any pesticide-related risks. A 1996 report by the US National Academy of Sciences concluded that both synthetic and naturally occurring pesticides are consumed at such low levels that they pose little threat to human health.

Herbs and spices, in general, do not pose a high risk with regard to the presence of pesticide residues basically because the total daily intake is very small. Further, usage of spice and herbs at home and by food manufacturers involves such processes as washing, peeling, cooking, canning, freezing and drying which decrease the residue levels. Above all, usage of pesticide chemicals in agriculture and storage of most spices are very limited. Today, most food manufacturers monitor farmers' use of pesticides to ensure the raw ingredients they buy meet strict quality assurance standards.

2.3 Analytical methods for detecting pesticide residues

Monitoring and measuring residue levels is a critical stage in the control of chemical residues. Pesticides and other chemicals occur in spices and herbs only in trace levels (generally at concentrations of parts per million). Measuring such small amounts in the presence of enormous amounts of other chemicals that occur naturally in them is a challenge, because these plant chemicals may interfere with measurement. A variety of analytical methods are currently used to monitor pesticide residues, all of which contain the following basic steps:

- sample preparation: by chopping, grinding, or separating herbal plant parts
- extraction: removal of a pesticide residue from other herbal components
- clean-up (isolation): removal of constituents that interfere with the analysis of the pesticide residue of interest; this step includes partitioning and purification
- determination-separation: separation of components, i.e., individual pesticides, and sample co-extractives, based on differential partitioning between a solid and non-volatile solvent or between a liquid and gas carrier that moves through a column (liquid and gas chromatography) or along a coated plate (thin layer chromatography)
- determination-detection: production of a response that measures the amount of components moving through the column, allowing detection and quantification of each pesticide.

The first step in analysing a spice/herb sample is to chop and grind the sample. The samples must be handled in such a way as to avoid loss of volatile pesticide residues and to prevent contamination of the sample with other pesticides or interfering chemicals. Chopping or grinding followed by blending and mixing are manipulations designed to produce a homogeneous composite sample from which sub-samples can be taken, and to disrupt the gross structural components of the sample to facilitate extracting pesticides from the sample. Once the sample is prepared, extraction is performed with a solvent to remove the pesticide residue of interest from other components of the sample. In most analytical laboratories, a solvent such as acetone or acetonitrile is used to extract pesticides from 250 grams or less of the spice/herb to be analysed. The solvent is blended with the sample, and smaller amounts are further homogenised using an ultrasound generator. Salts, such as sodium chloride or sodium sulphate, can be added to absorb water. Or, additional water is added, so that the resulting aqueous solution can be partitioned with a water-immiscible solvent in a subsequent cleanup step.

Extraction times vary from a few minutes to several hours, depending on the pesticide to be analysed and the sample type. After putting the sample through an alumina packed column, solvent is added to elute the pesticides off of the packing in the column. The cleanup step is often a limitation in pesticide residue methods because it generally consumes a large amount of the total analysis time and restricts the number of pesticides that are recovered in some cases, as a result of losses in chromatography, partitioning, and other cleanup steps. Problems that occur during the extraction process include incomplete recovery and emulsion formation. Incomplete recovery generally can be remedied by selecting a more efficient solvent. Emulsions are the production of a third phase or solvent layer that confuses the partitioning process. They can usually be broken down by adding salt to the sample/solvent combination.

Super-critical fluids (SCFs) provide a new technique for extracting pesticides. They are fluids that are more dense than gases but less dense than liquids. They are not yet used in regulatory methods to analyse pesticide residues in food, but are gaining favour for their ability to extract a wide variety of chemicals from many sample types. Solid phase extraction (SPE – also known as accumulator or concentrator columns) is another technique that can speed up cleanup as well as extraction. The SPE packing materials or cartridges retain the pesticide. These cartridges also have the advantages of batch sample processing capabilities, small size, adaptability to robotic technology, low cost, and ready availability from many sources. SPEs have the disadvantages of being unproven for many pesticides, inability to handle large sample sizes, and generally ineffective for extracting water soluble pesticides and metabolites. SPE is being used by industry and private laboratories, but is not yet routinely used by regulatory agencies to a significant extent. Some FDA laboratories use SPE to clean up extracts before the detection step to protect the column used in high-performance liquid chromatography (HPLC). After a pesticide has been extracted and isolated from the sample by a combination of the above-mentioned techniques, it is further separated from other co-extractives, usually by either gas chromatography or liquid chromatography or, less frequently, by thin layer chromatography.

Gas chromatography (GC) has been a dominant technique for separation, with at least 40 years of development and refinement. Most multi-residue methods (MRMs) used by the FDA and USDA and many single-residue methods (SRMs) are based on GC. In a gas chromatography setup, separation of pesticides and sample co-extractives

occur in analytical columns. Historically, five detectors have been used. They are the electron capture detector (ECD), Hall micro-electrolytic conductivity detector (HECD), thermionic detectors (NPD and AFID), and the flame photometric detector (FPD). ECD measures the loss of detector electrical current produced by a sample component containing electron-absorbing molecule(s). This detector is very sensitive for measuring halogenated pesticides, in the analysis of chlorinated hydrocarbon pesticides (organochlorines) such as aldrin, dieldrin and DDT. ECD is efficient for the analysis of poly chlorinated biphenyls (PCBs) as well. The HECD can measure chlorine (and other halogens), nitrogen, or sulphur. This detector is more selective than the ECD, though the ECD is more sensitive. The Hall electrolytic conductivity detector also has improved over the last few years, and has replaced the ECD in those laboratories where extreme sensitivity is not required. Both the NPD and AFID measure the presence of nitrogen and phosphorus atoms in the pesticide, with little response resulting from other types of atoms in the molecules.

Today, the flame photometric detector (FPD) measures sulphur or phosphorus, and is a rugged, highly stable, and very selective detector, since it does not detect compounds other than organophosphates and those containing sulphur. The flame photometric detector is less sensitive for phosphorus than the NPD and less sensitive for sulphur than the Hall detector. However, it is useful for the analysis of unclean crude herbal extracts. Conventional mass spectrometers (MS) have also been used by some pesticide residue laboratories as gas chromatography detectors, and as high-performance liquid chromatography detectors as well. MS is normally used when special techniques are necessary to confirm the identity of a particular pesticide, when conventional detectors cannot detect the pesticide. The use of MS is growing, especially with the development of the more portable and less costly mass selective detector (MSD). The MSD and ion trap detector (ITD) may become more routinely used for pesticide residue analysis, as improvements in their computer software are made and their scan parameters become more suitable for chromatography.

High-performance liquid chromatography (HPLC) for the analysis of pesticide residues is a fairly recent technology, but it is becoming the second most frequently used technique after GC. GC depends upon the volatilisation of the pesticide, whereas HPLC is dependent on the stationary phases that can selectively retain any molecular structure; polar, non-polar, ionic, or neutral. Separations can even occur as a function of molecular size (gel permeation) or chemical derivatisations (synthesis of a chemical derivative of the pesticide). HPLC is not as efficient as capillary gas chromatography for separator purposes because the chromatographic peaks are broader, though HPLC columns are more efficient than packed GC columns when columns of equal length are considered. HPLC columns usually last longer because they are not subjected to the extremely high temperatures that GC columns are. The HPLC detectors used for pesticide residue analysis are the UV absorption, fluorometer, conductivity, and electrochemical.

Many pesticides absorb UV light at the wavelength of mercury discharge (254 nanometres) and can be detected in very small quantities. Unfortunately, many food co-extractives do so as well, making this detector nearly useless for trace analysis in foods. An alternative is the variable wavelength detector, which can be tuned to a wavelength that is absorbed by the pesticide but not by the food co-extractives. The fluorometer is a highly sensitive HPLC detector for some pesticides, which is typically used for pesticides with aromatic molecular structures such as alachlor or paraquat. This detector, however, has limited application to the detection of most pesticides

(which do not fluoresce appreciably). For compounds having photo-ionisable functional groups, the photoconductivity detector is especially advantageous over UV detectors. It has been well studied and used by FDA and other laboratories for residue analysis. The electrochemical detector is also under study for its potential to improve detection of electro active functional groups.

The thin layer chromatography (TLC) technique is based on partitioning a pesticide between a solvent and a thin layer of adsorbent, which is usually silica or alumina oxide that has been physically bonded to a glass or plastic plate. Samples are applied, dissolved in a solvent, as spots or bands at one edge of the plate and the plate is then placed in a tank containing a solvent. The solvent migrates up the plate by capillary action, taking the pesticide with it, and depositing it at a given distance on the plate. The time required for TLC plate development ranges from a few minutes to several hours depending on the pesticide, the solvent, and the adsorbent. Following complete development, the plate is removed from the tank and the spots or bands left by the migration of the solvent are detected using any one of several techniques available such as visualisation under UV light, using reagents to produce colours resulting from chemical reaction specific for the pesticide/reagent combination. Amounts of pesticide can be judged semi-quantitatively by comparison with standards that are developed on the same plate as the unknowns. As a separator technique, TLC is much less efficient than either GC or HPLC because the resolution separated by TLC is approximately less than one-tenth of that found using a packed GC column to produce the same separation. Consequently, TLC as a separator technique has largely been replaced by GC and HPLC. On the other hand, interest exists in using TLCS to develop rapid, semi-quantitative methods.

For regulatory agencies like the FDA and the FSIS, the monitoring methods must provide results in a cost-effective, timely, reliable, and verifiable manner. These methods should also identify as many pesticides as possible in a range of food commodities because these agencies are responsible for monitoring all foods for all pesticides to keep the products containing higher levels from reaching the market. Analytical methods must also be able to detect pesticides at or below tolerance levels, and endure interfering compounds such as other pesticides, drugs, and naturally occurring chemicals. They should be insensitive to such environmental variations as humidity, temperature and solvent purity as well. There are different classes of methods that are used by the regulatory bodies, each method selected based on the need of the monitoring, type of sample, and sensitivity required. They are multi residue, single residue and semi-quantitative methods.

Multi-residue methods (MRMs) are designed to identify a broad spectrum of pesticides and their toxicologically significant metabolites simultaneously in a range of foods, and mostly meet the method needs of regulatory agencies. They are sensitive, precise, and accurate enough, and are economical or affordable. In addition, an MRM may detect, but not measure, a particular pesticide or metabolite, and also record the presence of unidentified chemicals, known as an unidentified analytical response (UAR). MRMs involve steps of preparation, extraction, cleanup, chromatographic separation, and detection. All MRMs used today in the USA are based upon either gas chromatography (GC) or high-performance liquid chromatography (HPLC) as the determinative step, while thin layer chromatography (TLC) is also used by several agencies in Europe. The basic weakness of MRMs is that they cannot detect every pesticide. For example, of the 316 pesticides with tolerances, only 163 could be analysed with FDA's five routinely used MRMs. Another weakness is that some

MRMs require a great deal of time to perform, thereby reducing the number of samples analysed and the speed of analysis. For example, certain foods, such as those with high concentrations of fats and oils, are difficult to analyse in a timely manner.

Single residue methods (SRMs) are another category that depend on a number of different techniques and vary widely in terms of reliability, efficiency, throughput (samples per day), degree of validation, and practicality for regulatory use. Because SRMs have been developed by the private sector for submission to EPA as part of the tolerance setting process, a method exists for every pesticide with a tolerance. Most SRMs, like MRMs, are based on GC using the full array of element specific detectors. Although less efficient than MRMs, SRMs are necessary to monitor those pesticides that cannot be detected by MRMs. SRMs are generally not considered adequate for routine monitoring by the regulatory agencies, though FDA uses them. To monitor one pesticide with an SRM is considered inefficient when an MRM can measure many pesticides using the same resources. In addition, SRMs vary widely even for chemicals of the same class, so a laboratory needs a wide array of glassware, evaporative devices, chromatography, and detectors to use the SRMs available.

There is a third class of methods, namely the semi-quantitative and qualitative methods, that range widely in their ability to quantify the chemical present in a sample. Semi-quantitative methods indicate the range of pesticide residue concentration in a sample, while qualitative methods show whether or not a particular pesticide exists above detectable limits. These methods use technologies like thin layer chromatography (TLC), enzyme inhibition, and immunoassay, all of which can be moved from the laboratory into the field without losing their ability to detect pesticides. The enzyme inhibition-based colour reactions make spots and bands of pesticide residues on thin layer chromatographic plates visible, in order to measure the pesticide residue either visually or with instruments. Such techniques are being used for cholinesterase-inhibiting insecticides and photosynthesis-inhibiting herbicides. Because sophisticated instrumentation is not required they are relatively inexpensive compared to quantitative methods. The benefits of these methods are their low cost, speed, or ease of use and more number of samples that could be analysed. Nevertheless, neither FDA nor FSIS is currently using these methods for pesticides. A drawback of semi-quantitative methods is that they do not provide the degree of accuracy necessary for enforcement action, as in a court of law. Violations found by a semi-quantitative method would have to be verified by a quantitative analytical method – or maybe two.

As techniques are improved by changes in instrument and hardware design, bringing about more sensitive, selective, and reproducible devices, their costs usually increase, particularly when automated sample handling and data manipulation are included. These additional costs translate into higher costs to implement contemporary pesticide methodologies for varied herbal samples. Supercritical fluid chromatography (SFC) is a new technique of chromatographic separation used in the regulatory analysis of pesticide residues in food. With super fluids as the solvent phase, SFC can chromatograph chemicals that cannot be handled by gas chromatography because of their non-volatility or thermal instability. Many detectors designed for GC can also be used in SFC, such as the flame ionisation, the nitrogen-phosphorus, and the atomic emission spectrometric as well as the UV absorbance detectors. New analytical methods are needed to expand the range of pesticide analytes that can be detected in plant derived food products like herbals in a more efficient process.

Some of these advanced technologies include gas or liquid chromatography/mass spectrometry (or tandem mass spectrometry), solid phase extraction, laser-induced

and/or time-resolved fluorescence, field-based instruments, immunochemical assays, biosensors, and other techniques. Direct sample introduction for gas chromatography/tandem mass spectrometry (DSI/GC/MS-MS) is a novel approach for the analysis of multiple pesticides in a variety of herbal food matrices. This approach has the potential to make a major impact in the analysis of many types of pesticides and other semi-volatile chemicals in a variety of matrices in food.

Tandem Quadrupole LC and GC/MS/MS is another new MRM system being used for multiple pesticide residues. This method involves a less selective extraction and cleanup, and is particularly applicable to complex food matrices of spices like ginger, garlic, and herbs, where the selectivity is sufficient to allow generic sample cleanup, apart from providing a good sensitivity up to 10 pg on column for most pesticide residues. A UPLC (ultra performance liquid chromatography) method is also available now where the cycle time can be halved, and improved efficiency coupled with high sample throughput could be realised through a combination of new technologies that offer enhanced chromatographic resolution and short analysis time. In addition, it can group MRM functions into time windows enabling the incorporation of confirmatory MRM traces, and switch rapidly between MRM channels and between positive and negative ionisation modes. The newly developed travelling wave (T-wave) technology can prevent cross-talk even at very short cycle times. The T-wave is produced by application of a transient d.c. voltage with opposite phase to alternate plates thus creating a square wave which travels along the length of the collision cell.

2.4 Control of pesticide residues in herbs and spices

Monitoring usage of chemicals and their residue levels in raw materials and finished products sets up strategies for controlling them at farm level. There are different ways of usage control at farm level that, grouped together, are termed as farm management systems. These are basically tools to achieve supply of quality agro-products through sustainable programs of agriculture and farmer development. These systems ensure quality at source through superior seed varieties, modern and sustainable agricultural practices, and provide consistent raw material quality to improve process efficiencies. There are many options open to herb and spice processors under these systems, like corporate farming through own land/leased land, and contract farming with large/institutional bodies and small/medium/large farmers. The rural farmers and small-scale entrepreneurs lack both reliable and cost-efficient inputs such as extension advice, mechanisation services, seeds, fertilisers and credit, and guaranteed and profitable markets for their output. Well-organised contract farming provides such linkages apart from providing the investors with the opportunity to guarantee a reliable source of supply, from the perspectives of both quantity and quality.

Contracting of crops has existed from time immemorial. In ancient Greece, the practice, known as *hektemoroi* or 'sixth partner' was widespread, in which specified shares of particular crops were contracted for paying tithes, rents and debts. Such sharecropping was also practised in China during the first century. In the USA, by end of the nineteenth century, sharecropping agreements had been drawn that allowed for a specific share to be deducted for rent payment to the landowner. In the first decades of the twentieth century, formal farmer-corporate agreements were established in colonies controlled by European powers.

Contract farming is an effective means to develop markets and to bring about transfer of technical skills in a way that is profitable for both the sponsors and farmers. To be successful it requires a long-term commitment from both parties. Exploitative arrangements by managers are likely to have only a limited duration and can jeopardise agribusiness investments. Similarly, farmers need to consider that honouring contractual arrangements is likely to be to their long-term benefit. Contract farming is becoming an increasingly important aspect of agribusiness, whether the products are purchased by multinationals, smaller companies, government agencies, farmer cooperatives or individual entrepreneurs. The approach is widely used, not only for tree and other cash crops but, increasingly, for fruits and vegetables, poultry, pigs, dairy produce and even prawns and fish. Contract farming of spices like chillies, ginger, nutmeg and vanilla as well as herbs like parsley, thyme, patchouli and stevia are wide spread, which makes the farm level quality improvement suitable to the international standards as set by country regulations.

This kind of backward linkage is market driven and hence is very competitive and effective. For sponsoring companies, contract farming may in many cases be more efficient than plantation production, and will certainly be more politically acceptable. It can give them access to land that would not otherwise be available and the opportunity to organise a reliable supply of products of the desired quality that probably could not be obtained from the open market. On the other hand, from the companies' perspective, contract farming is not without difficulties. Farmers may sell their outputs to outsiders, even though they were produced using company-supplied inputs. Conflicts can also arise because the rigid farming calendar required under the contract often interferes with social and cultural obligations. The essential precondition for a contract farming project is that there must be a market for the product that will ensure profitability of the venture.

There is a range of other factors that affect the success of contract farming ventures. These include the physical, social and cultural environments; the suitability of utilities and communications; the availability of land; and the availability of needed inputs. An essential precondition is that management must have the necessary competence and structure to handle a project involving many small-scale farmers. Another important requirement is government support. Contracts need to be backed up by law and by an efficient legal system. Existing laws may have to be reviewed to ensure that they do not constrain agribusiness and contract farming development and minimise red tape. Some major spice/herb producing countries like India have, unfortunately, not done much to the existing laws to bridge the gap.

In general, there are five basic models of contract farming; these are the centralised model, the nucleus estate model, the multipartite model, the informal or individual developer model and the intermediary model. Any crop product can theoretically be contracted out using any of the models, though certain products are more suited to certain approaches. Good management is a vital component of all contract farming models. It is essential to plan, organise, coordinate and manage production, including the identification of suitable land and farmers, the organisation of farmers into working groups, the supply of inputs, the transfer of technology and the provision of extension services. Above all, the quality requirement has to be clearly agreed upon and a detailed package of agro-practices needs to be designed and monitored. There has to be a harmonious management-farmer relationship throughout the implementation of the project, and promoters and sponsors of contract farming need to place particular importance on the monitoring of production. Companies should also monitor

the performance of their employees, particularly those in close contact with the farmers.

For spices, contract farming can be through small farmer groups. For example, a company that exports vanilla from Uganda works through groups of farmers organised into local associations. These associations play a leading role in selecting suitable farmers, recovering loans and bulking up the vanilla for purchase. Such farmer groups or associations control production, with the sponsor having direct contact with farmers only when conducting training programmes. In spices like chilli where processing (drying to control aflatoxin) is required immediately following harvest, there can be quality problems. In such cases well laid out quality checks and standards are to be agreed upon by the company and the farmer.

Successful chilli and marigold backward integration projects have been in operation continuously for the past ten years in the southern districts of India, run by the AV Thomas group's Integrated Spice Project. These projects are good examples of backward linkages, where the corporate sector works very closely with growers to meet global quality standards of produce. It is generally seen that the farmers accept new techniques only if the adaptations result in higher yields and/or improved quality and if the cost of such techniques is more than offset by higher returns. The introduction of technologies can cause cultural adaptation problems for smallholder farmers, even though these technologies are often the most important benefit of the contract. (Refer to Figs 2.1–8 as well as the flowchart (Fig. 2.9) of residue control for details of chemical controls at farms).

Field extension, monitoring of chemical applications/package of practices and recording of field data are very important to maintain traceability of the produce. Extension staff have the responsibility to schedule the sowing of seed beds, the transplanting of seedlings, and the cultivation and harvesting of the contracted crop within a defined climatic season and in harmony with the farmers' own cropping regimes. At the beginning of each season, management, extension staff and farmers should discuss and confirm all planned activity schedules. Managers should present

Fig. 2.1 Improving post-harvest processing/drying by use of clean sand beds for quick and complete drying to arrest mould growth and to avoid use of fungicides.

Fig. 2.2 Improvement of post-harvest handling/picking by using clean containers to arrest mould growth and to avoid use of fungicides.

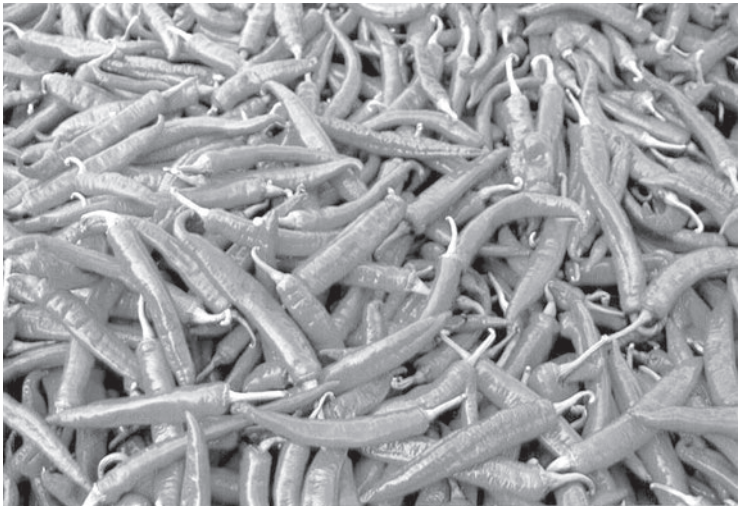

Fig. 2.3 Use of chemicals is strictly prohibited at least a month before harvest in the backward integration projects of AVT McCormick to reduce chemical loads on dry pods to the barest minimum.

the sequence and timing of each crop activity to farmers before the first sowings. Pest control packages and usage schedules are often discussed before sowing, and are monitored by the field extension staff at specified intervals. A routine analysis is carried out to ensure that current and future production remains within the quality and quantity parameters required.

Deterioration of quality can have serious and far-reaching consequences for any business venture. Quality controls are especially critical for spices that are more prone to usage of chemicals like pesticides. Here, an approved list of chemicals to be used is released by the company. IPM strategies to be followed are also described in

Fig. 2.4 Use of IPM strategies such as pheromone traps and border cropping to control insects in a marigold field under the backward integration operations of AVT Natural products.

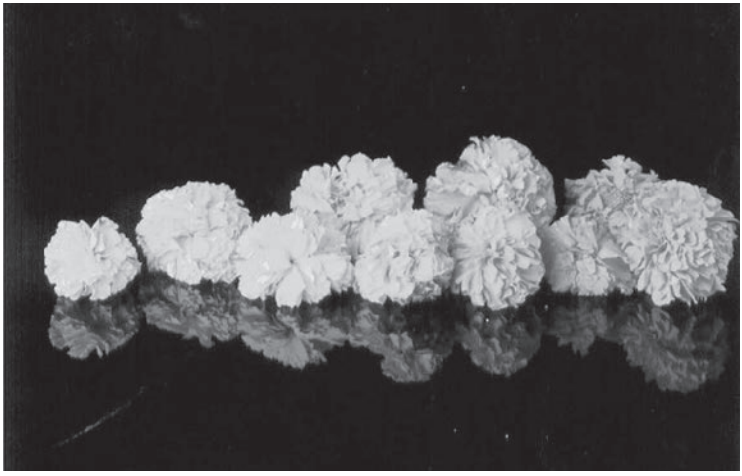

Fig. 2.5 Marigold flowers for quality checking at the laboratory.

detail. Each venture must develop quality control and monitoring systems suitable for its operation. Management must prioritise monitoring procedures and decide how often they should be carried out, in what locations and what should be inspected and assessed. Checking product quality can take place before, during and immediately after harvesting as well as at the time farmers grade their own production and when the products reach the company's processing or packaging facilities. Quality controls may start as specifications in a written contract or as verbal explanations of quality standards given in both pre-season and pre-harvest farmer-management meetings. Some of the major causes of poor quality are failure to apply fertiliser, ineffective weed and insect controls, disease, immature harvesting and indiscriminate grading and packaging.

Fig. 2.6 Scientific farming practices based on GAP and IPM in a marigold field by AVT Natural products.

Fig. 2.7 Post-harvest drying by natural methods under hygienic and controlled environment to reduce mould infestation and usage of fungicides by AVT McCormick.

2.5 Integrated pest management and organic production

Integrated pest management (IPM) is a more or less total solution to controlling chemical residues in agriculture of spices and herbs. Any contract farming model for quality improvement of spices and herbs should essentially contain an IPM module suited to that crop. Though used to control insect and mite pests, herbs are prone to attack themselves from a wide range of pests. Aphids, two-spotted spider mites, caterpillars such as armyworms and cabbage loopers, leaf miners and whiteflies are some of the examples. Each spice/herb variety harbours its own spectrum of pests, and managing these depends on specific host-parasite relation. IPM options

Fig. 2.8 Strict quality checking for chemical residues and other customer requirements.

available to control them are limited, as much research has not been conducted in this area.

The Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) regulates the pesticides being used to control insect and mite pests of herbs and spices in the USA. According to this law any chemical used for controlling pests must be registered by the Environmental Protection Agency (EPA). Section 2ee of the FIPRA not only allows use of pesticides as per specific instructions on the pesticide label, but also requires that the target must also be specified on the product label. This law dramatically limits the number of insecticides that are allowed to be used on herbs since few products contain mention of this 'minor crop' thus technically controlling, by default, use of many pesticides on herbs.

An ideal IPM strategy for spices and herbs should include use of *Bacillus thuringiensis* for control of armyworms (1st and 2nd instars), loopers and salt marsh caterpillars; insecticidal soaps (e.g. M-Pede®) for control of whiteflies, aphids, leafhoppers, plant bugs, spider mites and thrips; azadirachtin to control a variety of insects including leaf miners, fungus gnats, gypsy moths, western flower thrips, mealy bugs, armyworms, aphids, loopers, cutworms, leaf rollers, leaf hoppers, webworms, spruce budworms and sawflies. Products containing azadirachtin (extracted from neem tree) are considered to be 'insect growth regulators', which work by interfering with the insects' key moulting hormone, ecdysone, to prevent them from moulting from one life stage to the next. Egg and adult stages of insects are not affected by azadirachtin application and have anti-feedant properties. Garlic water is also used to prevent pests like ants, aphids, grasshoppers, 'leaf loopers', leaf rollers, spiders, spider mites, thrips and whiteflies, though claims are made that this product kills insects and mites.

Whenever using a herbal product or product mixture for the first time on a plant or plant's growth stage, a few plants should be tested and observed for several days to determine if that spray will harm the plant in any way. Leaf yellowing, burning, deformation or drop are some of the symptoms to be looked for. Plants in flower or in stress are more likely to display such phototoxic reactions. There may be other products that are specifically used on one or more herbs. For example, numerous

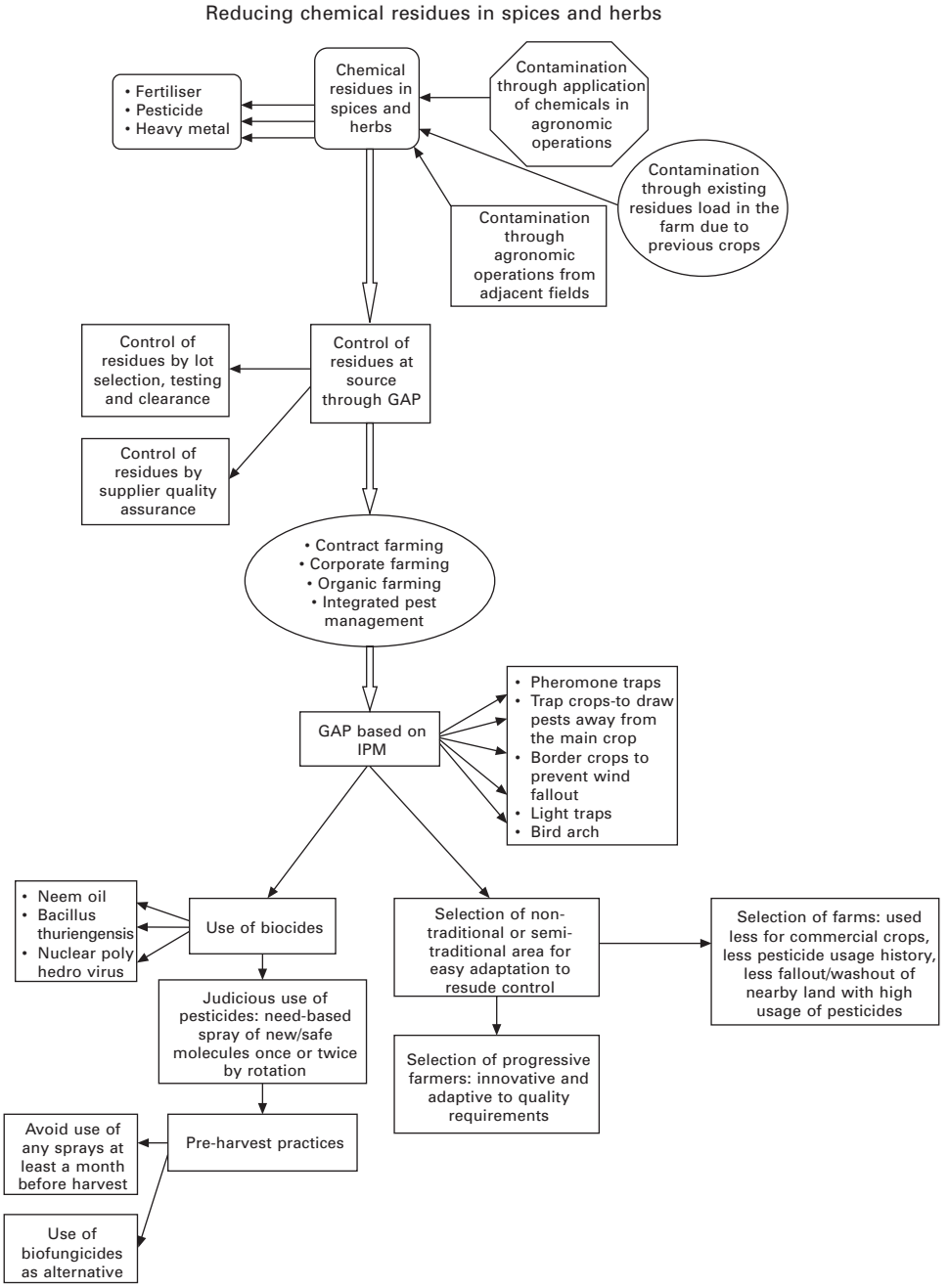

Fig. 2.9 Reducing chemical residues in herbs and spices.

products are reportedly registered for peppermint, spearmint and parsley. There may also be products, particularly 'organic' products, generally registered for insect and mite control with no particular site listed on the product label. Diatomaceous earth products are not registered insecticides for use on herbs. Use of this material and other 'home made' sprays, dusts or similar treatments must be practised with care. Other IPM approaches that can be used in herbs include the following:

- Start with clean plants and use pest resistant/tolerant species/varieties if available.
- Practise good sanitation in the production area based on good agricultural practices (GAP), remove heavily infested material and clippings, eliminate weeds and *pet plants* in and around the production area.
- Use exclusion techniques such as screens and other physical barriers ('no-thrips' screen, bug bed environmental screening, econet – anti-insect net and others).
- Hand pick pests and/or use high-pressure water sprays (water-wand, jet-all or others).
- Practise biological control methods including conservation of natural enemies and augmentive releases (applications) of predators (predaceous mites, green lacewing larvae and others), parasites (Encarsia and Trichogramma wasps and others) and insect-predaceous nematodes (Bio-Safet™, Entonem®, Ecomaskn™, Scanmaskn™, Larvanem® and others).

Pest suppression approaches for herb production are often labour intensive and expensive. The price of the final product must include the added cost of pest control. Sharing information regarding registered products and their sources would be an effective way.

Organic production is a system that uses a combination of management techniques to maintain soil quality and fertility, and control weeds, pests and diseases. Crop rotation plays a big role in achieving these goals. Conventional chemical fertilisers, herbicides and pesticides are eliminated, although organic products are generally allowed, subject to compliance with the organic standard. This system of agri-production is a total way of controlling use of chemicals in agriculture. Of late, many organically grown spices and herbs are readily available in the market. Organic agriculture is the strictest of the environmentally sound agricultural practices. Its main focus is on minimising environmental damage and on sustaining or building up soil fertility. Organic agriculture is commonly perceived as refraining from the use of chemical inputs, such as synthetic fertilisers, pesticides and herbicides or defoliants. More environmentally sound alternatives are employed to replace chemicals, such as crop rotation, particularly incorporating legumes, careful management and use of manure and crop wastes, use of appropriate cultivation techniques, natural and biological pest and disease control measures and mechanical and other non-chemical weed control techniques. In many regions of the world, agricultural systems equivalent to what is now defined as 'organic' farming have existed for centuries, especially in third-world countries, where agriculture is often 'organic by default' as no money is available to buy chemical fertilisers and pesticides.

The EU market for organic spices and herbs grew rapidly in the 1990s. There is growing demand for organic spices and herbs in Europe. In order to make agricultural products from organic sources easily recognisable to consumers, EU 'organic' labels have been introduced. Organic production and labelling is covered by Council Regulation (EEC) No 2092/91 as a means of providing consumers with a guarantee of origin, preparation, processing, and packaging of products.

Each country should try to evolve a process to develop a regulated national standard, that growers must follow. There are several organic certification organisations. The standards of these organizations may vary, in part, due mainly to different interpretations of products of restricted use. Consumers often interpret certified organic produce as merely pesticide free but this is not the case. Organic pesticides may have been used in some instances. Organic crops must always be produced according to accepted guidelines of the organic standard being followed, including soil management practices.

The transition time to convert from conventional to organic production generally requires a minimum of three years and is set by the certification agency. Weed management is often a challenge, especially during transition, but may be less so in horticultural or herb crops that are grown in rows. Most importantly, record keeping is vital for organic certification of crops.

The main principles for organic production at farm level and the rules that must be followed for the processing, sale and import of organic products were established by the passing of Council Regulation EEC 2092/91 and its supplement EC 1804/99. This regulation is very complicated and difficult, which makes it necessary for an exporter to the EU to consult experts on this matter. Use of the term 'organic' is now limited in the European Union to products derived according to the principles of production and the rules of processing defined in the regulation. IFOAM (International Federation of Organic Agriculture Movements) was a major contributor to the organic standards of the EU adopted in Regulation 2092/91. Agricultural units, the processors as well as their products, must be certified by the EU recognised control bodies to confirm that they meet the required EU or specific national standards, before their products can be offered for sale in EU markets. Important inspection agencies are SKAL, Ecocert, Soil Association, etc.

It should be noted that a number of these organisations have their own inspectors in some developing countries. Under EU regulation, the marketing of organic produce from third-world countries is permitted only where the Commission is satisfied that the imported goods have been produced according to rules equivalent to those of the European Union and where the producer has obtained a certificate of inspection from a competent EU recognised authority. Exporters from non-EU member states can indicate their interest in obtaining certification for organic production by contacting either an international inspection organisation, or a national organisation from an EU member state, designated as a competent authority under Regulation No 2092/91.

The EKO quality label is the label in The Netherlands that guarantees the organic origin and quality of agricultural products and food products. The organisation SKAL is the holder of the officially registered EKO quality symbol. Internationally, SKAL is a member of IFOAM (International Federation of Organic Agriculture Movements). It provides services in the field of inspection and certification, both nationally and internationally, acting as an independent third party. Other important EU inspection organisations operating internationally include BCS and Naturland (Germany), Ecocert (Germany, France, Belgium, and Italy) the Soil Association (United Kingdom) and KRAV (Sweden).

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3

Irradiation to decontaminate herbs and spices

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3.1 Introduction

Spices are natural plant or vegetable products, or mixtures thereof, in whole or ground form, and are used for seasoning and imparting flavor, aroma and pungency to food (Pruthi, 1980). Spices and condiments play an important role in the economies of several exporting and importing countries of the world. The terms spices and condiments are often used synonymously, but the latter refers to a mixture of one or more spice(s) along with flavor potentiators, added to food after it has been served on the table. Herbs are actually weak-stemmed plants or plant parts, used raw in powdered form or for the extraction of biologically active principles, used extensively in the alternative system of medicine including ayurveda and unani. Herbal medicine is becoming increasingly popular around the world. China and India are the largest producers of medicinal herbs. Globally, the herbal medicine market is valued at around $60 billion.

The majority of spices and herbs are grown in tropical countries of the world. Since these are high-value commodities they are an important source of valuable foreign exchange for many countries. Though data on medicinal herbs is lacking, figures for spice production and export are well documented. India alone produces more than three million tonnes of spices annually. There are more than 112 plant species that are used as spices and vegetable seasonings. In India about 53 spices are included in the official list of the Indian Spices Board, of which 12 are spices of major economic importance to the country. Most of these spices are consumed within the country. Only about 10% of the produce is exported. India accounts for about 50% of the world export of spices. During their journey from farm to table spices exchange several hands. Improper conditions like drying in open and inadequate packaging and storage result in contamination of spices with soil, excreta of birds, rodents, and insects.

3.2 Quality considerations

Spices and herbs are normally used for their volatile aromatic oils and biologically active principles. They do not contribute much to the nutritional value of food. The volatile oil in spices could vary from negligible to 13%. Most of the spices and herbs have a high level of moisture, varying from 16–88% at the time of harvest. After harvest spices and herbs are dried locally by the farmers and collectors. Spices are generally produced by small farmers, where traditional systems of cultivation and drying are used. The moisture content of spices and herbs may vary from 6–12% depending on the extent of drying and climatic conditions. Often the produce is not adequately dried, cleaned, graded or packed. It is often the middlemen who collect, pool, clean, grade and bag the spices, before selling them to traders and exporters. Ideally, for storage stability, moisture in spices should be below 12%. But often farmers do not dry the produce to the required extent often unintentionally, but some times intentionally, to get the advantage of weight.

Open air drying leads to contamination of spices and herbs with soil and dust. The biotic factors responsible for the deterioration of spices and herbs and the consequences to consumers are shown in Table 3.1. Contamination of spices and herbs with biotic agents not only risks the spoilage of the valuable commodity but also poses risks to human health due to the presence and outgrowth of pathogens and toxin-producing molds.

Due to low moisture in dry spices and herbs the water activity is often less than 0.60. Thus these commodities are inherently stable during storage. Spices contain a number of microorganisms as shown in Table 3.2, however, the actual number of bacteria present may vary from spice to spice (Table 3.3). Spices contain a high load of spores of bacteria and fungi (Table 3.3). These spores are mainly mesophilic aerobes, mesophilic anaerobes, and flat sour thermophilic aerobes (Pruthi, 1980). Spices may also contain human pathogens. This is indicated by the presence of coliforms and *E. coli* (Table 3.4), the organisms known as indicators of fecal contamination and thus the hygiene of the commodity (Farkas, 1988). The presence of human pathogens such as *Salmonella*, *E. coli*, and *Bacillus cereus* has been well documented in spices (Pruthi, 1980). Due to low water activity spices and herbs are inherently resistant to bacterial spoilage.

Fungal contamination and spoilage of spices and herbs could occur either during drying when the process is slow or if the drying is inadequate, or during post-harvest storage, especially when relative humidity during storage is high and the temperature

Table 3.1 Biotic factors in quality deterioration of spices and herbs

- Storage insects
  - Infestation and deterioration
  - Loss of marketability
  - Risk unethical use of harmful insecticides
- Contaminating microbes
  - Potential spoilage of spices
  - Potential spoilage of food
  - Potential human pathogen
  - Potential toxin producer
  - Loss of marketability
  - Risk unethical use of chemicals

Table 3.2 Common microorganisms in spices and herbs

Molds	Bacteria	Pathogenic bacteria
Aspergillus flavus	*B. subtilus*	*B. cereus*
A. niger	*B. lichniformis*	*Clostridum perfringens*
A. fumigatus	*B. megaterium*	*E. coli*
A. glaucus	*B. pumilus*	*Salmonella sp.*
A. tamarii	*B. brevis*	
A. terreus	*B. polymyxa*	
A. versicolor	*B. stearothermophilus*	
Absidia sp.	*B. firmis*	
Mucor sp.	*S. faecalis*	
Penicillium		
Rhizopus sp.		
10^2–10^6	10^3–10^8	

Table 3.3 Total bacterial and spore counts in pre-packed ground spices

Spices	Total bacteria CFU/g	Total bacterial spore	Total fungal spores
Pepper	3×10^6	2×10^6	5×10^2
Chili	4×10^6	2×10^6	6×10^5
Turmeric	1×10^6	8×10^5	1×10^3
Coriander	2×10^5	2×10^5	2×10^3

Table 3.4 Frequency of occurrence of coliforms and *E.coli* in spices

Range	Coliforms		*E. coli*	
	Samples	%	Samples	%
10–10^2	31	14	22	10
10^2–10^3	21	9	10	4
10^3–10^4	20	9	4	2

is conducive to fungal growth. Fungal infection, particularly with toxin-producing fungi like *Aspergillus flavus* and *Aspergillus parasiticus*, may result in accumulation of mycotoxins that pose a significant health risk to consumers (Table 3.2).

The microbial load on common Indian whole and ground spices varies from sample to sample as shown in Table 3.3 (Sharma *et al*., 1984, Munasiri *et al*., 1987). Some spices may contain potent antibacterial and antifungal principles that are abundant in certain spices like clove, cinnamon and ajowan (Sharma *et al*., 1984, 2000). Spices are frequently infested with insect pests (Padwal-Desai *et al*., 1987). The insect pests normally encountered in spices are given in Table 3.5. These include common storage insects such as cigarette beetle, confused flour beetle, saw-toothed beetle and Indian meal moth. Insect infestation of spices is a major storage problem. Spices may also contain a large amount of antioxidants and radioprotective agents (Gautam *et al*., 1998). These agents, along with the antibacterial and antifungal compounds, are primarily responsible for the medicinal and nutraceutical value of spices.

The addition of heavily contaminated spices even at 0.1% level in a prepared or

cooked food can significantly increase its microbial load and lead to spoilage, particularly of canned food. The pathogens may grow and increase in number when the spice is added to food and if the food is allowed to incubate. Thus the presence of microorganisms in spices could severely affect not only the keeping quality of food but also increase the risk of human foodborne illnesses. The loss of quality and marketability of stored spices can result in huge economic losses.

3.2.1 Quality standards

Quality has been given the utmost importance by both exporting and importing countries. In India, The Export (Quality Control & Inspection) Act 1963 was amended in 1984 to streamline inspection and testing procedures before export. The Bureau of Indian Standards in collaboration with the International Standards Organization has laid down standards for spices. However, it is not always that the export consignments conform to the standards of quality and quarantine. Leading importing countries have stringent quality control inspection. Exceeding permitted defect levels could invite stringent punishment in the form of rejection of the consignment or even black listing of the export house. The microbial standards for spices proposed by the ICMF are shown in Table 3.6.

Culinary practices in India, usually adding spices before or at the time of cooking, may serve as safeguards against poor microbiological quality of spices for home consumers. However, infested and molded spices are pushed in as ingredients of powders and condiments. Besides being contaminated with the excreta and body parts of insect pests, these spices may carry heat resistant mycotoxins such as aflatoxin. Storage insects cause major losses to farmers as well as traders. Very often traders resort to unethical practices by using banned chemicals to store their spices for

Table 3.5 Predominant storage insects found in Indian spices

Insect	Whole spices					Ground spices		
	Chili	Turmeric	Ginger	Cardamom	Coriander	Chili	Turmeric	Coriander
Lasioderma serricorne	+	+	+	–	–	+	+	–
Oryzaephilus surinamensis	+	–	–	–	–	+	–	–
Sitophilus cerealella	+	–	–	–	–	+	–	–
Tribolium castaneum	+	+	+	+	+	+	+	+

Table 3.6 Recommended microbiological specifications for spices

Test	Limits/g			
	N	C	m	M
SPC	5	2	10,000	1000,000
Molds	5	2	100	10,000
E. coli	5	2	10	1,000

From N samples analyzed; C samples may exceed; m but none may exceed; M (ICMF, 1974).

longer periods. The presence of invisible microorganisms, many of which could cause, disease poses risks to human health, especially when spices are added to food after cooking.

At present fumigants like methyl bromide, ethylene dibromide and ethylene oxide are used to treat spices and herbs for insect disinfestation and microbial decontamination. Besides being less effective, these fumigants leave chemical residues on spices that are harmful to human health. These fumigants are also harmful to the environment as halogenated hydrocarbons deplete ozone in the atmosphere. Many countries have banned the use of fumigants. Other countries, including India, are planning to ban them.

3.2.2 Quality improvement by irradiation

Radiation processing offers a very effective and safe alternative for disinfestation and microbial decontamination of spices and herbs. It is a cold process, sometimes also referred to as cold pasteurization, therefore it does not affect the delicate aroma and flavor compounds in spices. Radiation processing can be carried out in pre-packed spices without running the risk of post-treatment contamination. The process is very effective compared to fumigants and does not leave any harmful residues on spices.

Table 3.7 shows the effect of irradiation on bacterial and fungal microflora of spices. It is clear that a radiation dose of 5 kGy can eliminate fungal microflora, whereas, a dose of 10 kGy destroys all bacterial contaminants, making spices commercially sterile. Table 3.8 shows the microbial profile of irradiated and non-irradiated spices during storage. It is evident that irradiated spices retain their

Table 3.7 Effect of gamma radiation on total bacterial and fungal population in spices

Spice	Total bacteria (CFU/g) Dose (kGy)				Total fungi (CFU/g) Dose (kGy)				
	0	5	7.5	10	0	1	5	7.5	10
Pepper	1×10^7	1×10^3	4×10^2	0	9×10^2	1×10^1	0	0	0
Nutmeg	4×10^4	6×10^2	0	0	8×10^3	8×10^1	0	0	0
Cinnamon	2×10^3	1×10^2	0	0	3×10^2	0	0	0	0
Cardamom	1×10^4	3×10^2	2×10^1	0	1×10^3	5×10^1	0	0	0
Clove	9×10^2	4×10^2	0	0	9×10^2	4×10^1	0	0	0

Table 3.8 Total bacterial count of non-irradiated and irradiated spices during storage at ambient temperature

Spice	Dose (kGy) Storage period (mo) 0			5			10		
	0	3	6	0	3	6	0	3	6
Pepper	3×10^6	3×10^6	3×10^6	2×10^3	2×10^3	5×10^2	0	0	0
Chili	4×10^6	2×10^6	1×10^3	2×10^4	2×10^3	2×10^2	35	0	0
Turmeric	1×10^6	9×10^5	8×10^2	2×10^2	2×10^2	0	0	0	0
Coriander	1×10^5	1×10^5	1×10^5	2×10^2	1×10^2	1×10^2	0	0	0

microbiological quality during storage. A comparative study was undertaken to evaluate the effectiveness of radiation processing by six research laboratories in India. As shown in Table 3.9 all six laboratories reported the effectiveness of radiation processing in eliminating microbial load on three major spices. Table 3.10 shows the comparative effectiveness of irradiation with fumigation on some spices and dry vegetable seasonings (Farkas, 1988). It is evident that irradiation at 10 kGy was much more effective than ethylene oxide at 800 g/6h.

It has been shown in several studies that the quality parameters of spices do not change appreciably after radiation processing. Table 3.11 shows the color power of turmeric (curcumin content) during storage at ambient temperature. It is also evident that the color power of irradiated (10 kGy) stored turmeric was better in comparison with the control. Table 3.12 shows that the active ingredients of spices are not affected by irradiation. In the case of chili the extractable color, capsanthin, was not found to be significantly affected by irradiation and storage (Table 3.13). Table 3.14

Table 3.9 Microbiological analysis of spices

Spice		CFU/ g*					
	LAB**	1	2	3	4	5	6
Pepper	W***	7×10^5	2×10^6	2×10^4	3×10^5	2×10^8	3×10^6
	G	1×10^5	7×10^4	3×10^4	9×10^5	3×10^8	3×10^6
	IW	NIL	30	NIL	9	NIL	NIL
	IG	NIL	30	NIL	20	NIL	60
Chili	W	6×10^4	5×10^4	1×10^4	2×10^5	1×10^5	2×10^4
	G	5×10^4	5×10^4	2×10^4	3×10^5	7×10^5	3×10^5
	IW	NIL	30	NIL	90	NIL	10
	IG	NIL	30	NIL	NIL	NIL	20
Turmeric	W	6×10^4	5×10^5	3×10^4	3×10^5	1×10^8	2×10^6
	G	6×10^5	1×10^6	2×10^4	1×10^6	4×10^5	3×10^6
	IW	NIL	30	NIL	36	NIL	10
	IG	NIL	30	NIL	NIL	NIL	20

* CFU: Colony forming units, each value represents readings average of four replicates from samples.
** Laboratories:
1 Bhabha Atomic Research Centre, Mumbai.
2 Analytical Quality Control Laboratory, CFTRI, Mysore.
3 Central Food Laboratory, Kolkata.
4 Central Food Laboratory, Pune.
5 University Department of Chemical Technology, Mumbai.
6 Food Research & Standardization Laboratory, Gaziabad.
*** W – Whole, G – Ground, IW – Irradiated whole, IG – Irradiated ground.

Table 3.10 Comparison of efficacy of irradiation and fumigation

Spice	Log CFN/g Dose (kGy)				
	0	4	8	10	ETO 800 g/6h
Pepper	5.5	2.9	1.2	0	3.8
Paprika	3.2	1.0	<1.0	0	1.9
Onion powder	2.8	<1.0	0	0	<1.0
Garlic powder	4.1	3.3	2.0	0	3.8

Table 3.11 Color power of turmeric (curcumin content) during storage at ambient temperature

Storage period (mo)	Curcumin content %	
Treatment	Control	Irradiated 10 kGy
1	2.82	2.73
6	2.82	2.96
8	2.82	3.04

Table 3.12 Extractable color (capsanthin content) in chili during storage at ambient temperature

Component	Content % W/W Control	Irradiated (10 kGy)
Piperine	4.9 ± 0.4	5.0 ± 0.4
Gingerol	1.0 ± 0.1	1.0 ± 0.1
Curcumin	1.5 ± 0.1	1.5 ± 0.1
Capsaicin	0.06 ± 0.01	0.07 ± 0.01
Color value chili	12159 ± 286	12120 ± 264

Table 3.13 Chemical quality of irradiated spices

Storage period (mo)	Absorbance at 460 nm	
Treatment	Control	Irradiated 10 kGy
2	0.455	0.430
8	0.320	0.305

Table 3.14 Microbial stability of pasteurized tinned pork with irradiated spice mix (Farkus, 1998)

Storage temp °C	Dose to spice mix in kGy		
	0 Shelf-life (days)	7.5	10
0	>180	>180	>180
15	96	>180	>180
20	15	~30	>90

shows the consequences of adding non-irradiated and irradiated spices to canned food. As is evident the shelf-life of canned pork was significantly enhanced by using irradiated spices (Farkus, 1998).

It is therefore clear that, as far as microbial decontamination is concerned, all spices, whole or ground, need to be given a dose of 5 kGy and above. Most countries have approved a dose of 10 kGy. Some countries like the USA allow even higher doses (30 kGy). For insect disinfestation however, a dose of 1 kGy would suffice for all spices. All dry spices, whole, ground as well as blends require similar doses.

3.3 Application of ionizing radiation

Radiation processing involves controlled application of the energy of ionizing radiations such as gamma rays, X-rays, and accelerated electrons to food commodities including spices for achieving one of the following objectives:

- disinfestation
- shelf-life extension
- hygienization
- sterilization.

The technology holds considerable promise because in many cases it has an edge over conventional methods. It could be applied judiciously where conventional methods are inadequate, uneconomical, or pose potential health risks. It can also be used as a complementary process with many new and emerging technologies. The process helps in keeping the chemical burden on the commodities low and also increases the packaging possibilities. These benefits accrue mainly from the cold nature of the process and the high penetrating power of ionizing radiation. Being a cold process the technology is particularly appropriate for spices that are valued for their delicate aroma and flavor constituents.

Radiation technology offers several advantages for processing spices. These advantages are listed below:

- It is a physical, non-additive process, causing minimal change in spices and herbs.
- It is highly effective compared to chemicals and fumigants.
- It does not leave harmful residues.
- It can be applied to bulk as well as prepackaged commodities.
- It is a cold process and preserves spices in natural form.
- It does not destroy the heat-labile aroma and bioactive constituents of spices and herbs.
- The process is safe to workers and friendly to the environment.

3.3.1 Ionizing radiations

Ionizing radiations are a part of the electromagnetic spectrum. They have relatively short wavelengths and high energy. These radiations can eject electrons from an atom of a molecule in food to form electrically charged species known as ions. The ejected electrons cause further ionizations. Due to the short wavelength and high energy associated with ionizing radiations, they are highly penetrating and effective. Therefore, unlike other methods, foods for radiation processing can be pre-packed and treated to get the desired effect.

In accordance with international regulations such as Codex General Standards for Food Irradiation, the ionizing radiations that are permitted for irradiating foods are limited to:

- Gamma rays from radioisotope cobalt-60 or cesium-137
- X-rays generated from machine sources operated at or below an energy level of 7.5 MeV
- Electrons generated from machine sources operated at or below energy level of 10 MeV.

3.3.2 Sources of ionizing radiations

The sources of ionizing radiations can be classified into two broad categories, namely, radioisotopes, and machines.

Radioisotope sources

It is a general practice to use cobalt-60, however, cesium-137 can also be used. The broad characteristics of the two sources are given in Table 3.15. While cobalt-60 is produced in nuclear power reactors by bombardment of cobalt-59 with neutrons, cesium-137 is a fission product and has to be extracted from the spent fuel of a nuclear reactor through reprocessing. Though cobalt-60 is the preferred choice, cesium-137 offers advantage in building portable or modular types of irradiators.

In the case of radioisotopes, emission of radiation results in conversion of the isotope into a stable atom. This results in reduction in the number of radioactive atoms over a period of time. The time required by a set of radioactive atoms to display half of its original activity is called half-life. The energy of radiation emitted by a radioisotope is fixed, however, in the case of machine sources variable energies can be obtained. Radioisotopes also provide much lower dose rates compared to machine sources.

With a half-life of 5.27 years, an annual replenishment of 12.3% is needed to maintain the source strength. A basic design of a gamma irradiation facility is shown in Fig. 3.1. For use as a radiation source, cobalt-60 pellets are encapsulated in stainless steel and these pellets or slugs are loaded in stainless tubes to form a pencil. Several such pencils are then mounted on a rack to make the final source of radiation in a radiation processing facility. Goods to be irradiated are conveyed to the irradiation chamber through a labyrinth, which prevents radiation from reaching the work area and operator room. When the facility is not in operation, cobalt-60 is stored in the source rack under water at a depth of about six metres. The water column thus absorbs the radiation and acts as a shield to prevent radiation being present in the cell area when the source is idle. During the processing of a commodity, the source rack is brought up to the irradiation position after activation of all safety devices. The

Table 3.15 Characteristics of different types of ionizing radiation

Radioisotope sources

Characteristics	Radionuclide	
	Co-60	Cs-137
Typical source form	Metal	Cesium chloride pellets
Half-life	5.3 years	30 years
Specific activity	1–400 Ci/g	1–25 Ci/g
Gamma energy	1.17–1.33 MeV	0.66 MeV
Dose rate* (10 kCi)	0.953 kGy/h	0.221 kGy/h

Machine sources

Characteristics	Mode	
	EB	X-rays
Power	Variable	Variable
Energy**	10 MeV (max.)	7.5 MeV (max.)
Penetration	3–4 cm (water equivalent)	30–40 cm

*At a distance of 30 cm in a material of 20 cm thickness.
**Machine to be operated at the energy level permitted.

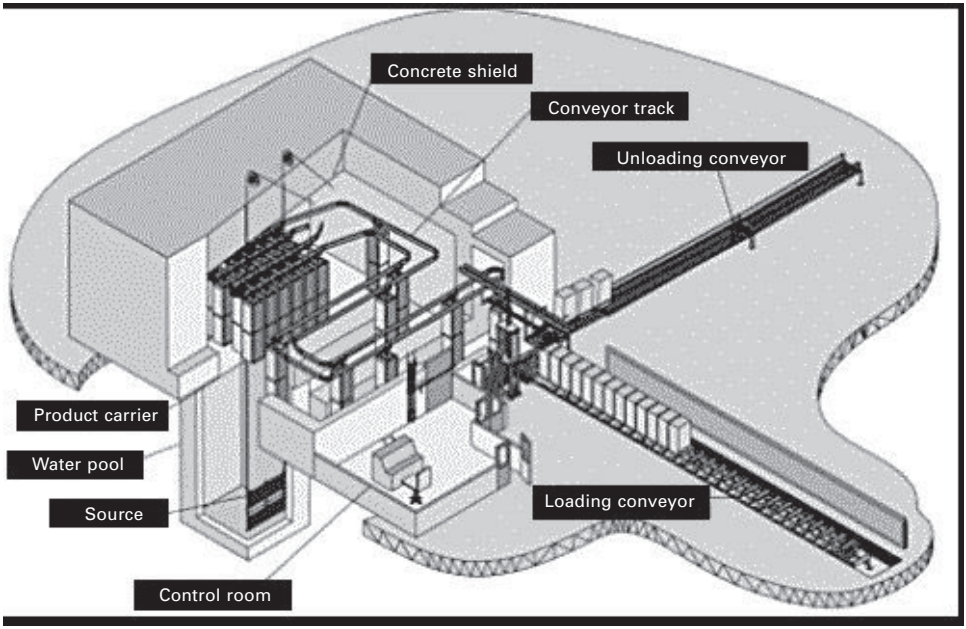

Fig. 3.1 A typical gamma irradiation plant.

irradiation chamber is shielded with concrete walls usually about 1.5–1.8 metres thick. The goods in aluminum carriers or tote boxes are mechanically positioned around the source rack and are turned around their own axis so that the contents are irradiated from both the sides.

The absorbed dose is determined by the dwell time of the carrier or tote box in the irradiation position. The dwell time can be preset after taking into consideration the dose rate, which in turn would depend upon the source strength. The absorbed dose is measured by placing dose meters at various positions in a tote box or a carrier. In Fricke's dose meter radiation-induced oxidation of ferrous ions in a 0.4 M sulfuric acid solution to Ferric ions is measured at 304 nm.

Machine sources

Machine sources used in food irradiation include various types of electron accelerator. The electron beam emerging from the accelerator can be either used directly or converted into X-rays. Both DC (direct current) accelerators and microwave or radio-frequency linear accelerators (LINAC) are used. In both types electrons are accelerated close to the speed of light in an evacuated tube. Electrons emitted from an electron source are pushed from the negative end of the tube and are attracted by the positive end. The higher the potential difference, the higher the speed attained by the electrons. A scanning magnet at the end of the accelerator tube deflects the mono-energetic electron beam onto the material being irradiated. In LINACS, pulses of electrons produced at the thermionic cathode are accelerated in an evacuated tube by driving radio-frequency electromagnetic fields along the tube. The LINAC electrons are mono-energetic but the beam is pulsed. As the electron beam can be directed at the product, the efficiency of electron accelerators is about 20% higher then that of gamma sources. Energy and current determine the output capacity of an electron accelerator.

Because of the lower depth of penetration (5 mm/MeV in water), electron beams cannot be used for irradiation of thick chunks of food commodities or bulk packages.

This difficulty can be overcome by converting electrons into X-rays by fitting a water-cooled converter plate to the scanner. The electrons, upon striking the metal plate, are converted into X-rays. The conversion efficiency depends on the material of the converter plate and the energy of the striking electrons. The X-rays are as penetrating as gamma rays.

3.3.3 The choice of an irradiator

A number of aspects are considered during the choice of an irradiator. These include:

- the type of commodity
- whether loose bulk or packages
- throughput required
- thickness and shape of the product
- the size and shape of the container
- the packaging density of the product
- techno-economic feasibility
- socio-political implications.

3.3.4 Process control

During irradiation processing the aim is to expose the material to at least the minimum required dose that governs the effectiveness of the process. Correct measurement of dose and dose distribution in the product ensures that the radiation treatment is both technically and legally correct. Application of an experimentally established dose for the purpose of radiation processing of a specific food is important both technologically and economically. Dose and dose distribution are determined by product and source parameters. Product parameters are primarily the density of the commodity and packages. The source parameters vary with the facility or the type of radiation being used.

3.3.5 Mechanism of action of ionizing radiations

Ionizing radiations bring about the desired effects by different mechanisms in different foods, depending upon the dose of radiation. The ionizing radiations cause these effects by causing changes in bio-molecules, primarily DNA, either by direct deposition of energy or indirectly through production of radiolytic product of water, that interact with the bio-molecules. The extent of radiation effect depends on the radiation energy absorbed. It increases linearly with the dose in the dose range normally employed in food irradiation. Water is an abundant component of food. Therefore, interaction of water with radiation has a major role to play during irradiation. The radiolytic products of water such as hydroxyl radical, hydrated electrons, hydrogen atoms, and peroxides are highly reactive and play a major role in bringing out the effects of irradiation. The effects brought about by the interaction of radiolytic products of water with bio-molecules are also called indirect effects.

3.4 Nutritional and safety aspects

No other method of food processing has been subjected to such a thorough assessment of safety as radiation processing. The various aspects of wholesomeness and safety

of radiation-processed foods have been studied in great detail (WHO, 1994; Diehl, 1997). These include:

- possibility of induced radioactivity
- microbiological safety
- safety of chemical changes
- nutritional adequacy
- animal feeding
- human trials.

At the energies of the gamma rays from Cobalt-60 (1.3 MeV) and those recommended for X-rays (5 MeV) and accelerated electrons (10 MeV), no induced radioactivity has been detected. The microbiological aspects of radiation-processed foods have been studied in detail. None of these studies have indicated that foods preserved by radiation pose any special problems in relation to microflora. It has been found that there is no unique radiolytic product formed and free radicals in the system disappear depending on the nature of the commodity and its post-irradiation storage and treatment. In fact, the chemical differences between radiation-processed foods and non-irradiated foods are too small to be detected easily. Though the rough composition of the food remains largely unchanged, some losses in vitamins may be encountered. However, these losses are often minor and could be made up from other sources.

Animal feeding studies have been the most time-consuming and expensive feature of wholesomeness testing of irradiated foods. None of the short- or long-term feeding studies, as well as the mutagen testing studies conducted with several irradiated foods in several species of laboratory animals, has shown any adverse effect on these animals. Similarly, no adverse effects have been found in human volunteers fed irradiated food (WHO, 1994).

3.5 International approval

In 1980 a joint FAO/IAEA/WHO Expert Committee on Food Irradiation (JECFI) reviewed the extensive data on wholesomeness collected up to that time and concluded that irradiation of any commodity up to an overall dose of 10 kGy presents no toxicological hazards and introduces no special nutritional or microbiological problems. An expert group constituted by WHO in 1994 once again reviewed the wholesomeness data available till then and validated the earlier conclusion of JECFI (WHO, 1994). In 1997 a joint FAO/IAEA/FAO Study Group constituted by WHO affirmed the safety of food irradiated to doses above 10 kGy (WHO, 1999). In view of this recommendation the Codex Committee on Food Standards of The Codex Alimentarius Commission has also revised the Codex General Standard for Irradiated Foods that now allows use of doses higher than 10 kGy in case of a technological need. In this context it may be noted that the USFDA has approved a dose of 30 kGy for sterilization of spices, herbs and vegetable seasonings.

3.6 SPS application to boost international trade

One of the major problems of international trade in spices and herbs is the presence of exotic insect pests and microbes. This invites quarantine restrictions and hinders

free movement of plant products from one country to another and sometimes from one state to another within a country. Therefore, in order to be competitive in international market effective quarantine treatment of food and agricultural produce is necessary.

Fumigation of food and food ingredients with such chemicals as ethylene dibromide (EDB), Methyl Bromide (MB), and ethylene oxide (ETO) either has been banned or is being increasingly restricted globally. According to the Montreal Protocol, by the end of this decade all the above fumigants will be phased out in the advanced countries. The developing countries have been given some grace period to phase them out by the middle of the next decade. The obvious alternative to business and trade is therefore radiation processing. The effectiveness of irradiation as a broad-spectrum quarantine treatment first recognized by the North American Plant Protection Organization (NAPPO) in 1989 is irradiation. In the USA USDA/APHIS first approved in July 1997 the use of irradiation for quarantine treatment of fresh papaya, lychee, and carambola fruits from Hawaii.

The agreements on sanitary and phytosanitary (SPS) practices and technical barriers to trade (TBT) under the World Trade Organization (WTO) have provided a distinct incentive for the adoption of irradiation as an SPS measure in international trade under the principle of equivalence. Thus, irradiation can be applied to overcome quarantine barriers, and to hygienize products for international trade. These agreements are administered under the standards, guidelines, and recommendations of the international organizations such as the Codex Alimentarius Commission, International Plant Protection Convention, and The International Office of Epizootics. The governments that impose regulations more strictly than those recommended by the above organizations would be required to justify their positions to the WTO. This should encourage application of radiation for improving international trade in agro-horticultural foods among the WTO member states. The Plant and Animal Health Inspection Service (APHIS) of the USDA issued a Final Rule on 'Irradiation Phytosanitary Treatment for Imported Fruits and Vegetables' in 2002. Similarly, the International Plant Protection Convention has also included irradiation as a quarantine treatment. These regulations have opened up the market for irradiated commodities.

Today, more than 40 countries have approved the use of radiation processing technology for different food commodities including spices and herbs. A bulk of nearly half a million tons of food commodities that are processed around the world is comprised of spices and herbs. International regulations require that the irradiated commodities be labeled with the internationally recognized 'radura' symbol and a statement describing the treatment. The countries that have approved irradiation of spices include EU, Argentina, Australia, Belgium, Brazil, Canada, Chile, China, Croatia, Czech Republic, India, Indonesia, Iran, Israel, South Korea, Malaysia, Mexico, New Zealand, Poland, Pakistan, Peru, South Africa, Thailand, UK, USA, Vietnam, and Yugoslavia.

3.7 Detection of irradiated spices and herbs

Because of the small amount of energy involved in radiation processing, no significant differences can be observed in terms of appearance, smell or taste of irradiated commodities. It is difficult to detect small changes by simple chemical tests. Detection of irradiation treatment may, however, be necessary for obtaining legal remedy in case of disputed samples. A number of sophisticated techniques can detect spices or

herbs hygienized by ionizing radiation. These include detection systems based on electron spin resonance spectroscopy, luminescence, and GC/MS. The only way for consumers to know that the commodity has been irradiated is by the label that the product carries clearly declaring the treatment in words, with a symbol or both.

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4

Other decontamination techniques for herbs and spices

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4.1 Introduction

Quality is one of the most important and critical factors in the world food market, and herbs and spices are no exception. Importers and buyers place increasing importance on 'clean' herbs and spices rather than 'cleaned' herbs and spices, and will not import herbs and spices that are still contaminated after cleaning. In order to supply clean herbs and spices, there are a number of dos and don'ts which should be strictly followed during production and processing.

National food laws and regulations aim to protect citizens from health hazards. These laws and regulations include the quality of herbs and spices people consume, and many governments have specified maximum permissible limits of possible contaminants. The contaminants include extraneous matter, microbial infection, insect infestation, insect, bird and animal excreta, mycotoxins, pesticide residues and heavy metals. Some of the specifications prescribed by importing countries are so stringent that it is safest to aim at zero levels to avoid the risk of rejection. Contaminants generally make herbs and spices deteriorate in physical and intrinsic quality, and may also cause diseases, some of which are very dangerous. Table 4.1 lists the harmful effects of contaminants.

Consumers are becoming increasingly quality conscious, and farmers, traders, processors and exporters must maintain the quality of their products at every stage during production, processing and handling. The steps required for quality assurance have become an integral part of the production and supply strategies for herbs and spices, particularly in developed countries.

The decontamination techniques for herbs and spices described in this chapter are divided into two main areas, prevention of contamination and decontamination. The section on preventive measures explains how to avoid contamination, and how to clean herbs and spices to bring the contaminants down to within permitted limits is discussed under decontamination. Irradiation of herbs and spices is dealt with in Chapter 3.

Table 4.1 Contaminants found in herbs and spices and their harmful effects

Contaminant	Harmful effects
Extraneous matter (other parts of the same plant or other plants, sand, stones, etc.)	Reduces physical quality.
Moulds and bacteria	Moulds change flavour. Some produce toxins. Bacteria cause ill health and disease.
Insects (in the field and during storage)	Carry disease-causing organisms, damage the product, affecting physical quality, and leave excreta. Some insect parts are harmful.
Spiders, mites and psocids	Harmful to health. Excreta of some spiders is toxic.
Rodent, animal and bird excreta and detritus	Carry disease-causing organisms and reduce quality of product.
Pesticides and chemicals	Extremely harmful to health, may cause cancer.
Heavy metals, e.g., arsenic, zinc, copper, lead, mercury, etc.	Toxic to humans, may cause cancer.

4.2 Preventive measures against contamination

A code of hygienic practices for herbs and spices (whether whole, broken or ground, mixes and blends or processed products) must be followed precisely to ensure quality. It covers the minimum hygiene requirements during production, harvest and post-harvest activities. Post-harvest technologies include curing, bleaching, drying, winnowing, grading, processing (extraction of essential oil and oleoresin, freezing, freeze-drying, dehydration, etc.) packing, transportation, storage and microbial and insect disinfestations. The necessary measures are described below.

4.2.1 Production

- Seed material should be from a reliable source and devoid of pests and diseases.
- Contaminated crop residues and materials from animal, domestic and human sources should not be used in crop production.
- Irrigation water should not be polluted or contaminated.
- Control measures for pests and diseases involving chemicals or biological agents should only be undertaken with expert advice and with thorough knowledge of the potential dangers to health.

4.2.2 Harvesting and on-farm processing

- Curing (drying) may be done in sunlight or mechanically. If carried out mechanically, all surfaces and machinery used should be clean to prevent contamination.
- The area used for drying crops in the sun should be a raised platform or concrete floor inaccessible to domestic animals, and where dust contamination (by wind) is minimized.
- Precautions should be taken to prevent contamination by rodents, birds, insects and other animals during drying and handling in the open.

- For most herbs and spices, the moisture levels should be reduced to below 11% to prevent fungal infection.

4.2.3 On-farm storage

- Properly dried and cleaned spices should be packed in hygienic and moisture-proof containers. Previously used containers, such as jute bags, polyethylene bags, bins and tins, can be reused only after thorough cleaning. Empty fertilizer, pesticide and fungicide bags should never be reused, even after washing.
- Jute bags and bamboo bins should be lined with plastic film if necessary to prevent dried products from re-absorbing moisture, which could lead to growth of mould.

4.2.4 Processing factory

- Factories or other buildings used for processing herbs and spices should be on elevated land with adequate drainage facilities to drain rain and effluent water efficiently.
- The building should preferably be located in an area free from objectionable odours, smoke, dust or other contaminants.
- The building should be designed and constructed to enable easy and thorough cleaning inside. Entry of pests and rodents should be prevented as much as possible. Mice can enter through holes as small as 5 mm, and, if necessary, ultrasonic rodent devices may be installed. Electrical control devices for flying insects, consisting of an electrified grid and a tray for collecting the dead insects, should be installed at strategic points, especially near the entrances to the processing and storage areas.
- Floors, walls and ceilings should be waterproof, non-absorbent and without cracks and crevices. They should have smooth surfaces for quick cleaning and disinfestation. The angle between the walls and the floor inside factory and storage facilities should not be 90° but should form a concave surface, to discourage movement of rodents and facilitate easy cleaning.
- Doors, windows and other openings should be designed so as to avoid accumulation of dust and dirt. Doors should have self-closing devices and close perfectly, and may also be insect-proofed. Internal windowsills, if unavoidable, should be sloped to prevent dust accumulation. External windowsills should also be sloped to minimize dust accumulation and also to prevent birds from alighting on them.
- Living quarters, toilets and areas where animals are housed should be completely separate and distant from the processing facility and storage area.
- The water supply should meet World Health Organization (WHO) standards for sufficiency, pressure and temperature.
- There should be an efficient effluent and waste disposal system, and it should be kept well-maintained.
- Conveniently located changing facilities and toilets should be provided for workers. The facilities should be designed to ensure smooth removal of waste materials and kept clean and well-maintained.
- Washing facilities with hot and cold water, toilet soap and clean towels should be provided adjacent to the toilets. Where paper towels are used, there should be dispensers and waste receptacles provided near the washing facility notice directing

personnel to wash hands with soap after using the toilet should be displayed if this practice is not prevalent. Separate conveniences should be provided for males and females.

- Hand washing and drying facilities should be provided in the processing area if the process makes this necessary. Cloth or paper towels should be provided for hand drying and receptacles for their disposal.
- Adequate lighting should be provided in the processing area. The intensity of light should not be less than 540 lux (50 foot candles) at the inspection points, 220 lux (20 foot candles) in work areas and 110 lux (10 foot candles) in other areas. Bulbs, tube lights and fixtures should have casings that protect processed products from contamination if the bulb breaks, and also prevent dust accumulation.
- Proper ventilation should be provided to prevent excessive heat and to ventilate out contaminated air.
- All equipment and utensils should be designed and constructed so that thorough cleaning and disinfection are easily possible.
- Cleaning and disinfection of equipment and utensils should be carried out regularly, at the end of each day or at such intervals as may be appropriate.
- Buildings, equipment, implements, utensils and all other physical equipment and facilities, including drains, should be regularly checked and maintained.
- Each facility should have a permanent cleaning schedule drawn up to ensure that all areas are cleaned and maintained in a timely and appropriate manner. It is desirable to make one member of staff responsible for cleaning and hygiene, either for a particular area or the entire establishment, and make sure that he has sufficient knowledge about the possible contaminations and health hazards.
- There should be an effective and continuous programme for disinfestation and pest control in the processing facility and storage areas.
- Waste material should be collected in a systematic manner and removed from handling and working areas as often as necessary, and at least once at the end of the day.

4.2.5 Packaging

- All packaging materials should be stored in a clean and sanitary manner.
- The material should be appropriate for the product to be packed and for the expected storage conditions.
- Packaging material should not transmit any proscribed substance to the product beyond acceptable limits (which may vary according to the importing country).
- Packaging material should be sound and capable of preventing contamination.
- Containers should not have been used before, which could affect the quality of the product to be packed.
- Containers kept at the facility should be inspected periodically, and definitely immediately before use, to ensure that they are in a satisfactory condition.
- Packaging material that is required for immediate use should only be kept in the filling or packaging area.
- Packaging should be carried out under hygienic conditions that prevent contamination of the product.

4.2.6 Storage

- The product/s should be stored at moisture levels low enough to prevent the development of mould and deterioration by oxidation or enzymatic changes. An environment with a relative humidity of 55–60% will protect quality and prevent mould growth. Where this is not possible, the product should be packed in waterproof and air-tight containers and stored in a cool place.
- If allowed by the regulatory authority, fumigation may be carried out using safe chemicals prior to storage. Stored products should be inspected periodically and fumigated again if infestations are found. When necessary, infected herbs and spices may be removed for separate fumigation or destroyed, depending upon the seriousness of infestation.
- Bags containing herbs and spices should be neatly stored in accessible lots away from walls, in rows and with sufficient space for movement between stacks. This will allow refuse and spilled products to be cleaned easily, and also facilitate checking for infestation.
- Infested products should be isolated from the rest of the stock to prevent contamination.
- Bags should be kept on wooden pallets to prevent moisture re-absorption and mould growth.

4.2.7 Hygiene and health requirements for personnel

- The health, cleanliness of dress and behaviour of the personnel working in the processing facility and storage area should be monitored. Every effort should be made to motivate personnel to adopt healthy and hygienic practices. There should be periodic training sessions in hygienic handling of the products.
- Staff who come into contact with the products should have a thorough medical examination prior to employment. Workers should undergo periodic medical examinations to detect and deal with any communicable diseases.
- All staff working in the handling and processing area should maintain a high degree of personal cleanliness and wear aprons, head covers and appropriate footwear. If necessary, workers should wear gloves and other protective devices, such as masks. These articles should be cleaned after every use.
- Any practice considered unhygienic in the workplace, such as eating, chewing, smoking or spitting, should be strictly prohibited in the handling and processing areas.
- The entry of visitors into the processing and storage facilities should be regulated to avoid contamination. If visitors are allowed, they should be provided with protective clothing, masks and caps before entry.

4.2.8 Transportation

- The products should maintain their integrity during transportation. Carriers should be weather-proof, clean, dry and free from infestation, and sealed to prevent entry of moisture, rodents or insects.
- Loading, transportation and unloading should be done in a way that protects the products from damage.
- Insulated carriers or refrigerated trucks may be used, depending upon the nature of the product/s and packaging.
- In warm, humid weather, products should be allowed to reach ambient temperature before being exposed to external conditions.

4.2.9 Sampling and laboratory analysis

- There should be a quality evaluation laboratory with at least the minimum equipment for analyzing the common contaminants.
- Approved sampling and analytical procedures should be used.
- The laboratory technicians should be qualified and trained adequately to carry out analysis accurately.
- The common tests carried out are determination of moisture, mould growth, plate count, insect infestation, etc. (George 2001a).

4.3 Organic production

Applying organic farming methods for the production of herbs and spices is catching up in some countries, including India, Sri Lanka, Indonesia and Guatemala. Herbs and spices produced by organic methods are gaining popularity in Europe, the USA and Japan because they are produced by environmentally friendly farming systems and are regarded as particularly safe by consumers. Organic cultivation does not permit the use of fertilizers, pesticides, fungicides and hormones of chemical origin, which means that herbs and spices produced in this way, are free from chemical residues. Over 100 countries are members of the International Federation of Organic Agricultural Movements (IFOAM), which promotes organic farming and is supported by UN agencies such as the Food and Agriculture Organization (FAO) and the International Trade Centre (ITC) (George 2001b).

4.4 GAP, GMP, ISO 9000, HACCP and ISO 22000

Measures such as good agricultural practices (GAP), good manufacturing practices (GMP), quality management systems under International Standards Organization (ISO 9000) and hazard analysis and critical control points (HACCP) help reduce or eliminate contaminants in herbs and spices (Steinhart *et al.*, 1996). Many processing units in exporting and importing countries have already been certified under one or more of these quality systems.

Certification under HACCP is very important as herbs and spices are food products and there must be no risk of contamination beyond permissible limits at any of the critical control points. The HACCP system is based on seven steps which outline how to establish, implement, maintain and assure quality. They are the following:

1. Conduct a hazard analysis. Prepare a list of processing steps where significant hazards can occur, including purchase of raw materials, and detail preventive measures.
2. Identify critical control points (CCPs) in the process by studying the entire process in depth.
3. Establish critical limits for preventive measures for each identified CCP.
4. Monitor CCPs and use the results to define procedures and subsequently adjust or improve processes to maintain controls effectively.
5. Introduce proper corrective action/s to be taken when monitoring indicates a deviation from an established critical limit.
6. Set up effective record-keeping to document the HACCP system.
7. Institute procedures to verify that the HACCP system is working correctly.

An HACCP system should be implemented by a multidisciplinary team, including the top management. It is specially designed for food safety and very effective if implemented properly (Varman and Evans 1991).

On 1 September 2005 the International Standards Organization published a single standard to encompass all the needs of the market place and designated it as ISO 22000. This standard ensures a safe food supply chain worldwide (Anon. 2005).

4.5 Decontamination techniques

Decontamination or cleaning is very important in the production of herbs and spices because of the large number of small farmers involved, and also because on-farm processing is often carried out in the open air. Cleaning in a factory fitted with modern equipment is very important to ensure that the end-product is of sufficient quality. The foreign materials found in herbs and spices supplied by farmers are surprisingly varied, and include sand, stones, dust, nails, bailing wire, pieces of jute, cotton threads, nuts, bolts, cigarette stubs and packs, pieces of charcoal, toys, rodent droppings, and dead and live insects.

Cleaning equipment must take into consideration the size, shape and density of the herbs or spices to be cleaned. Most often, the cleaning process is based on shape and density. If the shape and density of the foreign materials are similar to those of the product, it is very difficult to remove them. It is impossible to separate out all foreign materials completely with the technology and equipment available; hence there are permitted levels of foreign materials and/or contaminants which will not adversely affect the desired quality or create a health hazard. The equipment commonly used for removing foreign materials includes magnets, sifters, air tables, de-stoners, air separators, indent separators and spiral gravity separators. Cleaning operations are expensive considering the cost of equipment and labour, and the likely loss of the product during the operation.

4.5.1 Magnets

Magnets remove iron particles and pieces of metal. The cleaning machinery should have magnets at as many points as possible. In addition to removing metals, magnets help protect grinding equipment from damage. There are different kinds of magnets, typically in bar and plate forms, but none are 100% efficient. Since the iron particles are removed only if they come into contact with the magnet, the flow of the product past the magnet should not be too dense. The magnets must also be cleaned periodically, according to a schedule designed around the quality requirement. It should be noted that even well-designed magnets can attract and hold only a limited quantity of metal. A system that allows the product to flow over two or three magnets is much more effective than using a single magnet.

4.5.2 Sifters

Sifters remove particles of the wrong size from the product via a set of vibrating screens. The sifting operation is not totally efficient as the products are not uniformly round or spherical, some may be oval in shape and occasionally pieces of leaves are admixed with them. In such situations, the sifting operation becomes very difficult and does not clean the product effectively.

4.5.3 Air tables

Air tables separate light and heavy materials, and are commonly used for cleaning spices. The spice is put on a wire mesh screen fixed to the table and a stream of air is passed through the screen. Light materials are suspended higher in the air than heavy materials, and very light particles are thrown out by the force of the air. Rotational vibration of the screen is adjusted so that the heavy particles are tapped and pushed up the screen by repeated tapping. This separates the heavy particles from the lighter particles. The tilt and rotational vibration of the screen, and the air flow, are adjusted to standardized levels according to the specified requirements for the particular spice being cleaned.

4.5.4 De-stoners

De-stoners work according to the same principles as air tables. De-stoners are generally smaller than air tables. They are used to remove heavy stones and pieces of rock, while air tables separate the product into as many groups as necessary. The air flow, the inclination and vibration of the screen, and the type of screen used, are adjusted according to the materials being separated.

4.5.5 Air separators

Air separators also work on the same principle as that of air tables. The difference here is that a thin stream of the herb or spice is made to fall through a horizontal air flow. Heavier particles fall straight to the bottom, while lighter particles are blown to the side. Some air separators operate with a vertical flow of air, but the principle of operation is the same.

4.5.6 Indent separators

Indent separators work on the difference in shape between the herb or spice and the foreign materials. The herb or spice is fed into one end of a revolving drum. The outer edge of the drum has rows of uniformly shaped indentations designed to fit only the herb or spice being separated. As the drum revolves, the centrifugal forces hold the desired material in these cavities, while foreign materials remain in the centre of the drum. The rotational forces move the herb or spice out of the machine and into a collection trough. Foreign materials are collected separately and disposed of. Different herbs and spices require different drum designs, depending on their size and shape.

4.5.7 Spiral gravity separators

Spiral separators are used to separate spherical spice seeds from non-spherical extraneous material. They can also be used to separate spherical extraneous matter, including other seeds and rodent excreta. The spiral separator consists of a U-shaped trough that curves downward into a spiral. Spherical spice seeds (e.g. black or white pepper) are fed into the top of the separator. They gain speed as they roll down the chute, and as they pick up speed, the centrifugal forces drive them up the side of the chute. Particles that are not spherical or have lower density do not roll and cannot attain the same speed, so they slide down to the centre of the chute. A divider at the bottom of

the chute separates the spice seeds at the side of the chute from the foreign materials in the centre of the chute. Spiral separators do not require a motor or blower, as gravitational forces are sufficient to achieve the separating effect (Tainter and Grenis 2001).

4.6 Sterilization of herbs and spices

Herbs and spices often have a high microbial population when they are harvested. A number of factors lead to an increase in the microbial population, including delays in drying, incomplete drying, contact with infested surfaces, re-absorption of moisture during storage, faulty packing, etc. Hence it is not unusual to find total plate counts in the range of about ten million or more colonies per gram in certain spices, such as black pepper. A high microbial load can reduce shelf-life, as well as lead to the risk of phytotoxins being produced by harmful species. Thus herbs and spices should be subjected to sterilization or microbial treatment.

Food sterilization treatments that can be used effectively to combat moulds and other microorganisms in herbs and spices are essentially sterilization by heat, steam or chemicals, use of low temperature, dehydration, desiccation, lyophilization, modification of acidity, application of chemical preservatives or irradiation (Bourgeois and Leveau 1995). Steam sterilization or chemical fumigation appears to be better for processed or ground herbs and spices, as these processes are easy and cheap to carry out, especially compared to irradiation, which requires highly sophisticated and expensive equipment. The chemicals permitted for sterilization in herbs and spices are ethylene oxide (ETO) and propylene oxide (POP). The subject of irradiation is dealt with in Chapter 3.

4.6.1 Ethylene oxide (ETO)

This chemical has been used to reduce microbial population in herbs and spices for many years. It is very effective for reducing the microbial population significantly. However, the process itself is not easy to carry out because of potential health hazards to workers and pollution risks. ETO is reported to be carcinogenic by inhalation, but not when herbs and spices treated with it are consumed. Regulations in the USA permit an ETO residue of not more than 50 ppm after treatment. However, European countries do not permit the use of ETO because of the possible health hazards.

Treatment with ETO is also effective for killing insects at various instars, particularly in seed spices, such as coriander, cumin, fennel, fenugreek and celery, which carry insect eggs laid inside or on them while reaching full maturity in the field. Treating these spices with ETO destroys the eggs and prevents them from hatching. The material to be treated is placed in a sealed chamber, the air inside is evacuated and pure ETO or a mixture of ETO with other gases is passed through the chamber. After a specified time, the remaining ETO in the chamber is carefully removed by evacuation until the residual level of ETO is brought down to desired levels. Blends of herbs and spices can also be treated with ETO, but they must contain no traces of common salt, which will react with ETO and form toxic chlorohydrins.

By carefully selecting time, temperature and concentration of ETO, it is possible to achieve a significant reduction in microbial population. The material may then have a plate count as low as 50,000 colonies, yeasts and moulds 500 colonies and

coliforms 10 colonies per gram. The nature of the material will determine what level of microbial load will be present after treatment. For instance, ETO-treated coarse ground black pepper will have lower counts than fine-ground black pepper. This is because the ETO gas penetrates more effectively among the coarse ground black pepper particles than among the fine particles. Raw materials with lower initial counts can achieve much lower levels after treatment than those with higher initial counts.

The type of container used for the raw material during ETO treatment also influences the reduction in microbial load. For example, if the raw material is contained in burlap bags, ETO gas penetration is excellent and the reduction of the microbial population is very good. If the same raw materials are packed in heavy polythene bags and placed in corrugated boxes, which can withstand the evacuation of air, ETO will not have free access to the materials and microbial destruction will be limited.

4.6.2 Propylene oxide (PPO)

This chemical occurs in the form of a liquid with a low boiling point of 34.5 ^{o}C. It has been used as a food sterilizing agent since 1958, but it is not as effective as ETO. However, it has been approved for the microbiological treatment of herbs and spices. Many spice processors in California had switched over to PPO for paprika and chili peppers because of the problems associated with ETO. PPO also has insecticidal properties.

The basic equipment for fumigation is a vacuum chamber and a volatilizer, similar to that used for ETO treatment. The raw materials are loaded into the chamber at a vacuum of 26 inches of Mercury and vaporized PPO is released. After four hours, the gas is removed by air washing. Use of PPO for food fumigation is governed by CFR 40 Part 185.15 of the US FDA and US EPA regulations. The residue tolerance for PPO in herbs and spices is 300 ppm. Though this chemical does not yet face the same threat from the US Environmental Protection Agency (EPA) regarding treatment of herbs and spices as ETO, it is likely that it will also eventually be phased out once the use of ETO is banned.

4.6.3 Steam sterilization

Steam sterilization is ideal for herbs and spices because no chemical residue is left on account of this treatment. Steam sterilization can be applied to both whole and ground herbs and spices. However, special equipment is required because steam must be applied under pressure if the treatment is to be effective, and the treatment requires high precision. The pressure must be kept at the required level as otherwise the temperature of the product will rise and essential oil will be lost. The moisture brought in by the steam should be removed fully as soon as treatment is over, to prevent clogging and mould growth (Anon. 1991 and 1999). Steam sterilization equipment is expensive and only a few processing factories use it.

4.7 Detoxification

Herbs and spices can be infected by different fungi, some of which produce toxins that are harmful to health. The most common and dangerous mycotoxin found in

herbs and spices is aflatoxin, produced by the fungi, *Aspergillus flavus* and *Aspergillus parasitics*. Ochratoxin, produced by *Aspergillus ochraceus,* is sometimes found. These fungi infect herbs and spices when moisture levels remain high after harvesting, for instance if drying is delayed.

Aflatoxin is more commonly found in chili, ginger and nutmeg, among others. There are four types of aflatoxin – B1, B2, G1 and G2. B1 is the most dangerous among the four. Aflatoxin is carcinogenic and is not destroyed by cooking. The upper limits in herbs and spices prescribed by the European Union are 5 ppb for B1 and 10 ppb for B1+B2+G1+G2. The USA permits a higher level of 20 ppb for all the fractions together. The European Union is considering introducing the maximum permissible limit of ochratoxin.

Some work has been done on hydrogen peroxide and ammoniation treatments to remove aflatoxin from peanut protein, milk, cotton seed and other materials, but has not been found to be effective. These techniques have not been tried on herbs and spices. The best way to produce aflatoxin-free herbs and spices is through timely harvest, immediate drying and good storage practices (Palle 1987).

It is also impossible to remove completely any pesticide residues or heavy metals in herbs and spices, or even to reduce them to permissible limits, with presently available techniques. Pesticide residues found in these products are mainly of three kinds, organo-chlorine compounds, organo-phosphorus compounds and carbamates. Some pesticides are systemic and their residues will be in the products until they are denatured. Heavy metals are stable and their poisonous effect may continue indefinitely. The best way to avoid health hazards in herbs and spices is to ensure that any chemical pesticides used in crop production are applied in accordance with the manufacturer's instructions and government regulations for their use. Herbs and spices should be grown on soils where heavy metal levels are low, and any possible contamination with heavy metals during pre-harvest and post-harvest operations should be avoided.

4.8 Sources of further information and advice

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5

Packaging and storage of herbs and spices

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5.1 Introduction

Food products undergo numerous physical, chemical and microbiological changes during storage. The stability of food is a function of changes occurring in the food components, such as food proteins, lipids, carbohydrates and water due to environmental and processing factors (exposure to light, moisture, temperature, etc.). The protective coating or barrier provided during processing, storage and handling not only retards deterioration of food, but may also enhance its quality. Suitable packaging can slow the deterioration rate and also may extend product shelf life. In recent years a wide variety of packages and approaches have been employed to interact with the food and provide desirable effects. Examples of these include incorporating oxygen, moisture and ethylene scavengers for oxygen, moisture or ethylene sensitive foods, use of carbon dioxide or ethylene emitters in other foods, flavour imparting or scavenging chemicals and antimicrobial agents for microbiological safety of food. Physical incorporation of these chemicals or other agents may be made into the package material, on or between the package and the food. Such approaches, designed to perform some desirable function other than providing an inert barrier, are called active packaging, interactive packaging and intelligent packaging.

The use of plastics in the packaging of foods has been increasing at an accelerated rate. The reason for this is the reduction in the cost of packaging materials due to technological innovations and the inherent properties of plastic films, which makes them very well suited to food packaging. Active packaging technology is a relatively novel concept designed to provide interaction between food and packaging material, while sustaining the microenvironment within. It is aimed at extending the product shelf life, maintaining its nutritional and sensory quality, as well as providing microbial safety.

5.2 Consumer trends driving innovation

Consumers are adding spice to their lives with meals infused with flavours of Asia, Latin America, Africa, Indian, Carribean and the Mediterranean. Ethnic foods, with

their multidimensional flavour and texture profiles, are becoming the trend, especially cuisines that feature a variety of spices, seasonings and condiments. Ethnic foods are no longer thought of as being ethnic, they have formed part of the mainstream consumption pattern.

The key factor driving this trend includes immigration, global travel, media coverage, environmental interest and perception of freshness. Spices and seasonings build flavours and set apart one cuisine from another. Consumers' knowledge of spices and their technology including how to store them effectively in order to reduce product deterioration becomes of paramount importance. Spices are typically available in a variety of forms including fresh, dried, frozen, whole, ground, crushed or pureed; or as pastes, extracts or infusions.

Fresh spices such as ginger, cilantro, galangal, lemongrass, sweet basil, chilli peppers or curry leaves are frequently used by chefs and consumers. Their fresh taste is a result of their overall flavour, aroma and texture. Using fresh spices in commercial applications presents significant problems since if they are not seasonal and processed immediately they have short shelf life and stability. Fresh herbs and spices are available in another product format that uses a patented formulation to provide the fresh product, with a 90-day refrigerated shelf life.

Dried spices are available throughout the year, are cheaper in costs but lack the aromatic properties of their fresh counterparts. Volatile oils are lost or oxidized during drying, curing, crushing, grinding or other processing methods. Dried spices become more concentrated in their nonvolatiles, which can result in bitterness, increased pungency and unbalanced flavours.

Spice extracts are produced by grinding or crushing the spices and extracting them with steam distillation, solvent extraction or other methods. Most of the volatile and nonvolatile components that give each spice its flavour are concentrated forms of spices used for uniformity and consistency of flavour, colour and aroma. The volatile portions include essential oils and typify the spice aroma. The nonvolatiles include the oleoresins and aquaresins and include fixed oils, gums, resins, antioxidants and hydrophilic compounds that contribute to taste or bite. Since oleoresins frequently lack volatile compounds, both oleoresins and essential oils are needed to drive a more complete spice profile. To ensure spices and herbs maintain the flavour properties for as long as possible appropriate storage and packaging techniques need to be utilized. The types of techniques will be unique to the type of herb and spice and will be discussed in more detail later in the chapter.

5.3 Herb and spice product formats and packaging techniques

5.3.1 Examples of fresh and dried packaging formats

To pinpoint the most appropriate packaging to be used for fresh or dried herbs and spices it is critical to understand the following factors:

- Light sensitivity. Spices containing carotenoids or chlorophyll are highly susceptible to deterioration by light. The light will cause changes in the colour of all spices and cause the colour to fade.
- Flavour sensitivity. As soon as spices are harvested their inherent essential oils begin to deteriorate. Some varieties of spices will deteriorate in flavour more rapidly than others due to the highly volatile compounds.

- Moisture and oxidation sensitivity. The smaller the particle size in ground spices, the larger the surface area exposed to atmospheric conditions and the more susceptible the product is to moisture penetration and oxidation. Increasing the moisture content of the spices can also lead to problems with insect damage and possibly potential microbial risk factors if the water activity of the product medium reaches high levels. In order to reduce the oxidation reactions it is important to avoid high-temperature storage, utilize packaging with low oxygen permeability and gas flush with controlled atmosphere or modified atmosphere conditions.
- Grinding. As soon as dried or fresh spices are size reduced this increases the surface area exposed to atmospheric conditions. In doing this it can cause increased susceptibility to oxidation reactions, moisture increases and flavour loss. To limit these reactions it is important to control the temperature, contact with oxygen, the humidity surrounding the product and reduce the contact with light.

Grinding of spices is an important processing step during which loss of volatile aromatics occurs. The technology used at present for grinding spices has inherent disadvantages of high heat generation, loss of volatile oil and low efficiency. This method of grinding is not desirable for materials of plant origin including spices with high heat sensitivity, high fat and fibre contents. Loss of volatile oil could be overcome partly by lowering or maintaining the mill temperature as low as possible (less than the boiling temperature of the volatile constituents of the spices volatile oil). The shelf life of ground spices is about three to four months at refrigeration temperature and whole spices will have about one and a half years of shelf life.

5.3.2 Herbs and spices (refrigerated formats)

Herbs are found in a variety of forms including fresh herbs, fresh herbs and other ingredients packaged in a tube format, herbs in a paste format that have been heat treated and dried herbs.

Fresh herbs

Fresh herbs are mainly sold in bunches with an elastic band holding the product together. In this format the level of deterioration that occurs during storage will vary depending on the type of herb being analysed and the environmental conditions. Most herbs are unlikely to be satisfactory after one week of storage in refrigerated conditions, some will last up to two weeks providing the environmental conditions are acceptable. Fresh herbs can also be stored in plastic bags but this procedure requires more attention to the type of plastic used and the atmosphere present within the bag in order to ensure product quality and safety is maintained.

Fresh herbs and spices in a tube packaging format

Herbs are also sold in a patented formulation that uses fresh herbs in combination with other ingredients to maintain a high-quality product that ensures the essential oils and the microbial safety remain acceptable during storage. This unique product format is now located world-wide in Australia, New Zealand, USA, Canada, Europe and Asia. This product format uses innovation in the packaging to provide consumers with a tube format that allows individual choice as to the amount of herb or spice added to a food as well as a single-serve packaging format to provide consumers with convenience. The tube format incorporates a higher level of technology in the packaging

so as to provide consumers with a format that is easy to use as well as protecting the product from environmental influences.

5.3.3 Herbs and spices (dried)

Whole or ground herbs and spices
The natural whole spices are much more robust and deteriorate much slower than spices that have been sized reduced. Ensuring the surface of the spices remains unaltered assists in minimizing both a chemical and microbiological change in the spice. Many spices are still packed under conventional types of packaging materials and there is great scope for improving the type of packaging materials. Twill or gunny bags are used depending on the value of the spices. The weave clearance of the different types of twill and gunny bags is 1–2%, 3–5% and 4–6% respectively, which prevents spillage but also restricts insect movement in the bags. Sometimes double bags are used to get a better physical barrier. Polyethylene lined gunny bags and HDPE woven sacks are used to restrict moisture ingress during storage. Besides the conventional jute bags, multi-wall paper bags, plastic sacks and paperboard boxes can offer better protection and appeal.

Coriander
Ground coriander can be stored for up to six months in aluminium foil bags and is the preferable storage mechanism. Jute bags lined with polythene are ideal for storage for large quantities of powder. Paper, polyethene and cotton bags are not suitable for storage. It is critical that ground spices are protected adequately from oxygen as the large surface area exposed will increase the rate that flavour deterioration occurs. Coriander seed can be stored in polythene bags or cotton bags for six months with minimum loss of flavour. After a period of 12 months there was a loss of 20–25% volatile oils. Flexible plastic films and foil laminates with better physico-chemical properties need to be used for necessary protection to the product against loss of volatile oil, seepage of fats and ingress of moisture during storage. The seeds should be stored under cool, dark and dry conditions so as to prevent browning, loss of flavour and ingress of moisture. Under good storage conditions it is reported that coriander seed will retain flavour and colour for 6–9 months.

Garlic
Among the most important garlic products are garlic flakes, dehydrated garlic powder, garlic paste and garlic salt. Garlic harvested for dehydration is brought to a dehydration plant in large bulk bins or open mesh bags. The bulk is broken into individual cloves by passing between rubber-covered rollers, which exert enough pressure to crack the bulb without crushing the cloves. The loose paper shell is removed by screening and aspiration. The cloves are washed in a flood washer, at a time the root stabs are floated off. Garlic is sliced and dehydrated in a manner similar to that used for onions. Garlic is commercially dried to about 6.5% moisture. Dehydrated garlic is sold commercially as garlic powder, granules, sliced, chopped or in a minced form.

Garlic powder is obtained by drying the garlic at temperatures of 50–70 °C for five to eight hours and results in losses of volatile flavour up to 30–35%. The non-enzymatic browning reactions result in a yellowish brown powder which is undesirable. Clumping is also an issue as the powder is highly hygroscopic and must be maintained in packaging that maintains low water vapour transmission.

Cinnamon

Cinnamon is prepared from removing the inner and outer bark. The inner bark curls naturally into quills, which are joined to increase their length, and filled with smaller quills and pieces to make a near solid cylinder. First-quality quills are smooth, uniform yellowish brown. Smaller quills, bark pieces are sold separately and a proportion chopped or ground for local sale or distillation. Cinnamon quills should be stored in sacks, and highest quality bark is wrapped in new sacking or corrugated cartons. Quills are pressed into cylindrical bales of 100–107 cm, weighing 45–50 kg for shipment. Bark is packaged into individual containers that are based on individual recognized standards.

Turmeric

Turmeric is a plant of the Zingiberaceae family. The rhizome of this plant when dried and ground provides a yellow and flavourful powder, used for centuries as a natural colouring agent in food, cosmetics and textiles, and as a flavouring agent. Turmeric is usually dried by sun drying or artificial drying and is then ground into a powder to be predominantly used as a colouring.

Cumin

The current method of grinding spices including cumin involves the use of a grinder that subjects the spices to elevated heat levels. Grinding cumin at chilled temperatures increases volatile oils and improves the fineness of the particles and sensory qualities. Aluminium and polyethelene pouches and a storage condition of 37 °C and 70% relative humidity are an ideal storage condition.

Nutmeg

Following collection the seed (nut) with surrounding aril is separated from the fruit and the aril (mace) is detached. After drying, nuts are shelled and become the spice nutmeg. Nutmeg trees produce three main products including nutmeg and mace used directly as spice, nutmeg and mace oils and oleoresins used as spice and flavourings and leaf oil and other derivatives. Nutmeg and mace are the most important domestic products but oils and oleoresins have becomes more common within industrial applications.

Nutmeg and mace should not be ground until required as the organoleptic qualities rapidly deteriorate, mainly through loss of volatile oils. Incorrectly stored nutmeg oil may also undergo significant composition changes if exposed to a high ambient temperature. Unprotected powders and oil can absorb unpleasant odours. Powders, oils and oleoresins should be stored in full, sealed, preferably opaque glass containers until required. Nutmeg is sold as whole nutmeg in importing countries and is further ground to a distinct mesh size for spice powders. The whole nutmeg is packaged in bags while the nutmeg spice can be sold in a range of packaging but most commonly glass or high-barrier plastic packaging film to protect the quality of the product.

Mustard

Mustard produces seed, the most important product, and has an oil content of 30%. It is critical during drying and storage that the seed is not overheated as this can cause rancidity and loss of quality. The seed received at storage has a moisture content of 10% and 25% from standing crops and between 10% and 15% from windrowed. Clean and dry seeds store well due to the hard outer surface, but appropriate packaging

is required to prevent the round seeds running freely. The bulk mustard seed is stored in sacks or in bulk. The mustard meal is manufactured by grinding dry, whole seeds and should be kept fresh or sealed, using opaque containers in a cool environment. This product is seldom used as a food-based product and is utilized more in the medicinal industry.

Pepper
Pepper is one of the most prominent spices found in the world. There are various forms of pepper that are manufactured. After harvesting, the pepper berries are separated from the spike by rubbing between the palms or trampling under the feet. The green pepper is then dipped in hot water for one minute and dried in sunlight for uniform colour and speedy drying for 5–7 days to obtain a moisture content of 10–11%. The dried pepper is cleaned to remove stems, husks and pinheads. The white pepper is the product obtained from berries that are fully ripe. They are picked and piled in heaps to ferment or are soaked in water for 5–7 days, the pulp and the outer costing of the seed are then removed. White pepper is yellowish grey in colour and has a smooth surface. It is also prepared from black pepper by grinding off the outer parts by machinery.

Ginger
Ginger powder stored in glass jars at 4 °C showed a significant decrease in gingerol content after eight weeks storage (23%) and after 16 weeks storage the level had further decreased (37%). This compares to ambient storage at 23 °C where the gingerol decrease was 30% after eight weeks storage and 37% after 16 weeks storage.

Heat treated herbs and spices in glass or plastic jars
There are a variety of herb pastes that incorporate the raw herb or spices with other ingredients and the product is heat treated. This treatment will reduce the level of quality deterioration but also reduces the level of natural essential oils that provide the key flavour attributes of the product. These products are typically packaged in glass or plastic jars. This type of packaging medium is quite common in the herb and spice industry.

5.4 Essential oils

Essential oils are a complex mixture of volatile compounds responsible for the aromatic characteristics of the spice. They are comprised of two basic groups which are hydrocarbons including terpenes, sesquiterpenes and diterpenes and oxygenated hydrocarbons such as alcohols, esters, aldehydes, ethers and ketones.

Basil
During the drying process the essential oil composition of herbs and spices changes. The results on basil indicated a 19% overall loss of essential oil after drying and three months of storage in aluminium polyethylene polyamide bags. After six months storage in the bags there was a 62% loss in essential oils. During storage there was a decrease in the total quantity of essential oils as well as volatile essential oils. Methylchavicol and eugenol decreased linearly and some other components disappeared altogether.

Ginger
Ginger oil is produced by steam distillation of the freshly ground ginger. The ginger oleoresin contains the volatile oil and the pungent extracts. The oleoresin can be encapsulated to present it in a dried form. This can be achieved by spray drying and can then be easily incorporated in food products.

Cinnamon
Cinnamon bark produces two oils, a superior type derived from the inner bark and a lower quality from broken quills, chips and bark. Cinnnamon oil is frequently sold as unrefined crude oil in 200-litre drums, or refined in 50-litre and 200-litre drums. The oils should be kept cool and should be stored in containers that have the minimum allowable oxygen headspace to minimize oxidation and loss of product quality.

Turmeric
Turmeric is an important spice which is used in curries and as a natural colouring. Among the cuciminoid pigments responsible for the colour of turmeric, curcumin is a major pigment. The curcumin content continued to decrease during storage up to a period of ten months and after this period the level of decrease was minor.

Nutmeg
Nutmeg oil is mainly used as flavouring in a range of edible food products and must be stored in opaque containers and in a cool environment to protect the product from oxidation.

Mustard
Mustard oil is obtained by extracting whole seeds to obtain an oil content of 25–35% and is mainly used as cooking oil. To maintain the product quality it should be packaged in opaque glass or high-protection barrier plastic to prevent oxidation and maintain quality.

5.5 Oleoresins

Spice oleoresins are a liquid, semi-solid or solid residue obtained by solvent extraction and possessing the full character of natural spices. The main components of an oleoresin include essential oils, fixed oils, pigments, pungent constituents and natural antioxidants. The process for obtaining oleoresins is designed around extracting both essential oil and non-volatile components that are desirable and contribute largely to the flavour profile. The solvent is removed by using a vacuum and the concentrated extract is the oleoresin. The physical characteristics of oleoresins range from viscous oils to thick, tacky pastes. This makes it difficult to add these components directly to the food. The most suitable method for utilizing the oleoresins is to use a carrier and options that are utilized are as follows:

- emulsions prepared by blending essential oils with gum arabic or other emulsification agents
- essences developed with ground spices and ethanol and the addition of essential oils or oleoresins
- solubilized spices are blended with essential oils and/or oleoresins mixed with a polysorbate ester or other agent

- Dry soluble spices are prepared by dispersing an essential oil and oleoresins onto a carrier such as salt, dextrose or other types of ingredients.
- Encapsulated spices are prepared by spray drying pre-made emulsions using gum arabic or starches. Once dried these flavours are encapsulated, the flavour is released when added to water.

Oleoresins have a high stability in storage and have a minimum shelf life of one year, without any loss in quality. There are also other advantages including reduced space storage requirements as they require only 1–10% of the space required by ground spices. Controlled-atmosphere storage (temperature and humidity) is generally not required.

5.6 Storage requirements for fresh and dried herbs and spices

Most herbs are marketed in the dried form, since a high concentration of water will cause product deterioration over time. The changes in the volatiles depend on factors such as the drying method, the biological characteristics of the plants and their volatile composition. Oven drying and freeze drying applied to dill and parsley leads to a significant loss in volatiles. This compares to the effect on drying bay leaf which is much less.

5.6.1 Main factors that cause deterioration of foods during storage

- climatic influences that cause physical and chemical changes (UV light, moisture vapour, oxygen and temperature changes)
- contamination (by micro-organisms, insects or soils)
- mechanical forces (damage caused by impact, vibration, compression or abrasion)
- pilferage, tampering or adulteration.

5.6.2 Selection of packaging materials

Packaging of food is usually utilitarian and protective. The primary purpose of packaging is to preserve the flavour and keep the product in good condition until it reaches the consumer. A large number of factors must be considered in detail when choosing a suitable packaging material for food that provides flavour. The factors can be grouped into basic factors and consumer acceptance factors. Basic factors include:

- price of packaging
- protection of product from contamination
- resistance to impact injury
- effectiveness of interior surface
- absence of handling problems
- space and other storage requirements
- special features relating to the performance of the package.

Consumer acceptance factors include:

- size
- ease of opening
- reseal features

- pouring qualities
- space saving of consumer's premises
- protection from light
- transparency
- tamper-proof construction
- physical characteristics of outside surface including appearance
- ease of disposal
- special features relating to performance for consumer.

5.7 Types of packaging material

The various materials suitable for packaging of foods include paper products, polyethylene flexible films, aluminium foils, glass, tin, hessian and timber. The selection of packaging material intrinsically will depend on the nature of the product and other considerations.

5.7.1 Paper and cardboard cartons

These are the least expensive unit packages for whole spices. They have good advertising potential and can be folded into any shape. Wax coating on the outside improves attractiveness as well as resistance to water. Polyethylene coating inside gives extra protection as well as sealability. Paper and cardboard are unsuitable for ground spices, owing to their high permeability to flavour components and gases. This disadvantage can be overcome by an inner pouch of polyethylene.

5.7.2 Aluminium foil

This offers excellent potential for packaging ground spices. It is not transparent and is ideal for spices that need protection from light. Its resistance to gas transmission is essential to protect the delicate flavour of many spices. It is subject to puncture, but this can be overcome by laminating the outside with paper. Heat sealability can be accomplished by coating the inside with a heat sealable film such as polyethylene. Aluminium is also used as the barrier material in laminated films to metallize flexible films and to make collapsible tubes for viscous products.

5.7.3 Glass

Although glass can be made into a variety of shapes, particularly for marketing high-value products such as liquors and spirits, simple cylindrical shapes are stronger and more durable. Glass surfaces may be treated with titanium, aluminium or zirconium compounds to increase their strength and enable lighter containers to be used. Glass can be made in a variety of colours including green, amber and blue.

5.7.4 Flexible films

Since a single film does not fulfil all the functional requirements, a combination of films can be used to obtain the desired effect. This can be achieved by lamination, coating or co-extrusion.

5.7.5 Single films

The most important types of film for food packaging are described below.

Cellulose films

Plain cellulose is a glossy transparent film which is odourless, tasteless and biodegradable within approximately 100 days. It is tough and puncture resistant, although it tears easily. It has low-slip and dead folding properties and is unaffected by static build up, which makes it suitable for twist wrapping. It is not heat sealable and the dimensions and permeability of the film vary with changes in humidity. It is used for foods that require a complete moisture or gas barrier, including fresh bread and some types of confectionery.

Oriented polypropylene is a clear glossy film with good optical properties and a high tensile strength and puncture resistance. It has a moderate permeability to moisture, gases and odours, which is not affected by changes in humidity. Biaxially orientated polypropylene has similar properties to orientated polypropylene but is stronger.

Polyethylene terephthalate (PET) is a very strong transparent glossy film which has good moisture and gas properties. It is flexible at temperatures from –70 °C to 135 °C and undergoes very little shrinkage with variations in temperature and humidity.

Low density polyethylene (LDPE) is used as a copolymer in some tubs and trays. It is heat sealable, chemically inert, odour free and shrinks when heated. It is a good moisture barrier but has relatively high gas permeability, sensitivity to oils and poor odour resistance. Low slip properties can be introduced for safe stacking or, conversely, high slip properties permit easy filling of packs into an outer container. It is the least expensive of most films and is therefore widely used.

High density polyethylene (HDPE) is stronger, thicker, less flexible and more brittle than low density polyethylene and has lower permeability to gases and moisture. Sacks made from 0.03–0.15 mm HDPE have a high tear strength, tensile strength, penetration resistance and seal strength. They are waterproof and chemically resistant and are used instead of multi-wall paper sacks for sipping containers. Other types of film structures include uncoated polyvinylidene chloride (PVdC), polystyrene and ethylene vinyl acetate (EA).

Coated films

Films are coated with other polymers or aluminium to improve their barrier properties or to impart heat sealability. A thin coating of aluminium produces a very good barrier to oils, gases, moisture, odours and light. Metallized film is less expensive and more flexible than foil laminates which have similar barrier properties. Metallized polyester has higher barrier properties than metallized polypropylene, but polypropylene is used more widely as it is less expensive.

Laminated films

Lamination of two or more films improves the appearance, barrier properties and/ or mechanical strength of a package. Laminates typically include nylon-LDPE, nylon-PVdC-LDPE and nylon-EVOH-LDPE for non-respiring products. The nylon provides strength to the pack, EVOH or PVdC provides the correct gas and moisture barrier properties and LDPE gives heat sealability. PVC and LDPE are also used for commonly respiring MAP products.

Coextruded films
Coextrusion is the simultaneous extrusion of two or more layers of different polymers to form a single film. Coextruded films have the following advantages over other types of film:

- They have very high barrier properties, similar to multi-layer laminates but are produced at a lower cost.
- They are thinner than laminates and closer to mono-layer films and are therefore easier to use on forming and filling equipment.
- The layers cannot separate.

The main types of compounds used in this application are:

- olefins (low-density and high-density polyethylene and polypropylene)
- styrenes (polystyrene and acrylonitrile-butadiene-styrene)
- polyvinyl chloride polymers

Edible and biodegradable films
There has been a paradigm shift imposed by growing environmental awareness to look for packaging films and processes that are biodegradable and therefore compatible with the environment. The concept of biodegradability enjoys both user-friendly and ecofriendly attributes, and the raw materials are essentially derived from either replenished agricultural feedstocks or marine food processing, and therefore capitalize on natural resource conservation with an underpinning on environmentally friendly and safe atmosphere.

Biopolymers from agricultural feed stocks and other resources have the ability upon blending and/or processing to result in appropriate packaging materials. Their functionality can be better expressed by using in combination with other ingredients such as plasticizers and additives. The potential uses for such biopolymeric packaging materials are:

- use and throw, disposable packaging materials
- routine consumer goods for day to day use
- disposable personal care
- lamination coating
- bags for agricultural uses.

Two types of biomolecules (hydrocolloids and lipids) are used in combination for the preparation of biodegradable packaging films or composites. Individually they lack structural integrity and characteristic functionality. Hydrocolloids, being hydrophilic are poor moisture barriers, a property compensated by adding lipids, which are very good moisture barriers. Composite films are a mixture of these and other ingredients in varying proportions, which determine their barrier (to water, oxygen, carbon dioxide and aroma compounds) and other mechanical properties.

Synthetic polymers are gradually being replaced by biodegradable materials especially those derived from replenishable, natural resources. More than the origin, the chemical structure of the biopolymer determines its biodegradability. Use of biopackagings will open up potential economic benefits to farmers and agricultural processors. Bilayer and multicomponent films resembling synthetic packaging materials with excellent barrier and mechanical properties need to be developed. Cross-linking, either chemically or enzymatically, of the various biomolecules is yet another approach of value in composite biodegradable films. Innovative techniques for preserving food

safety and structural-nutritional integrity as well as complete biodegradability must be adopted as these provide the means for sustained environmental management.

5.7.6 Active packaging technologies

The choice of type of active packaging is based on three broad considerations. Most important is the requirement of the food, followed by the packaging format and the requirement of the active agent. The main types of active packaging are oxygen scavenging, carbon dioxide scavenging or release, packaging to remove odours and antimicrobial packaging.

5.7.7 Modified atmosphere packaging

Controlled atmosphere and modified atmosphere are terms implying the addition or removal of gases from storage rooms, transportation containers or packages in order to manipulate the levels of gases such as oxygen, carbon dioxide, nitrogen and ethylene, etc. Modified atmosphere is more commonly used and is used to extend the shelf life of food products and to prevent any undesirable changes in the wholesomeness, safety, sensory characteristics and nutritive value of foods. MAP achieves the above objectives based on three principles:

- reduction of undesirable physiological, chemical/biochemical and physical changes in foods
- control of microbial growth
- prevention of product contamination.

5.8 Printing

5.8.1 Bar codes and other markings

The Uniform Code Council (UCC) or EAN International provide companies with a bar code number to enable consumer products to be scanned by lasers in checkouts. The Uniform Code Council (UCC) and EAN International have changed their names. GS1 is the new name for EAN International and the UCC has also changed its name to GS1 US. The UPC or EAN code consists of a manufacturer number which is combined with a product number and a digit assigned by bar code software systems. The UCC Company prefix is provided by the Uniform Code Council (now GS1 US or GS1) and the manufacturer creates the individual product number. When the prefix number and product number are entered in the software it automatically generates the check digit.

There are currently five versions of UPC and two versions of EAN. The Japanese Article Numbering (JAN) code has a single version identical to one of the EAN versions with the flag characters set to 49. UPC and EAN symbols are fixed in length, can only encode numbers and are continuous using four element widths. The most frequently used UPC version has ten digits plus two overhead digits while EAN symbols have 12 digits and one overhead digit. The first overhead digit of a UPC symbol is a number related to the type of product while the EAN symbol uses the first two characters to designate the country of the EAN International organization issuing the number. UPC is a subset of the more general EAN code. Scanners equipped to

read EAN symbols can read UPC symbols. However, UPC scanners will not necessarily read EAN symbols. The bar code has restrictions on sizing in order to ensure the highest level of accuracy when being scanned. It is recommended to be 1.469 inches wide and 1.02 inches high. It is recommended to use black bars on a white background to provide the highest level of contrast in colours.

5.9 Microbiological safety of herbs and spices

The microbiological risks associated with herbs and spices vary dramatically according to the plant type and the process to which it is subjected. The main factors are:

- Growth habit. Herbs with curly or hairy leaves and stems are more likely to collect and retain bird droppings, dust and moisture than flat-leafed varieties, as are those with low growing habits. Root and bulb crops are obviously closely associated with the soil and are prone to contamination by poor irrigation techniques. All these factors are potential contributors to contamination with Enterobacteriaceae (including *Salmonella* and *E. coli*), yeasts and moulds.
- Harvesting and storage. Many herbs and spices are gathered from the wild and then stored in uncontrolled conditions. Ingress of insect and rodent pests can present major problems.
- Processing. High moisture content products are often air dried, open to the sun or under minimal cover.

Spices are used all over the world as flavouring agents in staple food items. The harvest of these crops predominately occurs in the warm, humid areas of the world where large numbers of micro-organisms are readily viable. The microbiological quality, load of total heterotrophs or Enterobacteriaceae in particular, often acts as an indicator of the hygienic conditions. Spices are exposed to a wide range of environmental contamination during collection and processing by dust, waste water and animal and even human excreta. Contaminated spices may cause a microbiological problem, depending on the end use. Cuisines that incorporate spices may pose a risk to public health because they are often added to foods that undergo no further processing or are eaten raw. Spices are the principal source of spore-forming bacteria in large volumes of foods such as soups, casseroles, stews and gravies. Under favourable conditions they germinate and multiply to infective and toxic levels. The key micro-organisms responsible for these are *Bacillus cereus*, *Clostridium perfringens*, *Escherichia coli*, *Salmonella* and toxigenic moulds.

The number of microbes on spices varies considerably according to the particular spice. Black pepper, capsicum spices, turmeric and allspice contained the highest microbial levels whilst cloves, nutmeg and cinnamon tend to be less contaminated. In many spices the microflora consists mainly of mesophilic spore formers that originate from the soil. Levels vary considerably but average counts in untreated products are in the region of 100,000 colony-forming units per gram. Spores may comprise more than 50% of the bacterial count. Spore formers capable of causing food poisoning when ingested in large numbers, such as *B. cereus*, *B. subtilis* and *C. perfringens*, are found in spices in low levels. Non-spore formers also form part of the microflora of spices. Studies show they are present in approximately 50% of untreated spices at levels between 10 to 100,000 CFU per gram.

Escherichia coli is found less frequently and usually in low numbers (<10 CFU per gram). Salmonella is found occasionally in a wide variety of spices and has been responsible for a number of outbreaks of salmonellosis. Enterococci are present in approximately 50% of spices, usually in low numbers and rarely exceeding 10,000 colony-forming units per gram. Moulds are frequently present on spices, usually at levels of less than 100,000 colony-forming units per gram, but yeasts are rarely found. The types of mould found include *Aspergillus spp*, *Penicullum spp*, *Rhizopus spp*. and *Spicara spp*. Some moulds capable of producing mycotoxins have been found in a range of spices including black pepper, ginger, turmeric, nutmeg, red pepper, cumin and mustard seeds. Nutmeg and red pepper is usually prone to aflatoxin production but levels are usually low, usually less than 25 μg/kg.

5.9.1 Strategies for reducing microbial load in herbs and spices

The food industry has long been aware of the risks associated with unprocessed herbs and spices and a variety of measures to eliminate or counteract microbial contamination have been used and are used today. However, many of these measures have, with time, been declared as dangerous (e.g., ethylene dioxide treatment) or unacceptable to most consumers (e.g., irradiation). Chemical preservatives in food items, such as sulphur dioxide, work by inhibiting the microbial uptake of oxygen thereby restricting their growth. Consumer reaction to E numbers has led manufacturers to remove preservatives where practical, thus placing further pressure on the herb and spice industry to supply ever-tightening microbiological specifications. Indeed, there is evidence that certain food preservatives such as nitrates and nitrites react in the presence of spices producing potentially carcinogenic nitrosamines.

Current strategies to process herbs and spices to reduce their microbial loads to acceptable levels include:

- Improved agriculture. The world's largest spice houses are working increasingly closely with growers and farmers and, by modifying irrigation and fertilizers, it is possible to produce a cleaner raw material for processing. This is effective in reducing microbial loads but cannot eliminate risks as the product is still largely processed in the open air.
- Product selection. Assessment of numerous batches of herb and spice products and the selection of the microbiologically cleanest can be effective.
- Irradiation. Ionizing radiation, which disrupts bacterial chromosomes to effect reduction of microbiological load, is highly effective but has a very poor public image.
- Heat treatment. Whole or ground herbs and spices are pasteurized by flash processing with steam or heated dry air.
- Flavour extraction or oleoresins. Steam distillation will extract the volatile flavour principles (essential oils), which due to the process are microbiologically sterile. Solvent extraction will additionally remove the non-volatile compounds. These extracts are known as oleoresins and contain the whole flavour character of the raw material.
- Encapsulation of herb and spice extracts. An encapsulation process converts liquid essential oils or oleoresin extracts into free flowing powders.

5.10 New packaging materials used in herbs and spices

The type of packaging materials predominately used in the herb and spice industry has not changed dramatically in the last 20 years. In recent years there has been an increased focus on utilizing the latest technology. New packaging materials such as coated BOPP films, nylon and polyester-based films, special laminates in combination with cellophane, polyethylene, polyester, multilayer coextruded nylon based films, coextruded films based on ethylene vinyl alcohol (EVAL) and PVDC-coated BOPP have a bright future for packaging spices. PET bottles with a unique shape and clarity may be considered a good possible alternative. Stand-up pouches are suitable for green and red pepper in brine solution and for ground spice powders. Fresh herbs and spices in a tube format bring innovation and convenience to the industry.

The product characteristics, storage and distribution conditions, dictate the required barrier properties of the packaging materials used for a specific application. Barrier properties include permeability of gases (oxygen, carbon dioxide, nitrogen, ethylene, etc.), water vapour, aromas and light. There are vital factors for maintaining the quality of foods. However, packaging materials cannot be chosen solely on the basis of their barrier properties. Factors such as processability, mechanical properties (tensile strength, elongation, tear strength, puncture resistance, friction, burst strength, etc.) migration/absorption and chemical resistance must also be considered. Environmental factors such as temperature, relative humidity and light intensity to which the product is exposed during storage and distribution must also be taken into consideration when selecting packaging materials.

5.11 Future trends

A continuing trend in food packaging technology is the study and development of new materials that possess very high barrier properties. High barrier materials can reduce the total amount of packaging materials required, since they are made of thin or lightweight materials with high barrier properties. The use of high barrier packaging materials reduces the costs in material handling, distribution and transportation and waste reduction.

Convenience is also a focus of manufacturing, distribution, transportation, sales, marketing, product development. Consumption and waste-disposal levels are also very important. Convenience parameters may be related to productivity, processibility, warehousing, traceability, display qualities, tamper-resistance, easy opening and cooking preparation. Safety is the third most critical element due to the risk of food bioterrorism. Food-borne illness and malicious alteration of foods must be eliminated from the food chain.

Consumers also want their food packaging to encompass all of the points discussed as well as being natural and environmentally friendly. The substitution of artificial chemical ingredients in foods and in packaging materials with natural ingredients is always attractive to consumers. The trend will be to move towards environmentally friendly packaging material that are more natural and contain more recyclable or reusable materials.

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6

QA and HACCP systems in herb and spice production

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6.1 Introduction

The herb and spice industry is a truly global industry. Raw materials from various countries around the world can become combined in products that are sold to consumers everywhere. A bottle of 'Herbs de Provence' sold in a small town in Canada could contain lavender from Canada, basil from Israel and rosemary from Italy. Caraway produced in the Canadian prairies could end up on the shelf of a cook in France, Coriander in a curry in India. Chamomile tea sold in Canadian supermarkets could contain Chamomile grown in the wild in Romania. Echinacea grown in Canada could end up in an extract in Italy. Many ethnic communities in Canada import herbal products to support the medicinal and culinary trends of those cultures.

The global availability of herbal material has provided consumers with an ever expanding choice of products. The question must be asked. Does this also expose consumers to an ever greater risk of food-borne illness? The answer is yes, and this risk must be controlled. In recognition of this need the Canadian herb and spice industry has evolved from small regionalized groups to a national forum. It was an effective process that brought together ten provinces and one territory. To understand the significance of this it is first necessary to understand the immensity and diversity of this country. Canada spans the northern half of the North American continent and touches three of the world's seven oceans. Her people range from the Atlantic fisheries-based east coast provinces, through an industrial and agricultural heartland through prairies, vast mountain ranges and on to the western coast on the Pacific Ocean. Canada extends north to the Arctic ocean and embraces communities that have become adapted to the harsh climate of the extreme north. In all of these regions people grow, collect and consume herbs.

In 2002 the Saskatchewan Herb and Spice Association, which represented herb and spice producers in western Canada from the prairie provinces to the west coast, explored the possibility of a national organization that tied the entire country together. Prior to 2002 the herb and spice industry was split between an organized but struggling western industry and many small distinct groups to the east and north. Key producers and manufacturers were contacted across the country and an inaugural meeting was

held. Everything came together at the right time. Federal and provincial governments were developing a focus on food safety and new regulations for natural health products, consumers were developing an awareness of the importance of safe products and farmers were working toward safe production practices and environmental sustainability. In 2004 the first national meeting of the Canadian Herb, Spice and Natural Health Product Coalition was held in Guelph, Ontario.

A mandate and strategic plan were developed. By 2005 the Canadian industry had moved from disjointed regional sectors to a unified coalition. They had developed a HACCP-based on-farm food safety model, an international plant identification practice, been an inaugural player in the development of the natural health product regulations and worked with the agriculture industry to develop a sector council for Agriculture. They also have been part of the development of national standards for traceability through the Cantrace initiative, are represented on the Special Crop Value Chain Round Tables and the medicinal crops working group for international harmonization of minor use products. They are part of the Natural Health Product Research Society and lead an initiative to guide the development of appropriate regulations for natural health products for animals.

They have successfully raised the profile of this industry and linked industry sectors together both at home and abroad. These connections have built business relationships and value chains across the industry. The scope of the coalition encompasses field and greenhouse production, wild harvesting, primary processing, manufacturing and finished products. The coalition also encompasses research, regulations, education, and includes the perspective of the consumer and practitioners in the industry. Visit the website at www.nationalherbspice.com for more information.

6.2 HACCP planning for herb and spice production

Food safety is one of the key issues facing the entire value chain of food production around the world. The safety and quality of any food product or raw ingredient begins at the source either with wild-harvesters or with the producers. By implementing good agriculture practices (GAP) on the farm, the safety at the beginning of the value chain can be optimized. For many products, buyers and manufacturers are coming to expect that their suppliers, including on-farm producers, have these practices in place. Two of the primary drivers of this initiative have been the issue of correct identification and the reduction of adulterants, both vital in the medicinal and culinary world.

The Canadian Herb, Spice and Natural Health Products Coalition (CHSNC) is one of over 20 national industry groups involved in the Canadian On Farm Food Safety Program (COFFS). These industries are developing voluntary HACCP-based On-Farm Food Safety Programs and Good Agriculture Practices (GAPs).The goal of the COFFS program is to use HACCP (Hazard Analysis and Critical Control Point) principles to enable a facility to provide protection from contamination of the food supply from the source to the consumer. Although it is technically impossible to remove all risk all the time, an accurate detailed on-farm food safety program using good agriculture practices will reduce risks and show due diligence. The herb and spice program covers natural health products, medicinal and culinary herbs (cultivated and wildcrafted) and spice crops. The end result is a government-recognized COFFS program for each industry group.

GAPs are really about 'saying what you do', 'doing what you say' and 'verifying that you did what you said you were going to do' on your farm or enterprise. Although GAPs require the development of consistent practices on your farm and formal record keeping, the real benefits for growers are traceability and safety assurances. Should manufactured product be recalled, GAPs provide growers with a recognized method of verifying whether or not their raw materials were part of the problem. Product can travel across the globe with traceable accountability that tracks back to the source. It also provides a mechanism for isolating problems on the farm from the rest of the production. The GAPs were developed to:

- assist with the process of risk identification
- aid in the development of appropriate solutions
- apply practices that eliminate or reduce these risks.

The HACCP based GAPs, once in place, will:

- protect human health by reducing food-borne hazards
- increase consumer confidence in the safety and quality of the products they consume
- enhance sector capacity to meet or exceed market requirements.

Establishing core principles

HACCP is a systematic approach to ensure food safety. It targets prevention rather than detection of problems. HACCP principles can be applied directly to the processing stage to ensure safety. HACCP is an internationally recognized system that uses sound principles in choosing corrective and preventive actions for food safety-related problems. The first step in developing a HACCP-based model is to work through a seven-point program and customize it to your operation. It would be unwieldy and virtually impossible for each producer or collector to develop individual HACCP programs. For this reason the CHSNC identified core principles that applied to the herb, spice and natural health product industry as a whole. These core principles are:

Principle 1: conduct a hazard analysis.
Principle 2: identify critical control points.
Principle 3: establish critical limits for each critical control point.
Principle 4: establish critical control point monitoring requirements.
Principle 5: establish corrective actions.
Principle 6: establish record-keeping procedures.
Principle 7: establish procedures for verifying the HACCP system is working as intended.

Three types of hazard

Under each of these core principles there are three types of hazard to be identified and addressed: biological, chemical and physical (BCP). Biological hazards are microorganisms that can directly cause illness or death, or create toxins in the food that could cause illness or death. These include pathogenic bacteria, yeast, mold, viruses and parasites. Chemical hazards are contaminants that may include residues from cleaners, agricultural chemicals, nitrates, heavy metals, lubricants and naturally occurring toxins known as allergens. Physical hazards are hazards that may cause physical injury to a consumer. Examples include glass, wood, stones, metals, wrong products in a tank or bin.

Traceability
Traceability is driven by many factors; some of the key ones are consumer confidence in the product, product credibility and market access, protection from brand fraud and adulterants, supply chain management, quality factors and the insurance system. Full traceability requires that producers are responsible for their product one level down from their operation and one level up, or one-step preceeding and one-step after. They need to know what they are buying or collecting, what goes into the production of it or the history of the land where the product is grown and where the product goes. Good record keeping is imperative to ensure this. Canadian Herb, Spice and Natural Health Product Coalition HACCP based GAPs were developed incorporating the CanTrace Standards for Traceability.

Auditing
The HACCP system developed for on-farm use is an auditable system. An audited system ensures traceability to the buyers. Producers must provide validation, either by third party or self-attestation ensuring that they can trace backwards at any stage and ensuring that all questions are able to be answered and documentation is in place to back this up. In a recall situation producers need to be able to isolate the problem if they are to prove it was not their product. If it is the producer's problem this system should be able to address the issue and enable them to fix the problem in a fast, effective and safe manner. A paper trail must be developed for an auditor, with farm maps, field history, input records, harvest and post-harvest records, storage data and sales information to confirm all processes and due diligence. Auditing is also a tool to ensure that producers are up to date and aware of regulations or changes to regulations that affect their operation. Auditing confirms that all processes are under control and that the producer/collector is providing a food or medicinal product that is safe and free from biological, chemical or physical contaminants.

Levels of GAPs
A GAP level relates to the significance of its impact in any given operation. The GAPs developed for this program fall into one of two levels; either 'must-do' GAPs or 'recommended practices'. A 'must-do' practice is relevant to all operations and has to be addressed. Any deviation from a 'must-do' practice must be recorded, explained and witnessed. 'Recommended practices' may be relevant to one operation and not to another. A risk assessment of the operation is vital to determine which practices should be implemented on a must-do basis and which are recommended to enhance the operation in ensuring quality, safety and traceability.

Critical limits and acceptable levels
A HACCP model identifies two things.

1. Points in the operation that have a critical impact on food safety (a critical control point) that can only be addressed through avoidance.
2. Points in the operation that recognize levels of food safety and that can be addressed through compliance with a designated procedure. These levels can be 'critical' or 'acceptable'.

In the CHSNC model they have not identified a critical control point but they have identified critical limits and acceptable levels.

Critical limits are defined as criteria that separate acceptable from unacceptable risks in relation to food safety. Critical limits are benchmarks for performance of the

preventive measures at control points. For management practices, the critical limit would be whether the preventive measure was complied with or not. A critical limit requires 100% compliance or an acceptable explanation of deviation from the program. For example, in our program one critical limit is the successful completion of the Plant Identification Practice prior to the sale of the product. Less than 100% compliance would be an unacceptable risk to anyone using the product.

Acceptable levels are defined as criteria that delineate a level of safety – whereas outside this range would be critical in relation to food safety. Acceptable levels must meet the criteria outlined in the monitoring procedure in an effort to minimize or reduce a non-critical hazard. They may require less than 100% compliance, although any deviation should be recorded. They must meet regulatory requirements. For example, acceptable limits for pesticide application follow label requirements that are set by law.

Monitoring the program

Monitoring procedures must be put into place to monitor the critical limits of a CCP or the acceptable limit of the 'must do' GAP to ensure control. The frequency of monitoring reflects regulatory requirements. For example, the monitoring procedure for pesticide application involves keeping a record of what is applied the rate and timing of application and the crop and pest that were targeted. The date of harvest of that same crop must also be recorded. Label instructions must be followed.

Deviating from standard procedures

Deviation occurs when a procedure addressing either a critical or acceptable limit does not follow the original program. Procedures that deviate from the original program may have a potential impact on food safety. A deviation procedure must clearly define the action taken to address the specific limit. It must reflect regulatory requirements. For example, the original procedure for pesticide handling may be to apply product immediately upon receipt and not to store it. A deviation procedure may be to store the product and talk to a local crop specialist or product buyer for recommendation. The deviation procedure does not alter the risk level. It does allow the latitude of addressing limits in more than one way.

Verifying what is done

The verification procedure is a process with two aspects:

1. Reviewing records for completeness. For example, in this document the verification procedure is to review sign-off documents relating to plant identification practice and confirm that the appropriate sample has been retained.
2. Maintaining records to verify that the HACCP plan has been adhered to. This procedure would identify the records to be in place for acceptable limits, monitoring and deviation procedures.

Good agricultural practices (GAPs)

There are eight good agricultural practices (including good wildcrafting practices) in this program:

1. plant/product identification
2. pest control products – purchase, storage, handling and application
3. purchasing
4. production – on farm and wild harvesting

5. post-harvest processing
6. personnel training
7. preventative maintenance
8. record keeping.

The two practices that are 'must-do' are plant/product identification and pest control products – purchase, storage, handling and application and records. The rest of the practices are 'recommended' and need to be applied appropriately depending on the risk and scope of the operation.

Each practice includes a written recommendation and a follow-up checklist to ensure that the practice has been completed. Standard operating procedures and a listing of other appropriate documents may also be required. It is important to note that the practices are outcome based. This allows producers to use existing practices, provided they meet the outcome, and avoids duplication. It is essential that the records of the existing practice be accessible and current.

6.3 Plant identification practice

Identified Plant/Product Identification is a 'must-do' practice. This practice was developed prior to the rest and is also a stand-alone practice. It was developed with input from experts throughout North America and with guidance from the WHO (World Health Organization). Proper plant identification is one of the keys to the development of an industry based on the safe use of high quality natural health products. Examples of misidentification, adulteration, and contamination of natural health products have been widely recorded both within Canada and around the world. Botanical identity is a key feature. Accurate plant identification is the foundation of the safe use of plant-based natural health products. Without proper botanical identification as a starting point, the safe use of quality products cannot be guaranteed. The goals were to:

- develop effective, practical tools for people growing and collecting to accurately identify medicinal herbs
- have this voluntary practice available to all to incorporate into good collection practices
- establish a tool both for cottage industries and large manufacturers to assure correct identification.

Since 1974, the WHO has asserted that the single greatest improvement in botanical quality would be the implementation of a program for the certification of botanical identity. The fact that after more than 25 years such a system had not yet been developed, even though the technical requirements are minimal, is indicative of the challenges involved. Two questions had to be answered:

1. How can a high degree of certainty be created that plant materials will be properly identified at the production end of the value chain?
2. What practices can be recommended that will be workable for producers and collectors?

The practices were developed by creating a plant identification working group with representatives from industry, government, and educational institutions including

producers, wildcrafters, first nations community, American Herbal Products Association, Herb Research Foundation and World Health Organization. The practices address one of the biggest issues facing the industry – accurate identity of plant material. They were developed within a government-recognized HACCP model and were based on an internationally recognized good agricultural practices model. The program respects traditional knowledge and skills. The practices help provide information for certificates of origin and disclosure of origin. They also help with identifying risks of pollution or contamination at collection sites and help isolate problems. They are a good basis for ethical methods and practices.

Steps in developing the plant identification practice

Step one – literature search: the first step in the development of the practices was to do a literature search to look for partially developed practices. No practices, complete or partial, were found.

Step two – outlining the practice: the practice was developed using a decision tree process where risk management was based both for the product and the people involved. This step encompassed the identification of the correct species, the correct variety or chemotype and the correct plant part.

Step three – including all aspects: it is essential that this practice include observation and documentation of the establishment, growth and harvest stages both for cultivated and wild-harvested plants.

Plant identification practice helps producers and collectors decide if they have the skills to identify their product (and what to do if they do not have the skills or information), how to identify their product, how to properly keep and take retention samples and voucher specimens. It also describes testing methods available (macroscopic/organoleptic, microscopic and chemical analysis).

Documenting the plant identification practice

As with every other GAP, verification through documentation is vital. The plant identification practice requires voucher labels with the retention samples, Certificate of Authenticity or Declaration of Identification. A Certificate of Authenticity must be signed by a recognized authority in botanical identification while a Declaration of Identification can be signed by a harvester or producer using their knowledge base and past experience to identify the products. This practice is used in situations where their qualifications meet the risks. For example a Certificate of Authenticity should be used and signed by a recognized authority when a plant that is difficult to identify is being harvested by someone without relevant experience, training and/or education. If an easy-to-identify product is being harvested by a person with adequate experience, training and/or education a Declaration of Identity is sufficient.

The plant identification practice is an internationally recognized practice that can either stand alone or be incorporated into any program that provides a concrete solution to an overarching problem throughout the industry! It can be found at www.saskherbspice.org. The practice was developed by a project team comprised of the following:

Connie Kehler, Executive Director, Saskatchewan Herb and Spice Association/Canadian Herb, Spice and Natural Health Product Coalition.

Dave Buck, Manager, Non-Timber Forest Products, Northern Forest Diversification Centre (Manitoba).

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Wanda Wolf, Lonewolf Native Plant and Herb Farm (Saskatchewan).
Dr Allison McCutcheon, President, Natural Health Product Research Society of Canada, ethonobotanist.
Edward Fletcher, American Herbal Products Association (North Carolina).
Jan Schooley, Ginseng and Medicinal Herb Specialist, Ontario Ministry of Agriculture, Food and Rural Affairs.
Al Oliver, Industry Specialist – Horticulture, BC Ministry of Agriculture, Food and Fisheries.
Dr Ernest Small, National Environmental Program, Biodiversity section, Agriculture and Agri-Food, Canada.
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Ross Wadell, Native Plant Society of British Columbia.
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Michelle Schröder, Centre for Non-Timber Resources, Royal Roads University.
Wendy Cocksedge, Centre for Non-Timber Resources, Royal Roads University.

6.4 Future trends

A successful industry that consumers can trust is an industry that practises systems that include safety, quality and traceability. In a global market where health and safety scares are occurring at an accelerated pace it is vital to minimize risk and to isolate problems as they occur. It is, however, daunting for producers who are facing more and more paper work and more need for several overlapping systems to be developed on farms. It is also difficult for producers who are having demands made of them by groups that do not understand how production or wildcrafting works, often resulting in demands that are not physically possible to meet. On top of this, producers will rarely get a premium for their efforts.

It is vital then that systems be developed as this one was, with industry developing the standards and requirements, with pilots to test the feasibility on farms, with outcome-based standards that recognize other systems and with collaboration of other systems further up the chain to ensure seamless integration throughout the chain.

6.5 Acknowledgement

We are grateful to the AAFC CARDS program under the Canadian On Farm Food Safety Program for funding.

6.6 Bibliography

OLIVER, A. *et al. A Good Agricultural Workbook for On-Farm Food Safety in the Herb, Spice and Natural Health Products Industry* **1.0**, 2005, Canadian Herb, Spice and Natural Health Products Coalition (www.saskherbspice.(org).

Part II

Herbs and spices as functional ingredients and flavourings

7

The range of medicinal herbs and spices

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7.1 Introduction

Medicinal herbs and spices have been important to human life for thousands of years. There is evidence for herbs, especially spices, being used by humans in the Middle East since 5000 BC. It is estimated that approximately 400 spices are used around the world, although only about 70 spices are officially recognized[8]. In the past decade, demand has increased for medicinal herbs and spices and their derived products for a variety of functions, such as herbal medicine, food flavorings, and cosmetics in the forms of tea, tablet, capsule, tincture, cream, syrup, and liquid. The worldwide herbal industry is now estimated to be more than US$10 billion dollars and increasing at a rate of three to four percent annually for reasons of increased consumption in processed foods and demand for ethnic foods, natural fragrances, and innovation in beverage products[53,54].

The largest markets, in terms of manufacturing and consumption, are in Europe, followed by Asia. World production and processing of medicinal herbs and spices remains concentrated in France and India. The North American market continues to be supplied by imports where quality and consistency of supply are in question[54]. Recently, Canada has become more active in the international herb and spice marketplace. Special crops production, including mustard, caraway, coriander seeds, and other herbs, has more than quadrupled since 1991–1992 as producers diversified into alternative crops to improve their income[52].

The properties of medicinal herbs and spices sometimes overlap. Medicinal herbs can be defined as plants which have health-promoting and curative properties. Spices are plants that are fragrant or aromatic and pungent to the taste from seeds, leaves, root, bark and flowers, and are used as food, food additives, flavorings, or to preserve food[42,63] via their antimicrobial properties[88]. Spices are important medicines and it has been speculated that more humans use spices as medicines than use prescription pharmaceuticals[89].

Many spices contain chemical components that have therapeutic value (Table 7.1) such as antioxidant and antiseptic activities, singlet oxygen quenching, cytochrome

Table 7.1 Major constituents and therapeutic values

Common name	Scientific name	Major constituents	Therapeutic values
Allspice	*Pimenta officinalis*	Eugenol, methyl eugenol, myrecene, chavicol, methyleugenol, eugenolmethylether, caryophyllene, (–)-∀-phellandrene.[9,84,88,90]	Analgesic, antibacterial, antioxidant, fungicidal. Treat hypertension, rheumatism. Relieve stomach aches, soothe sore muscles, toothache, menstrual cramps, antiseptic for teeth and gums.
Anise	*Pimpinella anisum*	Anethole, indole, fatty oil, coumarin, creosol, acetylinic, flavonoids.[1,82,84,85]	Antispasmodic, antifungal, diuretic, carminative relieve gas pains, dyspepsia.
Bay leaf	*Laurus nobilis*	Cineole, linalool, resin, ∀-pinene, ∀-terpineol, acetate, mucilage, tannin, eucalyptol.[1,2,38]	Indigestion, appetite, astringent, carminative, stimulant, stomachic, diuretic.
Caraway	*Carum carvi*	Carvone, limonene, flavonoids, polysaccharides.[3,4,5,85]	Inhibit carcinogenesis, relieve gas pains, antispasmodic, irritable bowel syndrome, cold, cough, bronchitis, antihistaminic activity, detoxicant, carminative.
Cardamom	*Elettaria cardamomum*	Essential oils, cineole, eucalyptol, linoleic and stearic acid.[22,86]	Antiseptic, dyspepsia, antimicrobial, digestive, aphrodisiac, astringent, antispasmodic, diuretic.
Cassia	*Cinnamomum aromaticum*	Cassia oil, trans-cinnamaldehyde, trans-cinnamyl, trans-2-methoxy cinnamaldehyde.[21,23,24]	Anti-mutagenic, astringent, carminative, antiseptic, excessive salivation, rheumatism.
Celery seed	*Apium graveolens*	Limonene, coumarins, bergapten, fatty acids.[3,6,7]	Carminative, gout, anti-rheumatic, hysteria, nervousness, weight loss, a detoxicant.
Chives	*Allium schoenoprasum*	Alliin, sulphoxide, sulphoeverman, linoleic acid, vitamin A, C, minerals, thiamin, niacin.[25,38]	Lower high blood pressure, antibacterial, inhibit immunodeficiency virus infection.
Cinnamon	*Cinnamomum zeylanicum*	Volatile oil, tannins, coumarin, cinnzelanin, cinnzelanol, eugenol, cinnamaldehyde.[26,27]	Antiseptic, astringent, balsamic, carminative, diaphoretic, febrifuge.
Cloves	*Eugenia caryophyllata*	Phytosterols, eugenol, campesterol, ascorbic acid, crataegolic acid, vitamin A, sitosterols, stigmasterol.[9,20,28]	Abdominal problems, callus, cancer, cough, diarrhea, gastritis, hernia, nausea, sores.
Coriander seed	*Coriandrum sativum*	Coriandrol, geraniol, borneol, carvone, linalool, vitamin A, C, ∀-pinene, terpinene, niacin, fiber, protein, rhiamin.[3,5,38]	A digestive tonic, carminative, sedative, anti-bacterial, lavicidal, anti-inflammatory, hypoglycemia effects.

Table 7.1 Continued

Common name	Scientific name	Major constituents	Therapeutic values
Cumin	*Cuminum cyminum*	Pinene, ∀-terpineol, ciminaldehyde.[3,5]	Relieves flatulence and bloating, stimulates digestive process.
Dill	*Anethum graveolens*	Carvone, limonene, flavonoids, cumarins, xanthones, triterpenes.[1,3,5]	Used as infant colic, cough, cold and flu remedies. Relieve digestive disorders.
Eucalyptus	*Eucalyptus citriodora*	Cineole, eucalyptol, caffeic, coumaric, gentisic, syringic, hydroxybenzoic, gallic, vanillic acids.[1,3,41]	Treat fevers and asthma. Externally for athlete's foot, dandruff, herpes.
Fennel	*Foeniculum vulgare*	Anethole, fenchone, fixed oil, perroselinic oil, oleic acid, linoleic acid, essential oils, vitamin A & C.[1,5,38]	Antispasmodic, stimulate mild flow and digestive disorders, a carminative, relieve infant colic, diuretic.
Fenugreek	*Trigonella foenum-graceccum*	Trigonelline, protein, linoleic, oleic, linolenic, and palmitic acids, choline, coumarin, nicotinic acid.[1,5,29,34]	Reduce cholesterol and triglycerides, blood sugar level, platelet aggregation in patients with coronary artery disease.
Garlic	*Allium sativum*	Phytosterol, alliin, choline, iodine, diallyl trisulfide, uranium, inulin-containing, allcin, polyoses, scordinins, selenium, saponin.[5,9,30,31,58,59]	Antibiotic, antifungal, anti-tumor activities. Treat anemia, arthritis, asthma, antioxidant, cough, diabetes, cold, hypotension, hypertension.
Ginger	*Zingiber officinale*	Essential oils, linalool, zingiberol, zingiberene, phellandrene, gingerol, camphene, citral, methylheptenone, nonylaldehyde, d-borneol.[32,33,34,35,74]	Anti-inflammatory, anti-tumor, stimulates gastric secretion. Effect on platelet aggregation in patients with coronary artery diseases.
Horseradish	*Armoracia rusticana*	Asparagine, resin, calcium, iron, vitamin B and C, potassium, phosphorus, mustard oil, asparagine, sinigrin.[3,38]	Arthritis, gout, and respiratory and urinary infections.
Licorice	*Glycyrrhiza glabra*	Glycorrhizin, mucilage, flavonoids, saponin, glycyrrhetinic acid, glabridin, tannic acid, glucuronic acid.[5,36]	Stomach, duodenal ulcers, cough remedy, anti-inflammatory, laxative, anti-allergic, anti-hepatitis.
Marjoram	*Marjoram hortensis*	Volatile oil, oleoresin, arbutin, calcium, iron, protein, Vitamin A & C, hydroxyquinone, minerals[37,38]	Rhinitis and colds for infants, rhinitis in toddlers, and gastritis. Oil for coughs, gall bladder complaints and gastrointestinal cramps.

Table 7.1 Continued

Common name	Scientific name	Major constituents	Therapeutic values
Mustard (black)	*Brassica nigra*	Allyl isothiocyanate, sinapine, mucilage, glucosinolates.[43,51]	Treat lung congestion and bronchial problems
Nutmeg, Mace	*Myristica fragrans*	Safrole, myristicin, lauric, oleic, stearic, hexadecenoic, linoleic acid, d-camphene.[2,11]	Internally for diarrhea, dysentery, vomiting, abdominal distention, indigestion, and colic.
Onion	*Allium cepa*	Quercetin, methylallin, dihydroallin, sulfides, spiraeoside, cycloallin, protocatechuic acid, phloroglucin.[9,21]	Aphrodisiac, diuretic, expectorant, emmenogogue, hypoglycemia, stimulant. Useful in flatulence and dysentery.
Oregano	*Origanum vulgare*	Carvacrol, galanigin, resin, magnesium, quercetin, sterols, ∃-carotene, thymol, flavonoids.[3,38,65,70,71,80]	Antioxidant, cancer chemopreventive, antispasmodic, antiseptic, stomachic, carminative effects.
Parsley	*Petroselinum crispum*	Apiole, myristicin, pinene, apiin, havonoids, phthalides, coumarins.[1,3,5]	Diuretic, stomachic, carminative, irritant, emmenagogue property.
Pepper (white, black)	*Piper nigrum*	Caryophyllene, canene, ∃-sitesterol, thiamine, riboflavin, volatile oil, piperine, pellitorine, piperidine, piperettine, humulene, fatty acids.[11,20,47,48]	Carminative, febrifuge, rubefacient, stimulant. Treat cholera, weakness after fevers, vertigo, coma.
Pepper (red, sweet)	*Capsicum annum*	Capsanthin, capsaicin, capsorubin, zeaxanthin, lutein, cryptoxanthin, ∀- and ∃-carotene.[19,20,26,27,76]	Counterirritant in lumbago, neuralgia, and rheumatic disorders, antioxidant.
Peppermint	*Mentha piperita*	Menthone, piperitone, isomenthone, tannin, neomenthol, menthol, fatty acids.[45,86]	A decongestant, antiseptic, carminative, stomachic, sudorific, relief neuroses, rhinosis.
Rosemary	*Rosmarinus officinalis*	Rosemanols, diosmin, eucalyptol, oleoresin, cineole, camphor acid, ursolic acid, apigernin, picrosalvin, romarinic acid, tannins, borneol.[1,3,5,62,66,67,79]	Carminative, antispasmodic, anti-rheumatic, liniments and ointments. An antioxidant.
Saffron	*Crocus sativus*	Crocine glycosides, ∃-carotene, phytoene, phytofluene.[1,5]	Emmenagogue properties, stomachic, antispasmodic.
Sage	*Salvia officinalis*	Thymol, borneol, cineole, camphor, malic and oxalic acids, salvin, eugenol, tannin, fumaric acid, vitamins A, B_1, B_2, C.[1,5,38,43,66,68,69]	Anti-oxidant, carminative, antiseptic, antifungal, astringent, diuretic, anti-diarrheal, anti-spasmodic, relieve fever, digestive.

Table 7.1 Continued

Common name	Scientific name	Major constituents	Therapeutic values
Star anise	*Illicium verum*	Trans-anethole, safrole, estragole, 1,4-cineole, ∃-farnesene, fatty acids, ∀-copaene, ∀-terpineol, hydroquinone.[1,10]	Anodyne, diuretic, anti-rheumatic, antiseptic, stimulant, carminative, stomachic, vermifuge.
Sumach	*Rhus glabra* *R. coriaria*	Tannins, astragalin, avicularin, myricetin, myricitrin, quercetin[18,89]	Antibacterial, anti-ulcer, antiseptic, anti-viral, dyspepsia, anti-inflammatory. For rheumatism, internal bleeding, diarrhea, enteritis colitis.
Tarragon	*Artemisia dracunculus*	Estragole, phelandrine, iodine, tannins, methyl coumarins, chavicol, rutin, flavonoids[12]	Diuretic, used as an appetite stimulant.
Thyme	*Thymus vulgaris*	Thymol, tannins, carvacrol, saponins, apigenin, luteolin.[1,3,5,62,73,81]	Antioxidant, anti-spasmodic, antitussive, relieve coughing.
Turmeric	*Curcuma longa*	Curcumin, ∃-carotene, thiamine, riboflavin, niacin, ascorbic acid, essential oils, ∃-sesquiphellandrene, curcumoids.[13,14,15,75]	Antioxidant, anti-gynecomastia, anti-cancer, anti-hepatotoxic activity, carminative, stomachic, improve liver function, treat ulcer.
Vanilla	*Vanilla planifolia*	Vinillin, fatty acids, p-hydroxybenzaldehyde, piperonal, vanillic acid, balsam, glucovanillin, glucovanillic alcohol[1,16,17]	Aphrodisiac, treat fevers and spasms, carminative, stimulant, vulnerary function.

and other enzyme inducers, reducing induction and advancement of cancer cell development[46,60,61,64]. For example, the pigments, carvone, curcumin, limonene, and lycopene are associated with reduced risk of cancer[49]. Oleoresin from rosemary can inhibit oxidative rancidity and retard the development of off-flavor in some products[85,88,89]. Capsaicin, the pungent principle in chilies, has been shown to reduce reactive oxygen species and thereby inflammation[50]. In Ayurvedic medicine, it is claimed that garlic lightens the blood, reduces tumors, and is an aphrodisiac tonic. This claim is confirmed by scientists with modern technology that garlic thins the blood, prevents cancer, and increases libidinous activities[89].

Medicinal herbs and spices also contain antimicrobial compounds, such as allicin (garlic), allyl isothiocyanate (mustard), cinnamaldehyde, eugenol (cinnamon), eugenol (cloves), thymol, eugenol (sage), and thymol, carvacrol (oregano)[43,72]. Thus, spices not only provide flavor and aroma to food and retard microbial growth, but are also beneficial in the prevention of off-flavor development. These attributes are useful in the development of snack foods and meat products[44].

7.2 The role of medicinal herbs and spices

In the past, essential oils, which contain volatile compounds (Table 7.2), derived from plants were used in cosmetics, perfumes and pharmaceuticals[56,77]. Today, aromatherapy is gaining overwhelming attention as an alternative healing modality entirely related to herbal medicine[39,40]. It was reported that cardamon, rosemary, and eucalyptus contain eucalyptol[22,41,62]. When it is administered topically as part of a massage, the direct touch stimulates sensory fibers in the skin, which triggers the parasympathetic nervous system, thus inducing relaxation and decreasing the perception of pain[41]. It is established that the functional effects of medicinal herbs and spice constituents include inhibition of cancerous growth, oxidative damage, stimulation of cytochrome enzymes, modulation of body temperature, counter-irritants, and prevention of oxidative damage to foods and the anti-nutritional effects of that damage[46].

Scientific literature supports the use of essential oils for insomnia; in addition, several randomized controlled clinical trials have demonstrated a reduction of pain medication for people with rheumatoid arthritis, cancer, and headaches[41]. Current research on the essential oils from herbs is concentrated or their chemical constituents and therapeutic value[77]. Pharmacological activities of pepper can be basically attributed to essential oils and amide alkaloids, especially content of piperine[47]. Essential oil from lavender has sedative and pain-relieving properties. It is believed to affect the amygdala by increasing inhibitory neurons containing g-amino butyric acid. Other claimed therapeutic values include anti-infectious, antispasmodic, mucolitic, and litholitic actions; expectorant and anti-parasitic qualities, stimulation of the immune system and antihistamine[41,46], anti-convulsive, and analgesic activities[48].

Essential oils extracted from either medicinal herbs or spices may cause such side effects as headache or contact dermatitis. Patients with hypertension should avoid using stimulating essential oils such as rosemary and spike lavender. Essential oil may be toxic if it is administered improperly and it should be stored away from children. It was reported that essential oil from pepper root is toxic based on studies of oral administration into mice, which died by convulsion[48]. Essential oils containing pharmacologically active ingredients may interact with medications[86]. This area of study is being investigated and more information has been published recently.

7.3 Major constituents and therapeutic uses of medicinal herbs and spices

The thirty-eight most popular spices were selected and are arranged alphabetically based on their common names with their major constituents, therapeutic values (Table 7.1), and essential oils (Table 7.2). The information in this chapter is primarily for reference and education. It is not intended to be a substitute for the advice of a physician. The uses of medicinal herbs and spices described in this chapter are not recommendations, and the author is not responsible for liability arising directly or indirectly from the use of information in this chapter.

Table 7.2 Major essential oils in herbs and spices

Common name	Scientific name	Essential oils
Allspice	*Pimenta officinalis*	Phenol-eugenol, cinool, laevo-phellandrene, caryophyllene, eugenol-methyl ether, palmitic acid[58].
Anise	*Pimpinella anisum*	Trans-anethole, methyl chavicol, cis-anethole, p-anisic acid, carvone, estragole, limonene, anisaldehyde, alpha- and beta-pinene, eugenol, camphene, sabinene, saffrol, myrcene, linool, cis-anethol[55,85].
Bay leaf	*Laurus nobilis*	Monoterpenoid, acetates, cineole, benzenoides, linalool, alpha-pinene, alpha-terpineol[55,56].
Caraway	*Carum carvi*	Carvone, limonene, carveol, phyllandrene, dihydrocarveol, dihydrocarvone, pinene, terpene[55,56,85].
Cardamom	*Elettaria cardamomum*	Terpinyl acetate, terpineol, terpene terpinene, sabinene, cineole, crystalline substance[58,85].
Cassia	*Cinnamonum aromaticum*	Cinnamic aldehyde with methyl eugenol, salicylaldehyde, methylsalicylaldehyde[55,56].
Celery seed	*Apium graveolens*	Limonene, phthalides, beta-selinene, selinene, apiol, santalol, sedanolide, lsedanic acid, citric, isocitric, fumaric, malic, and tartaric acids From seed oil: oleic, palmitic, paliloleic, petroselinic, petroselaidic, stearic, myristic, myristoleic acid[55,56].
Chives	*Allium schoenoprasum*	Cycloalliin, allicin[55,56].
Cinnamon	*Cinnamonum zeylanicum*	Leaf-eugenol, eugenol acetate, benzyl bezoate, linalool, safrol, cinnamaldehyde. Bark-cinnamaldehyde, eugenol, benzaldehyde, cuminaldehyde, pinene, cineol, phyellandrene[55,78].
Cloves	*Eugenia caryophyllata*	Eugenol, caryophyllene, alpha-humulene, alpha-terpinyl acetate, eugenyl, methyl eugenol, acetyl eugenol, naphthalene, chavicol, heptanone, sesquiterpenes, acetyl eugenol, methyl salicylate, pinene, vanillian[55,56].
Coriander seed	*Coriandrum sativum*	Linalool (coriandrol), alpha-pinene, terpinene, cymene, decylaldehyde, borneol, geraniol, carvone, anethole[55,56].
Cumin	*Cuminum cyminum*	Aldehydes, cumin ester, limonene, pinene, alpha-terpineol, cymene, phyllandrene, myrecene, camphene, borneol[55,56].
Dill	*Anethum graveolens*	Carvone, limonene, phyllandrene, eugenol, pinene, 3, 9-epoxy-p-menth-1-ene, 4,5-dimethoxy-6-(2-propenyl)-1,3-benzodioxole[55,56].
Eucalyptus	*Eucalyptus citriodora*	Citronellal, isopulegol, neoisopulegol, eucalyptol, pinene, limonene, alpha-terpineol, linalool, geraniol, pinocarvone, myrtenal, carvone, cineole, cuminaldehyde, citral, aromadendrene, globulol, eudesmol, eudesmyl acetate[55,56].
Fennel	*Foeniculum vulgare*	Anethole, fenchone, methyl chavicol, limonene, phyllandrene, pinene, anisic acid, camphene, palmitic, oleic, linoleic, petroselinic acids[55,56].
Fenugreek	*Trigonella foenum-graceccum*	Linolenic acid, oleic acid, palmitic acid[55,56].
Garlic	*Allium sativum*	Alliin, allicin, allypropyl, disulphide, sesquiterpene, allyl-propyl disulphide[55,58].
Ginger	*Zingiber officinale*	Sesquiterpenoid hydrocarbons, zingiberene, ar-curcumene, farnesene, alpha- and beta-selinene, camphene, neral, nerol, beta-sesquiphyllandrene, oxygenated monoterpenoids, 1, 8-cineole, beta-bisabolene, geranial, geraniol, geranyl acetate, alpha-copaene[55,56].

Table 7.2 Continued

Common name	Scientific name	Essential oils
Horseradish	*Armoracia rusticana*	Allyl phenylethyl isothiocyanate, 2-phenylethyl isothiocynate[55,56].
Licorice	*Glycyrrhiza glabra*	Monterpenoid, ketones (fenchone, thujone), coumarins (herniarin, umbeliferone)[57].
Marjoram	*Marjoram hortensis*	Terpenes, terpinene, terpineol, terpinenol-4, esters[58].
Mustard (black)	*Brassica nigra*	Allyl iso-thiocyanate, allyl thiocyanate, allyl cyanide, caarbon disulphide[55,58].
Nutmeg, Mace	*Myristica fragrans*	Oleoresin, alpha-, beta-pinene, alpha-, beta-terpinene, sabinene, myristicin, elincin, safrole, camphene, cymene, eugenol, linalool, pinene, safrole, terpineol[55,56].
Onion	*Allium cepa*	Dipropyl disulphide, methylalliin, cycloalliin, dihydroalliin, dipropyl trisulphide[55,56].
Oregano	*Origanum vulgare*	Thymol, carvacrol, beta-borneol, pinene, dipentene, cymene, caryophyllene, bisabolene[55].
Parsley	*Petroselinum crispum*	Apiole, myristicin, pinene, tetramethozyally benzene, apiol, phyllandrene, terpinolene[55,87].
Pepper (white, black)	*Piper nigrum*	Capsaicin, phellandrene, dipentene, sesquiterpene[55,58].
Pepper (red, sweet)	*Capsicum annum*	Capsaicin[19,55,56].
Peppermint	*Mentha piperita*	Menthol, menthone, menthofuran, acetaldehyde, dimethyl sulfide, isovaleric aldehyde, pinene, limonene, terpinene, beta-caryophyllene, neomenthol, 2,5-trans-p-methanediol, methyl acetate, methyl ethers, isomenthone, piperitone, pulegone[55,83,86].
Rosemary	*Rosmarinus officinalis*	1,8-cineole, camphor, camphene, borneol, alpha-pinene, olefinic terpenes, sesquiterpene, santene, tricyclene, thujene, fenchene, sabinene, myrecene, cerene, phyllandrene, limonene, terpinene, cymene, bornyl acetate[55,58,87]
Saffron	*Crocus sativus*	Pinene, safranal, cincole[55,56].
Sage	*Salvia officinalis*	Alpha- and beta-thujone, ocimene, borneol, cineole, camphor, linalool, linolenic acid[55,56].
Star anise	*Illicium verum*	*a*-pinene, phellandrene, cymene, cineol, dipentene, *l*-limonene, *a*-terpineol, methyl-chavicol, anise ketone, anethol[58].
Sumach	*Rhus glabra* *R. carnaria*	z-2-decenal, nonanal, ∀-pinene, ∀-terpineol, limonene[57].
Tarragon	*Artemisia dracunculus*	Estragole, ocimene, methyl chavicol, alpha- and beta-pinene, camphene, limonene, nerol, sabinene, myrcene, menthol, trans-anethole, anisole, anisic acid[55,56].
Thyme	*Thymus vulgaris*	Thymol, carvacrol, methylchavicol, cineole, borneol, cymene, terpinene, camphene, pinene, myrcene[55,56].
Turmeric	*Curcuma longa*	Sesquiterpene, zingiberen, turmeron, p-cymene, 1,8-cineole, alpha-phyllandrene, sabinene, borneol, ar-turmerone, alpha-atalantone, gamma-atalantone[55,56].
Vanilla	*Vanilla planifolia*	Vanillin, *p*-hydroxybenzaldehyd, *p*-hydroxybenzyl methyl ether[57].

7.4 Future trends

Medicinal herbs and spices have been important to human life for thousands of years. In the past decade, there has been a considerable surge of interest in medicinal herbs and spices and their derived products for a variety of functions for human health. The herbal industry is now estimated at more than US$10 billion dollars and is increasing at a rate of three to four percent annually.

The largest markets are in Europe and Asia. The North American market continues to be supplied by imports, although the United States and Canada have become more active in the international marketplace recently. Herb and spice production has more than quadrupled since 1991. To meet the surging demand, more scientific evaluation and research, proper regulation, quality control and education for the general public, herbal practitioners, and retailers are important to make this fragile industry both credible and sustainable.

7.5 Sources of further information

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8

Herbs, spices and cardiovascular disease

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8.1 Introduction

Herbs and spices have both been used as sources of flavour enhancers and pharmaceuticals since antiquity and their use continues undiminished today. The distinction between the two sources is blurred but it has been suggested that herbs tend to be of leaf origin and spices of stem, bark and seed origin. Full details of their origin, plant source, and culinary and medical properties can be found in the many past and also more recent herbals (Grieve, 1998; Bellamy, 2003). Currently, researchers in the pharmaceutical industry are aware of the fact that many of the remedies quoted in these books do have value in combating disease and are now analysing and testing the individual compounds present in a wide range of herbs and spices. Their aim is to be able to isolate single compounds that have specific roles in disease prevention. The attraction of such compounds is that they could be marketed under the heading of 'natural', which is seen as being more attractive than 'synthetic' by the potential customer.

However, plants are complex mixtures and where a pharmaceutical role has been identified, it is most likely to be achieved through a mixture rather than a single compound. This mixture of compounds may be a factor in giving individual herbs and spices a cure-all reputation. As an example, traditionally the herb thyme has been considered as an anthelmintic, antispasmodic, carminative, emmenagogue, expectorant, rubifacient, sedative, stimulant and tonic. The plant has been used in folk medicine against asthma, artheriosclerosis, colic, bronchitis, coughs, diarrhoea and rheumatism (Grieve, 1998). However, not all herbs are effective against cardiovascular disease, which would include atherosclerosis, hypertension and myocardial infarcation or heart attack. A literature search of those herbs and spices that do have a role in combating this disease revealed that rosemary, oregano, ginger, basil, cumin, tumeric, parsley, thyme and garlic are important.

In a health-conscious society, these plants are being advertised now for their medical as well as their flavour-enhancing properties (Rice-Evans, 2001) and in support epidemiological studies have suggested a positive association between the consumption of phenolic-rich foods and beverages and the prevention of disease

(Scalbert and Williamson, 2000). This approach makes the connection that in most chronic diseases there is a component of oxidative stress, which can lead to the production of damaging reactive oxygen and free radicals. In response to such damage a complex antioxidant defence has developed in which dietary oxidants provide an important role (Halliwell, 1996, 2000). Already it is possible to buy concentrated extracts of individual herbs and spices that claim to have specific medical benefits. Research is being undertaken to examine these claims (Vivekananthan *et al*., 2003) and also how the crude herbs and spices may achieve their effect specifically in cardiovascular disease (Blomhoff, 2005). The following is a discussion of the role selected herbs and spices play in delaying the onset of this important disease.

8.2 Chemical composition of herbs and spices

The chemical composition of selected herbs and spices that are thought to have a role in the delay or prevention of the onset of cardiovascular disease is described. For further details of these plants see herbals by Grieve (1998) and by Bellamy (2003).

8.2.1 Rosemary

Rosemary herb (*Rosmarinus officinalus* L.) is grown in many parts of the world as a six-feet-high evergreen shrub. Leaves and twigs are used as a flavouring as well as a treatment for a variety of medical conditions. It has pronounced anti-oxidant properties that may extend to the reduction of total cholesterol levels in serum and also in tissues such as the liver, heart and fatty tissue. The likely active compounds include six compounds with three different polyphenol skeletons, phenolic diterpenes (carnosic acid, carnosal, and 12-O-methylcarnosic acid), caffeoyl derivatives (rosmarinic acid) and flavones (isoscutellarein 7-0-glucoside and genkwanin). Only in the leaves are all six compounds present at the same time. Of the polyphenol compounds, rosmarinic acid showed the highest concentration and had the highest antioxidant activity (del Baňo *et al*., 2003).

8.2.2 Oregano

Oregano (*Origanum vulgare* L.) is native to northern Europe where it is cultivated commercially. Both fresh and dried leaves are used as a source of flavouring. At the same time it has been shown to have the highest anti-oxidant activity compared to the same amounts of fresh dill, thyme, sage and parsley. In general, fresh oregano on a weight for weight basis had three to 20 times higher antioxidant activity than the other herbs studied and in comparison to vegetables, oregano has 42 times more antioxidant activity than apples, 30 times more than potatoes, 12 times more than oranges and four times more than blueberries (Zheng and Wang, 2001). The most active component appears to be rosmarinic acid and thymol. As a measure of its antioxidant power oregano has demonstrated stronger antioxidant capacity than either of the two synthetic antioxidants commonly added to processed foods – BHT (butylated hydroxytoluene) and BHA (butylated hydroxyanisole) (Zheng and Wang, 2001). Kulisic *et al*., (2004) in an assessment of the components of the oregano essential oil, confirmed that the oil had remarkable antioxidant properties. It was suggested that the oil could be used as a potential source of antioxidants for the food industry.

8.2.3 Ginger

Ginger (*Zingiber officinale* Roscoe) is a perennial plant grown in hot moist climates. The tuberous roots are used fresh or as dried slices, preserved in syrup, as candy (crystallised ginger) or as a tea. It is thought to have a cholesterol-lowering and an anti-thrombosis effect through its antioxidant properties. The ginger contains volatile oils consisting of sesquiterpene hydrocarbons, predominantly zingeberene (35%) curcumene (18%) and farnesene (10%) with lesser amounts of bisabolene and b-sesquiphellandrene. A smaller percentage of at least 40 different monoterpene hydrocarbons are present with 1,8-cineole, linalool, borneol, neral, and geraniol being the most abundant. A sesquiterpene alcohol known as zingiberol has also been isolated (Govindarajan, 1982). The antioxidant properties are mainly due to its pungent constituents.

8.2.4 Basil

Basils (*Ocimum* spp) are a source of flavouring and of antioxidants. The species contain essential oils, mainly 1,8-cineole, estrageole and eugenol, flavonoids and anthocyanins. Assessment of the antioxidant capacity of the separate groups showed that most of the anti-oxidant activity was contributed by the flavonoids in the green basils and anthocyanins in purple basils, which had the highest antioxidant activity. In sweet basil, although the anti oxidant activity was low, the activity of the oil was the highest as this contained the highest amount of eugenol relative to the other samples. In comparison green tea, which is extremely rich in polyphenol compounds, contained 300 mg polyphenols/g of material whereas the Dark Opal variety of basil contained half the amounts of the tea sample at 126 mg/g material. The phenolic activity of the basil was similar to red and black raspberry and higher than rose hips (Juliani and Simon, 2002).

8.2.5 Cumin

Cumin (*Cumin cyminum* L.) is a small herbaceous annual plant cultivated extensively in Asia and the Mediterranean regions. The intact or powdered seeds have been used as a spice and medicine since antiquity. The main components in the volatile oil are cuminal and safranal (accounting for 32% and 24% respectively) and small amounts of monoterpenes aromatic aldehydes and aromatic oxides. The components in relatively small amounts are chiefly terpenes, terpenals, terpenones, terpene esters and aromatic compounds. When the anti-oxidant properties of cumin at 5% was compared with the common food additives (butylated hydroxyanisole, BHA), butylated hydroxytoluene, BHT and propyl gallate at 100 micrograms/g, BHA had greater and BHT less activity than cumin (Martinez-Tome *et al*., 2001).

8.2.6 Cinnamon

Cinnamon is the brown bark of the cinnamon tree which when dried rolls into a tubular form known as a quill. *Cinnamonum zeylanicum* (Ceylon cinnamon) and *Cinnamonum aromaticum* (Chinese cinnamon), often referred to as Cassia, are the leading varieties consumed. Cinnamon is available in either its whole quill tubular form (cinnamon sticks) or as a ground powder. The chief constituent is the volatile oil, which amounts to 1% of the bark. The principal constituent of the oil is cinnamic

aldehyde together with cinnamyl-acetic ester and a little cinnamic acid and eugenol. It has a variety of medical uses but its relation to cardiovascular disease is its anti-clotting effect. It is also a rich source of calcium and fibre, which are both able to bind to bile salts and remove them from the body. When bile is removed, the body must break down cholesterol to make new bile, which can help to lower the cholesterol levels. It does also have powerful antioxidant properties that far exceed those shown by anise, ginger, liquorice, mint, nutmeg and vanilla and is also more powerful than the chemical food additive BHA and BHT (Murcia *et al*., 2004).

8.2.7 Turmeric

Turmeric is a 5–6 ft plant (*Curcuma longa* L.), which sends out rhizomes that can be collected and either used fresh like ginger or dried and powdered. Aromatic tumerone is the major compound present in tumeric oil alongside curcumin. The spice is a powerful antioxidant where the antioxidant properties of the oil are thought to be due to the synergistic activities of the major components (Guddadarangavvanahally *et al*., 2002) of which the active components are a group of phenolic compounds including curcumin (Miquel *et al*., 2002).

8.2.8 Thyme

Thyme is a general name for the many herbs of the *Thymus* species all of which are small perennial plants found in Europe and Asia and which are now grown in the US. The leaves are used fresh and dried or extracted for the flavouring oil. The herb is also valued for its antiseptic and anti-oxidant properties. The major constituent is thymol but there appears to be a synergistic role for the other constituents of the oil, which are terpinen-4-ol, carvacrol, p-cymene, pinene, camphene, myrcene, 1,8-cineole, terpinene, d-linalool and flavonoids such apigenin, naringenin, luteolin and thymonin (Hudaib *et al*., 2002).

8.2.9 Garlic

Garlic (*Allium sativum*) has a very long folk history of use in a wide range of ailments. Daily use of garlic as in the Mediterranean diet is thought to contribute to the lower incidence of heart disease in these areas. The active components are the sulphur-containing compounds, alliin, iso-alliin and methin which, on tissue damage, release volatiles following breakdown by the enzyme alliinase. The volatiles are short lived and rapidly transform chemically to pungent sulphides and bisulphides (Block, 1996).

8.3 Herbs spices and cardiovascular disease

Analysis of the above herbs and spices has confirmed that they all contain a high concentration of antioxidants (Halvorsen *et al*., 2002). When included in the diet these antioxidants are thought to protect cell-based molecules from damage by oxidation which will occur during the normal process of metabolism. Further oxidative stress is created by over-strenuous exercise, chronic disease or exposure to environmental pollution. Free radicals produced as a result of oxidative processes are unstable and

if not eliminated these highly reactive free radicals will react with, and potentially alter, the structure and function of cell membranes, lipoproteins, cellular proteins, carbohydrates RNA and DNA. The function of the antioxidant compounds is to donate electrons to the free radicals thereby reducing their damaging effect. This is particularly important in such chronic ailments as cardiovascular disease (Blomhoff, 2005).

Cardiovascular disease is a major source of mortality in industrial societies including many below the age of 50. Atherosclerosis, which is the initial stage of the disease and can lead to hypertension and heart attacks, is a disease of the arteries where the inner layer becomes thickened by fatty deposits and fibrous tissue leading to a condition known as hardening of the arteries. Fatty streaks, which are the earliest indication of atherosclerosis, are areas of yellow discolouration on the inner surface of the artery but do not protrude into the lumen or disturb the blood flow. The streaks are characterised by the sub-endothelial accumulation of large foam cells filled with intracellular lipid. The foam cells, which are derived from macrophages and smooth muscle cells are the likely precursors of fibrous plaques, structures that form pale grey elevated lesions, which may project into the arterial wall and reduce the blood flow through the vessel. Calcification of the fibrous plaque leads to rigidity of the artery and hypertension while rupture of the plaque releases material into the bloodstream causing a thrombus to form. Occlusion of the vessel locally or following transport to distant sites can lead to myocardial infarctions or strokes (Bhattacharyya and Libby 1998).

8.3.1 Herbs, spices and cholesterol

The level of cholesterol in the blood is an important factor in the development of atherosclerosis. When fats are ingested as part of the diet, cholesterol and triglycerides are absorbed in the intestine and finally transferred to the venous circulation. These large molecules are hydrolysed by the enzyme lipoprotein lipase, which releases fatty acids into peripheral tissues while the metabolic remnants composed largely of cholesterol remain in the circulation. The liver in an endogenous cycle of cholesterol production and metabolism releases very low density lipoprotein (VLDL) into the circulation. Lipoprotein lipase acts on VLDL at muscle cells and adipose tissue to release free fatty acids into the cells as before and the residue, intermediate density lipoprotein (IDL), which contains esterified cholesterol remains in circulation. Further processing results in cholesterol-rich low-density lipoprotein (LDL) which is largely taken up by the liver. Cholesterol released back into circulation is transported by high-density lipoprotein (HDL) which returns the cholesterol to the liver via IDL and LDL for recycling into lipoproteins or excretion in the bile. The HDL appears to act in a protective role while elevated levels of LDL correlates with a high incidence of atherosclerosis. The level of cholesterol particularly LDL is critical (Bhattacharyya and Libby, 1998). One route to lowering the level of cholesterol in the body is through the increased intake of fibre (Brown *et al*., 1999). Herbs that are able to bind to bile salts through their fibre component and remove them from the body will stimulate cholesterol breakdown (Murcia *et al*., 2004; Zeng and Wang 2001).

The risk factor in atherosclerosis, such as high LDL or low HDL concentrations can lead to excess cholesterol available being taken up by the intimal layer which is the inner layer lining the lumen of the arteries. High LDL predisposes the arteries to endothelial dysfunction by making them more permeable to the transport of LDL. Once within the intima, LDL accumulates in the subendothelial space by binding to

components of the extracellular matrix. This trapping increases the residence time of LDL within the vessel wall where the lipoprotein may undergo chemical modifications. The LDL becomes oxidised by local free radicals and as oxidised LDL it attracts circulating monocytes to the vessel wall. The modified or oxidised LDL can be ingested by macrophages contributing to the development of foam cells.

Following oxidation of the LDL, the next stage is the attraction of leucocytes, primary monocytes and T lymphocytes. After the monocytes have adhered to the luminal surface they may penetrate into the subendothelial space by slipping between the junctions. Once localised beneath the endothelium, monocytes differentiate into macrophages, the phagocytic cells that are able to ingest oxidised LDL. The macrophages then become lipid-laden foam cells, the primary constituent of the fatty streak. More recently oxidised LDL has been recognised as playing a more important role in vascular dysfunction leading to atherosclerosis rather than native LDL (Battacharyya and Libby, 1998). Oxidation of the LDL is a key stage in the process of atherosclerosis. The antioxidant activity of herbs may have an important role at this stage of the disease.

8.3.2 Metabolic effect of antioxidants

Herbs and spices contain high levels of antioxidants which contribute to their pharmaceutical value (Dragland *et al.*, 2003). In the plants these compounds are necessary because they provide a protection against excessive input of solar energy during photosynthesis. Hazardous excess energy is eliminated and oxidative damage to the plant cell prevented. During the oxidative process of cellular metabolism reactive oxygen species and reactive nitrogen species are released. The most reactive are the free radicals of which the most oxidising and therefore the most reactive is the hydroxyl radical (OH^-) which can oxidise, i.e., remove an electron from almost any molecule and thus damage cell structures and cell metabolites. The function of the antioxidant system is to facilitate the donation of electrons to the free radicals thereby reducing the chemical energy of the hydroxyl radical or other reactive oxygen or reactive nitrogen species. The antioxidant itself then needs to be progressively reduced in a step-wise manner until the organic molecule is finally released as oxygen or carbon dioxide. Plants contain large amounts of many antioxidants compounds such as polyphenols, carotenoids, tocopherols, glutathione and ascorbic acid that can unite chemically and non-enzymically with an oxygen donor such as a free radical (Blomhoff, 2005). It is these compounds in herbs and spices that provide the essential antioxidant component in the diet of animals and humans (McCord, 2000).

In addition to the chemical non-enzymic protection of the antioxidant compounds, there is an anti-oxidant system that consists of a number of enzymes which are referred to collectively as phase 2 enzymes (Benzie, 2003). These enzymes remove the reactive oxygen species and catalyse the conversion of toxic metabolites to easily excreted compounds. The enzyme, superoxide dismutase, provides for the elimination of superoxide radicals and catalases and glutathione peroxidases for the elimination of hydrogen peroxide and organic peroxides. Members of the glutathione transferase family, γ-glutamyl cysteine synthetase and NAD(P)H:quinine reductase are also essential in antioxidant defence. Breakdown products of the sulphur-containing compounds from the Allium species may also induce phase 2 enzymes. It has been suggested that the anti-oxidant compounds and the phase 2 enzymes work together in sequence (Blomhoff, 2005). Antioxidant compounds such as quercetin may donate an electron

to a reactive oxygen or reactive nitrogen species then the oxidant radical which is formed in the reaction may then activate the gene expression of the phase 2 enzymes (Moskaug *et al*., 2004).

8.4 Measurement of antioxidants

A number of methods have been developed to measure the antioxidant concentration or capacity in dietary plants including herbs (Halvorsen *et al*., 2002). These are the 6-hydroxy-2,5,7,8-teramethylchroman-2-carboylic acid (Trolox) equivalent antioxidant capacity (TEAC) assay of Miller and Rice Evans (1996), the oxygen radical absorbance capacity (ORAC) assay of Delange and Glazer (1989) and the ferric reducing ability of plasma (FRAP) assay of Benzie and Strain (1996). The TEAC and the ORAC assays are based on the antioxidants' ability to react with free radicals while the FRAP assay measures the reduction of Fe^{+++} (ferric iron) to Fe^{++} (ferrous iron). The FRAP assay is the least selective and is therefore a good method for estimating the total antioxidant capacity of herbs in both water and fat soluble extracts. Using this method particularly it has been possible to draw up a table of anti-oxidant values for many types of foods including herbs and spices. Values of total anti-oxidant values in foods as determined by the ferric-reducing ability of plasma assay (mmol/100g) for cinnamon was 98.4, rosemary 66.9 and oregano 45.0 compared to the highest berries, dog rose 39.5 and blueberry 5.1, the highest nut, walnut 21.0, the highest fruit, pomegranate 11.3, the highest vegetable, kale 2.3 and fruit juice, blue grape 1.6 show that the herbs and spices are by far the richest source of anti-oxidants (Blomhoff, 2005).

Supplementing the bodies' defence mechanism by taking antioxidant supplements or eating a diet rich in anti-oxidants is regarded as a means of reducing the risk of cardiovascular disease. Support is sought for this approach by experimental modelling using organic molecules, cells and animals, epidemiological studies and finally by randomised intervention trials on human volunteers.

8.4.1 Model systems

Model systems are a direct method of establishing the anti-oxidant potential of herbs and spices. However, the experiments may only indicate an anti-oxidant effect, rather than one specifically related to cardiovascular disease. The anti-oxidant properties of seven dessert spices (anise, cinnamon, ginger, liquorice, mint, nutmeg, and vanilla) were compared with those of the common food anti-oxidants butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) and propyl gallate. Mint and cinnamon exhibited a higher percentage of inhibition of oxidation than the other spices and the food anti-oxidants analysed, as tested by the lipid peroxidation assay (Murcia *et al*., 2004).

Many cellular lipids and especially polyunsaturated fatty acids are vulnerable to attack by reactive oxygen species resulting in the formation of lipid peroxidases. The peroxidised lipids can cause cellular damage such as cross-linking of proteins and DNA. Also oxidised low-density lipoproteins can contribute to the formation of atheroslerotic plaques. Water and alcohol extracts of ginger have been shown to possess anti-oxidant activity on fats and oils and prevent lipid oxidation (Hirahara, 1974). In addition zingerone functioned as an effective scavenger of superoxide

anions as measured by nitro blue tetrazolium reduction in a xanthine-xanthine oxidase system (Krishnakantha, 1993).

In another model system involving garlic, the approach has been to use an *in vitro* system to show whether garlic supplement can prevent or reduce the oxidation of LDL. In the *in vitro* cell free system $CuSO_4$ was used to oxidise LDL and the product, thiobarbituric acid (TBARS), measured after 24 hours incubation in the presence and absence of the garlic supplement, AGE (Lau, 2001). The supplement exerted a concentration-dependent inhibition of Cu^{++} induced oxidation of LDL. All four water-soluble compounds derived from garlic, N-acetyl-*S* allyl cysteine, *S* allyl cysteine, alliin and allyl mercaptocysteine showed significant inhibition of LDL oxidation.

8.4.2 Animal studies

Animals have been used to establish the anti-oxidant potential of selected herbs and spices. These studies indicated the presence of compounds in ginger, which directly affected cholesterol metabolism. Activity of hepatic cholesterol–7-alpha-hydroxylase, the rate-limiting enzyme in bile acid biosynthesis was significantly elevated in ginger-fed rats. The conversion of cholesterol to bile acids is an important method of eliminating cholesterol from the body (Sambaiah and Srinivasin, 1991). In addition Tanabe *et al.* (1993) have recently isolated a new compound from ginger rhizomes, (E)-8b, 17-epoxylabd-12-ene-15,1 16-dial (ZT) that lowered plasma cholesterol levels in experimentally induced hypercholesterolemia in mice.

Oxidative modification of LDL is thought to play a key role in the pathogenesis of atherosclerosis. The lipid peroxidation lowering associated with ginger consumption was demonstrated in apoliprotein E deficient mice, i.e., mice that were prone to develop atherosclerosis (Fuhram *et al.*, 2000). Mice that consumed ginger (250 mcg of extract/day in their drinking water showed significant reduction in their basal concentration of LDL associated lipid peroxidases. The experimental data suggests a strong positive effect of ginger on plasma lipid composition that may be important for the prevention of atherosclerotic events. In a further study the oxidative stress induced by malathion (a pesticide) into rats was overcome by introducing ginger into the rats' diet. Ginger was able to lower lipid peroxidation in rats by influencing the enzymic blood level of the phase 2 enzymes, superoxide dismutase, catalase and glutathione peroxidase, known to be involved in antioxidant activity (Ahmed *et al.*, 2000).

The effect of dietary supplements of oregano essential oil was investigated on the performance of rabbits and the susceptibility of the produced raw and thermally treated muscle tissue to lipid oxidation during refrigerated storage (Spais *et al.*, 2004). A total of 96 weaned rabbits were separated into four equal groups with three sub groups. One group was given the basal diet and served as control, two groups were administered diets supplemented with oregano essential oil at levels of 100 and 200 mg/kg diet whereas the remaining group was given a diet supplemented with α-tocopherol acetate at 200 mg/kg. During the 42-day experimental period body weight and feed intake were recorded weekly and the food conversion ratio was calculated. Dietary oregano exerted no growth-promoting effects on rabbits. With increased supplementation of oregano essential oil, malondialddehyde values decreased in both raw and thermally treated muscles during refrigerated storage. This finding suggests that dietary oregano essential oil exerted a significant antioxidant effect. Dietary supplementation of oregano essential oil at the level of 200 mg/kg was more effective

in delaying lipid oxidation compared with the level of 100 mg/kg but inferior to dietary supplementation by 200 mg/kg α-tocopheryl acetate per kg. This study provided indirect evidence that anti-oxidant compounds occurring in oregano essential oil were absorbed by the rabbits and increased the antioxidant capacity of tissues.

8.4.3 Human studies

It is important that properly conducted trials be undertaken with human volunteers for the herbs and spices to be shown to have an effective role in diet. In a study of 20 males who had enhanced platelet aggregation following dietary supplements of 100 g of butter, 5 g of dried ginger twice daily significantly inhibited platelet aggregation induced by ADP and epinephrine (Verma *et al*., 1993). Not all studies were as conclusive. A randomised double blind study in eight healthy males tested the effects of daily doses of 2 g of dried ginger on platelet function. There were no differences in bleeding time or platelet aggregation between the ginger and placebo groups (Lumb, 1994). In a similar study of 60 patients with coronary disease a daily dose of 4 g of powdered ginger for three months did not affect ADP and epinephrine induced platelet aggregation (Bordia *et al*., 1997). These studies indicate that relatively large doses of ginger may be necessary to inhibit platelet function in humans.

In a small-scale study of the effects of garlic on LDL oxidation, a double blind placebo controlled crossover study involving eight subjects, four men and four women, mean age 68, four subjects took 1.2 g AGE three times a day for two weeks then two weeks of no garlic (washout period) followed by two weeks of placebo. The remaining four subjects took a placebo for the first two weeks followed by a two weeks washout and two weeks of 1.2 g AGE three times a day (Lau, 2001). Blood was drawn at the beginning of the experiment and at two, four, and six weeks and when the experiment was completed. Plasma LDL was isolated and the $CuSO_4$ test repeated. The use of garlic supplements was found to significantly increase the resistance of LDL to oxidation.

8.5 Complex mixtures versus single compounds

Epidemiological studies established that the Mediterranean diet which is rich in vegetables and herbs confirmed that the diet had health benefits for cardiovascular sufferers and could therefore delay the onset of the disease (Knoops *et al*., 2004). In order to identify the active components, the use of models and experimental animals have both shown a positive response to the use of specific supplements in stages of the disease such as oxidation of LDL. However randomised intervention trials that have been conducted to prove the anti-oxidant hypothesis for supplements in humans have not been convincing (Stanner *et al*., 2004). There is now evidence that there is no support for the use of anti-oxidant supplements such as alpha-tocopherol, carotene or ascorbic acid (Vivekanathan *et al*., 2003). One explanation for this is that in the complex mixtures that exist in plants there are large numbers of anti-oxidant molecules such as the polyphenols, whose role in the plant is to reduce oxidative stress by donating hydrogen to other compounds, are more effective than compounds such as ascorbic acid, β-carotene and α-tocopherol used in the supplements. The plant may employ many of these compounds in the multistage process of removing oxygen from the reactive oxygen species. Equally these compounds are available to animals

in their food to achieve the same objective. Consumption of the complex composition of the crude herb or spice would seem to have a distinct advantage over the use of single compound supplements.

8.6 Conclusions

Herbs and spices have been used as sources of flavourings and medicines for thousands of years and their use as flavourings continues today. Now the pharmaceutical industry is interested in these plants as a source of pharmaceuticals, particularly for their antioxidant value in the prevention and treatment of chronic diseases such as cardiovascular disease. One aim has been to isolate and identify single compounds from individual herbs or spice plants so that the compound can then be marketed as being of 'natural' origin. However, examination of the effect of single plant based compounds have not been as encouraging as the use of crude extracts. The difference rests on the fact that the plant contains a very large number of potential anti-oxidants that probably act synergistically. This is not surprising. In order to control the possibility of excess solar energy damaging cell metabolism, evolution has ensured that plants contain not one compound but a whole variety of compounds to achieve an anti-oxidant effect. It is not surprising therefore that animals including ourselves who depend on plants for food have co-evolved and have therefore the same requirement for multiple anti-oxidants rather than a single one. The conclusion that can be drawn from such an evolutionary view is that however commercially attractive and convenient it might be to take a single compound supplement, it is more sensible and beneficial to take a complex extract. It is far better to use the herbs and spices for their original purpose, which is to enhance the flavour of our food and at the same time ensure that we remain healthy on a balance of antioxidants.

8.7 References

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9

Herbs, spices and cancer

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9.1 Introduction

Cancer is a complex set of diseases and is one of the most devastating diseases worldwide. According to WHO statistics, the burden of new cases in 2000 was estimated to be 10.1 million in the world representing about 20% increase over the previous decade. Similarly about 56% of the estimated deaths from cancer occur in the developing world. The reason for the increased number of cancer deaths is due to the increased life expectancy achieved by combating life threatening diseases. In addition, tobacco consumption, newer infections, environmental degradation, change in lifestyle and diet and malnutrition are all contributing major factors for the increase in the burden. In males lung cancer is the leading cancer site both in terms of new cases as well as deaths, with almost equal contribution. The second most common cancer globally, is stomach cancer with almost two-thirds of the load contributed by the developing world, in particular China. This is followed closely by prostate and colo-rectal cancers. More than three-quarters of cases of prostate cancer and two-thirds of colorectal cancer occur predominantly in the developed world. In men, in the developing world the five leading cancer sites are lung, stomach, liver, oesophagus and head and neck. These sites accounted for more than 55% of the cases diagnosed and almost two-thirds of deaths due to cancer for the year 2000. Cancers of the breast and cervix are the two most important cancer sites and account for one-third of all cases diagnosed in the women of the developing world.

In order to combat diseases including cancer, human beings depended upon Mother Nature from time immemorial and a number of traditional health systems existed in the world. Herbs and spices have been used for generations by humans as food supplements and to treat some ailments. Each system has developed a number of effective herbal disease cure prescriptions, which spread throughout the world. The evolution of different systems of medicine was originally associated with cultural and religious history. An enormous number of medicinal plants were also recognized in each health care system and distributed in different parts of the world. About 250,000 plant species are recorded from different parts of the world from which about 80,000 plant species are medicinal. WHO also reported that over 30% of the

world's plant population has been used for medicinal purposes at one time or another. Most of the traditional health care systems of the world, like Egypt, the Middle East, India and China developed from 3000 BC onwards. India's use of herbal health care dates back close to 5000 years.

Scientific evidence has shown that many of these herbs and spices do have medicinal properties. A growing body of research has demonstrated that the commonly used herbs and spices such as garlic, black cumin, cloves, cinnamon, thyme, allspice, bay leaves, mustard, and rosemary, possess antimicrobial properties that, in some cases, can be used therapeutically. Other spices, such as saffron, a food colorant; turmeric, a yellow colored spice; tea, either green or black, and flaxseed do contain potent phytochemicals, including carotenoids, curcumins, catechins and lignan respectively, which provide significant protection against cancer.

9.2 What is cancer?

Cancer is a complex disease which may be caused by a toxic environment, devitalized food, lifestyle and lack of spiritual purpose in life, which in turn causes accumulation of toxic material that disturbs the balance of the basic elements. Each cancer is unique, the way it grows and develops, its chances of spreading, the way it affects the body and the symptoms produced. Several factors, including the organs it affects and how the cancerous cells grow determine the types of cancer.

All cancers, however, fall into four broad categories such as carcinoma, sarcoma, leukaemia and lymphoma. Carcinoma is a malignant neoplasm of epithelial origin and arises in the tissues of the body's organs like nose, colon, penis, breasts, prostate gland, urinary bladder and ureter. About 80% of all cancer cases are carcinomas. Sarcomas are tumors that originate in bone, muscle, cartilage, fibrous tissue or fat. Leukaemia is cancer of the blood or blood-forming organs. Lymphomas affect the lymphatic system, a network of vessels and nodes that acts as the body's filter.

9.3 Cancer therapy in modern medicine

In modern medicine cancers are treated by chemotherapy, radiation, surgical excision, biological therapy or by bone marrow transplantation (BMT). Chemotherapy is the use of certain drugs that treat the disease. At present chemotherapeutic drugs affect other normal fast dividing cells also such as those responsible for hair growth and replacement of epithelium cells in the intestine thereby causing adverse side-effects.

In radiation treatment, high doses of radiation are used that can kill cells or keep them from growing and dividing. Radiation therapy is a useful tool for treating cancer because cancer cells grow and divide more rapidly than many of the normal cells around them.

Surgery is the oldest form of treatment for cancer to remove the solid mass of a tumor along with a healthy margin of tissue. In addition to the tumor, other structures such as lymph nodes or blood vessels in close proximity to the tumor will be removed to aid in identifying the extent of the cancer and to remove optimally all cancerous tissues.

The general concept of biological therapy or gene therapy is defined as the transfer of genetic material (transgene) to a target cell for therapeutic reasons. Introduction of the transgene into a somatic or a germ cell can restore a lost function (gene substitution), initiate a new function (gene addition), or interfere with a gene's function (gene inhibition).

Autologous bone marrow transplantation (ABMT) has been increasingly used in the treatment of malignant diseases. When these preliminary results are analyzed, it is apparent that the outcome of this treatment modality is affected by several factors such as disease status or tumor burden at the time of ABMT, the anti-tumor effect of the pretransplant intensive therapy and the extent of bone marrow invasion by tumor cells.

9.4 Complementary and alternative medicines (CAM)

9.4.1 Cancer in Ayurveda

In Ayurveda, cancer is a disease that is caused by the involvement of the three body elements, i.e., *vata*, *pitta* and *kapha*. The cancer represents a negative life energy usually formed from an excess *apana* (the downward movement of air). Distension, constipation and diarrhea may be the basis of the symptom according to Ayurveda. Cancer cells, lacking oxygen (prana), represent a growth in the body outside the rule of the life force. In the *Vedic* system, cancer is caused by disruption of the aura allowing the entrance of negative astral force which will be cured by emotional cleansing, mantra and meditation.

According to Ayurveda, medicinal substances and living bodies are similar in composition. Hence herbs and drugs influence the body according to their nature and attributes. According to *Charaka*, medicines are those substances which, after entering the body, are eliminated through the gastro-intestinal tract within a specified period after their corrective role is over. Sometimes there will be overlap between foods and medicines, and foods also have medicinal properties and medicines have also tissue building action. Also, the human body is constituted of five basic elements, i.e., ether, air, water and earth which manifest as three basic principles or elements, 'tridosha', i.e., *Vata*, *pitta* and *kapha*. These three basic elements control all the biological, psychological and physiopathological functions of the body, mind and consciousness and when the balance of three elements disturbs, they contribute to disease processes. The basic constitution of the body (*prakruti* = nature) of an individual remains unaltered during the lifetime as it is genetically controlled and it is made up of a combination of all the three elements with a predominant tendency towards one or more. All Ayurvedic treatments attempt to establish a balance among the bodily elements, i.e., *Vata*, *pitta* and *kapha*.

Herbal therapies for the treatment of cancer are of different types. It may be an alterative or blood purifier, which destroy toxins. These herbs are used fresh along with a detoxifying diet. This category of herbs is claimed to cure lymphatic or skin cancer and are better for *Pitta* and *Kapha* varieties. The second category of herbs are circulatory stimulants that promote blood circulation and aid in the healing of tissues. Breast and uterine cancer are treated with these herbs which will affect all the three elements. The third category of herbs are immune strengthening tonics which are better in debility conditions and usually work on *Vata*. The fourth category of herbs include special expectorants. They are used for thyroid, neck or lymphatic cancer.

They work well on *Vata* and *Kapha* cancers. Besides these categories, there are herbs that are pungent or bitter and have fat reducing and toxin destroying properties.

Thus in Ayurveda, the herbs used in cancer treatment are categorized based on their action in different basic elements, i.e., herbs for *vata* are *Acorus calamus*, *Terminalia chebula*, *Commiphora wightii*, triphala formulation (*Terminalia bellirica*, *Terminalia chebula* and *Emblica officinalis*), etc.; in *Pitta*: Crocus sativus, *Rubia cordifolia* and *Curcuma longa*; vegetables, juice diets, etc.; in *Kapha*: *Piper nigrum*, *Piper longum*, *Zingiber officinale*, *Commiphora wightii, turmeric and trikatu formulation (Piper nigrum, Zingiber officinale, Piper longum*).

Complementary and alternative medicine (CAM) has gained popularity among cancer patients also worldwide. Among cancer patients, use of CAM ranges between 30 and 75% worldwide and includes dietary approaches, herbals and other biologically based treatments. Yamini *et al*. (2005) reviewed the use of complementary and alternative medicine (CAM) in developed countries, already in use as traditional medicines in various Asian countries. The Indian system of medicine, named as Ayurveda has an edge in this field. In 1998, the US Congress mandated the creation of the National Center for Complementary and Alternative Medicine (NCCAM) to conduct and support such research of CAM therapies in the USA (Richardson, 2001).

A study conducted in Israel (Pud *et al*., 2005) indicated that the key benefits from CAM reported by patients included improvement in emotional and physical well-being and increased ability to fight the disease. The most frequently used CAM method appeared to be herbal therapy, and the most commonly used herb was the stinging nettle in Turkey. Patients' responses indicated that 'the desire to do everything possible to fight the disease' and 'the idea that it may be helpful, at least it's not harmful' were the two most common reasons for using CAM (Algier *et al*., 2005). A study conducted at Michigan (Wyatt *et al*., 1999) showed that approximately 33% of older cancer patients reported using complementary therapies. Traditional Indian systems of medicine, such as Siddha, have been reported to benefit patients in India through herbal interventions for cancer (Srinivasan *et al*., 2004). A survey conducted in the US (David *et al*., 1998) showed that alternative medicine use and expenditure on it increased substantially between 1990 and 1997, attributable primarily to an increase in the proportion of the population seeking alternative therapies.

Cancer chemo-prevention by phyto-chemicals may be one of the most feasible approaches for cancer management. For example, phyto-chemicals obtained from vegetables, fruits, spices, teas, herbs and medicinal plants, such as carotenoids, phenolic compounds and terpenoids, have been proven to suppress experimental carcinogenesis in various organs. Phyto-chemicals may also be useful to develop 'designer foods' or 'functional foods' for cancer prevention (Nishino, 2000).

Gupta *et al*. (2002) studied the prevalence of use of CAM cancer therapies in leukaemia patients visiting haematology clinics of a north Indian tertiary CARE hospital. Prevalence of CAM use in leukaemia patients was found to be 56.6% and Ayurveda was the most commonly used CAM (33%). Most of the patients sought conventional medicine first, followed by CAM therapies.

Singh (2002) reviewed the Ayurvedic concept of cancer diathesis. A retrospective meta-analysis of observations on 85 plant drugs reported to have an anticancer effect indicates that herbs with *Katu* means bitter, *Tikta* means pungent *Kasaya Rasa* astringent taste, *Usna Virya* means hot biopotency and *Katu Vipaka* means catabolic active metabolites, and herbs with dry, coarse, light, and sharp biophysical properties have significantly greater possibilities of producing anticancer effects. Studies suggested

that *Withania somnifera* root has chemopreventive efficacy against forestomach and skin carcinogenesis and warrants the identification and isolation of active compounds responsible for its anticancer effects, which may provide the lead for the development of antitumor agents (Padmavathi *et al*., 2005).

9.5 Mechanism of action of herbs and spices

The use of herbs for medical benefit has played an important role in nearly every culture on earth. Herbal medicine was practised by ancient cultures in Asia, Africa, Europe and the Americas. The recent popularity in use of herbals can be tied to the belief that herbs can provide some benefit over and above allopathic medicine and allow users to feel that they have some control in their choice of medications (Wargovich, 2001).

Ayurveda pharmacology considers drug action to be mediated totally or partially through rasa (taste), vipaka (assimilation/fate of the drug), veerya (dosage) and prabhava (activity) of the drug. It is worth remembering that selection of a plant reported in classical texts for a particular disease alone is not going to help as Ayurveda is indeed a way of life. A holistic approach is required which would re-normalize the altered environment. Hence, in Ayurveda extracting the active principles from the crude drug as in the case of modern medicines is not recommended since it is believed that curative action of a crude drug is not due to one or two major constituents but because of synergistic action of a number of major and minor constituents present in the crude drugs. The process is different and much more complex than the simplistic model of the modern medicines (Ayyar, 1946). One of the most promising strategies for cancer prevention today is chemoprevention using readily available natural substances from vegetables, fruits, herbs and spices (Das *et al*., 2004).

There is considerable scientific evidence, both epidemiological and experimental, regarding vegetables and fruits as key features of diets associated with reduced risks of diseases such as cancers and infections. This has led to the use of a number of phytometabolites as anticarcinogenic and cardioprotective agents, promoting a dramatic increase in their consumption as dietary supplements. It is well observed that alteration of cell cycle regulatory gene expression is frequently found in tumor tissues or cancer cell lines, and studies have suggested that the herbal-based or plant-originated cell cycle regulators might represent a new set of potential targets for anticancer drugs (Singh *et al*., 2003).

An impressive body of data exists in support of the concept that Indian food ingredients can be used in preventive strategies aimed at reducing the incidence and mortality of different types of cancers because of their antioxidative, antimutagenic and anticarcinogenic properties. Vital ingredients used in Indian cooking include turmeric, cloves, ginger, aniseed, mustard, saffron, cardamom and garlic (Sengupta *et al*., 2004).

9.6 Evidence supporting the functional benefits of herbs and spices

Scientific evidence is accumulating that many of these herbs and spices do have

medicinal properties that alleviate symptoms or prevent disease. Saffron, a food colorant; turmeric, a yellow colored spice; tea, either green or black, and flaxseed contain potent phytochemicals, including carotenoids, curcumins, catechins, lignan respectively that provide significant protection against cancer (Hastak *et al*., 1997; Abdullaev, 2002; Lai and Roy, 2004).

Herbal products may act in a manner similar to pharmaceuticals yet without side effects. Natural anti-inflammatory compounds abound in the herbal world and are found in green tea, the spices turmeric and rosemary, feverfew and others. Because the use of nonsteroidal anti-inflammatory drugs (NSAID) is associated with a reduced risk for several cancers, it is at least plausible that natural NSAID should be explored for possible use as cancer preventives (Wargovich *et al*., 2001).

Adlercreutz (1995) studied the cancer-protective roles of some hormone-like diphenolic phytoestrogens of dietary origin, the lignans and the isoflavonoids. The plant lignan and isoflavonoid glycosides are converted by intestinal bacteria to hormone-like compounds with weak estrogenic but also antioxidative activity; they have now been shown to influence not only sex hormone metabolism and biological activity but also intracellular enzymes, protein synthesis, growth factor action, malignant cell proliferation, differentiation, and angiogenesis in a way that makes them strong candidates for a role as natural cancer-protective compounds.

Pharmacological studies (Das *et al*., 2004) have demonstrated many health promoting properties including radical scavenging, anti-mutagenic and immuno-modulating effects of Saffron (*Crocus sativus*, L.) apart from its use as a flavouring agent. Significant reduction in papilloma formation was found with saffron application in the pre-initiation and post-initiation periods, and particularly when the agent was given both pre- and post-initiation. The inhibition appeared to be at least partly due to the modulatory effects of saffron on some phase II detoxifying enzymes like glutathione-S-transferase (GST) and glutathinoe peroxidase (GPx), as well as catalase (CAT) and superoxide dismutase (SOD).

Several epidemiologic studies suggest that consumption of cruciferous vegetables may be particularly effective (compared with total fruit and vegetable consumption) in reducing cancer risk at several organ sites. Crucifers that are widely consumed are especially rich in glucosinolates, which are converted by plant myrosinase and gastrointestinal microflora to isothiocyanates which will be helpful in the production of proteins that exercise versatile, long-lasting and catalytic antioxidant protection (Paul and Jed, 2001).

Mantle *et al*., (2000) assessed various active compounds (or their semi-synthetic derivatives) derived from medicinal plants for their efficacy and tolerability in the treatment of breast cancer. Some of these plant species, including *Taxus baccata* (paclitaxel, docetaxel), *Podophyllum peltatum* (etoposide), *Camptotheca acuminata* (camptothecin) and *Vinca rosea* (vinblastine, vinorelbine) have well recognized antitumor activity in breast cancer. Antitumor activity derived from medicinal plants may produce results via a number of mechanisms, including effects on cytoskeletal proteins which play a key role in mitosis (paclitaxel), inhibition of activity of topoisomerase enzymes I (camptothecin) or II (etoposide), stimulation of the immune system (*Viscum album*), or antiprotease-antioxidant activity. Medicinal plant-derived antineoplastic agents may be used in single-agent or in combinational therapies, and have been used in first-line or second-line (including anthracycline-refractory patients) treatment of localized or metastatic breast cancer.

Srinivasan *et al*., (2004) studied the scientific basis for the antitumor property of

Semecarpus 'Lehyam' (SL) – a Siddha medicine – with respect to breast cancer and found SL to be a potent antitumor agent against the ER-negative breast cancer cell line. An extensive review by Premalatha (2000) described the phytochemical and pharmacological basis of anticancer properties of the species.

Anti-proliferative and antitumor effects of a herbal preparation termed PC-SPES (patent pending, US serial number 08/697, 920) which is a refined powder of eight different medicinal plants were studied (Tiwari *et al*., 1999; Marks *et al*., 2003). PC-SPES administered as a food supplement caused a dramatic decrease in prostate specific antigen levels in some prostate cancer patients with advanced disease. The study revealed the therapeutic benefit of this herbal food supplement and may be a useful adjuvant to conventional therapeutic modalities. Two marker compounds in PC-SPES are baicalin and oridonin, both of which exhibit antiproliferative effects. Bonham *et al*., (2005) studied the anticancer activity of *Scutellaria baicalensis*, a botanical constituent of the herbal mixture PC-SPES and purified four constituents that function in part through inhibition of the androgen receptor signaling pathway.

L-Canaline, the L-2-amino-4-(aminooxy)butyric acid structural analog of L-ornithine is a powerful antimetabolite stored in many leguminous Plants and this natural product was found to possess significant antineoplastic *in vitro* activity against human pancreatic cancer cells (Rosenthal, 1997).

Ovesna *et al*. 2004, investigated the antitumor and chemopreventive activities of plant-based diet (beta-sitosterol and taraxasterol) which were found to inhibit colon and breast cancer development. These compounds act at various stages of tumor development, including inhibition of tumorigenesis, inhibition of tumor promotion, and induction of cell differentiation and effectively inhibit invasion of tumor cells and metastasis.

A study by Zava *et al*. (1998) of about 150 herbs used traditionally by herbalists for treating a variety of health problems showed their relative capacity to compete with estradiol and progesterone binding to intracellular receptors for progesterone (PR) and estradiol (ER) in intact human breast cancer cells. It was demonstrated that many of the commonly consumed foods, herbs, and spices contain phytoestrogens and phytoprogestins that act as agonists and antagonists *in vivo.*

The rosemary extract (Herbor 025) and the extract of Provencal herbs (Spice Cocktail) showed good antioxidant activity in the Rancimat test, especially in lard (Aruoma *et al*., 1996). Both preparations promoted some DNA damage in the copper-phenanthroline and the bleomycin-iron systems. The two herbal preparations possess antioxidant properties that may make them useful in the food matrix.

Studies conducted using total extract, polar and non-polar extracts, and their formulations, prepared from medicinal plants mentioned in Ayurveda, namely, *Withania somnifera* (Dunal), *Tinospora cordifolia* (Miers), and *Asparagus racemosus* (Willd.), exhibited various immunopharmacological activities in cyclophosphamide (CP)-treated mouse ascitic sarcoma (Diwanay *et al*., 2004).

Mishima *et al*., (2003) reported that vaticanol C, a resveratrol tetramer, exhibits strong cytotoxicity against various tumor cell lines. They also reported the antitumor activity of the ethanol extract from the stem bark of *Vateria indica*, which has been traditionally used for health and healing diseases in Ayurveda in India.

Dietary administration of *Withania* root on hepatic phase I, phase II and antioxidant enzymes by estimation of its level/activity, as well as in attenuating carcinogen-induced forestomach and skin tumorigenesis in the Swiss albino mouse model showed that roots of *W. somnifera* inhibited phase I, and activated phase II and antioxidant

enzymes in the liver (Padmavathi, *et al*., 2005). Further, in a long-term tumorigenesis study, Withania inhibited benzo(a)pyrene-induced forestomach papillomagenesis, showing up to 60 and 92% inhibition in tumor incidence and multiplicity, respectively. Similarly, Withania inhibited 7,12-dimethylbenzanthracene-induced skin papillomagenesis, showing up to 45 and 71% inhibition in tumor incidence and multiplicity.

Another important traditional herbal medicine used for cancer therapy is *Cordyceps militaris* which has been used for patients suffering from cancer in Oriental medicine. The investigation of biochemical mechanisms of anti-proliferative effects by aqueous extract of *C. militaris* in human leukemia U937 cells were associated with the induction of apoptotic cell death through regulation of several major growth regulatory gene products such as Bcl-2 family expression and caspase protease activity, and *C. militaris* may have therapeutic potential in human leukemia treatment (Park *et al*., 2005).

Betel leaf (*Piper betle*) has many medicinal properties and is used in the Indian system of medicine (Chopra *et al*., 1954). Investigations have confirmed that the leaves contain a chemical called hydroxy-chavicol, a phenolic compound that exhibited suppression of induced mutagenesis (Amonkar *et al*., 1986).

Botany of some important herbs commonly used in cancer therapy are listed in section 9.7. A list of other medicinal plants reported to have anticancerous properties is presented in Table 9.1. Some of them may find importance in cancer therapy in future.

9.7 Botany of some important herbs in cancer therapy

9.7.1 *Aloe barbadensis* (Aloe)

Aloe belongs to the family Liliaceae. The plant is a perennial herb with condensed stem and succulent leaves arranged in a rosette shape. The exudates of the succulent fleshy leaves contain a number of therapeutically important compounds such as aloin, aloe emodin, etc. The species is native to Africa from where it has been introduced to India.

9.7.2 *Andrographis paniculata* (Kalmegh)

The plant belongs to the family Acanthaceae. It is an erect branched annual herb with simple leaves arranged in opposite manner. The whole herbage is bitter andtherapeutically important. The species is distributed throughout India, Sri Lanka and Malaysia.

9.7.3 *Asparagus racemosus* (Satavari)

It is a member of the family Liliaceae and the plant is a spiny, woody climber and much branched. Cladodes are 2–6 in number per node and arranged in a tuft. Leaves are modified into erect or sub-recurved spines. The fibrous root system is modified into fascicular roots for storage and used for medicinal purposes. The species is distributed throughout tropical and sub-tropical India, Sri Lanka, Australia and tropical Africa.

9.7.4 *Catharanthus roseus* (Periwinkle)

Periwinkle is a member of the family Apocynaceae. The plant is an erect annual or

Table 9.1 Other plant species having anticancer property

Plant name	Parts used
Abelmoschus esculentus L.	Seeds – against cancerous cell growth
A. moschatus Medic	Seed is antitumorous
Achillea millefolium L.	Essential oil is anticancerous
Actaea spicata L.	Against Ehrlich's ascitis tumors
Aerva pseudotomentosa Blatt. & Hall.	Plant is anticancerous
Agave americana L.	Leaf is anticancerous
Ailanthus excelsa Roxb.	Root bark – antitumourous and against lymphocytic leukaemia
Ajuga bracteosa Wall.ex Benth.	Leaf and root are anticancerous
Albizia lebbeck (L.) Willd.	Pod and root – anticancerous
Alhagi pseudalhagi (Bieb) Desv.	Plant is anticancerous
Allamanda cathartica L.	Leaf extract – against leukaemia and carcinoma
Alstonia scholaris (L.) R. Br.	Stem bark – anticancerous
Allium sativum L.	Bulb juice is antitumorous
Anamirta cocculus (L) Wt. & Arn.	Aerial part is anticancerous
Annona squamosa L.	Corydine from plant parts is anticancerous and used against malignant tumors
Anthemis nobilis L.	Nobilin is anticancerous
Aristolochia indica L.	Plant is anticancerous
Asparagus racemosus Willd.	Aerial part is anticancerous
Avicennia officinalis L.	Lapachol is antitumorous
Baliospermum montanum (Willd.) Muell.-Arg.	Plant is anticancerous
Berberis aristata DC.	Roots are anticancerous
B. asiatica Roxb. Ex DC	Roots are anticancerous
B. lycium Royle	Roots are anticancerous
Bergenia vulgaris L.	Rhizome is anticancerous
Bridelia retusa Spreng.	Stem bark – anticancerous
Buchnania lanzan Spreng.	Aerial part – anticancerous
Casearia zeylanica (Gaertn.)Thw.	Aerial part – anticancerous
Caesalpinia sappan L.	Stem is anticancerous
Calamus rotang L.	Aerial part is anticancerous
Calotropis gigantea (L.) R. Br.	Leaf and root are anticancerous
C. procera R.Br.	Leaf and root are anticancerous
Capparis grandis L.f.	Aerial part is anticancerous
Cassia fistula L.	Pod and stem bark – anticancerous
Castanopsis indica (Roxb.) DC.	Stem bark – anticancerous
Caucus carota L.	Against tumor
Citrus medica L.	Aerial part is anticancerous
Cleome gynandra (L.)	Plant is anticancerous
Cocculus pendulus (Forst.) Diels	Aerial part is anticancerous
Coix lachryma-jobi L.	Coixenolide showed anticancer activity
Colchicum luteum Baker	Rhizome and seed are anticancerous
Corchorus aestuans L.	Plant is anticancerous
Crotalaria retusa L.	Plant has antitumorous activity
Curculigo orchioides Gaertn.	Plant is anticancerous
Datura metel L.	Plant is anticancerous
Dipteracanthus prostratus (Poir.) Nees	Plant is anticancerous against epidermoid carcinoma of nasopharynx
Dolichos uniflorus Lamk.	Plant is antitumorous
Drimia indica (Roxb.) Jessop	Plant is anticancerous
Elephantopus scaber L.	Plant is anticancerous
Entada pursaetha DC	Plant is used in cancer
Euphorbia hirta L.	Anticancerous
Euphorbia tirucalli L.	Anticancerous

Table 9.1 Continued

Plant name	Parts used
Gardenia turgida Roxb.	Root – anticancerous
Gaultheria fragratissima Wall.	Essential oil is anticancerous
Jatropha glandulifera Roxb.	Aerial parts anticancerous
Jatropha gossypifolia L.	Plant – antileukaemic
Kaempferia rotunda L.	Roots – antitumorous
Kalanchoe spathulata DC.	Plant – anticancerous
Lannea coromandelina (Houtt.) Merr.	Stem bark and leaves – anticancerous
Leonotis nepataefolia (L) R.Br.	Plant – anticancerous
Luffa cylindrica (L) Roem.	Seeds – anticancerous
Mallotus philippensis (Lam.) Muell.-Arg.	Fruit – anticancerous
Manilkara hexandra (Roxb.) Dubard.	Aerial part – anticancerous
Melia azedarach L.	Bark is anticancerous
Moringa pterygosperma Gaertn.	Aerial part – anticancerous
Nigella sativa L.	Seed – anticancerous
Passiflora foetida L.	Aerial parts – anticancerous
Podophyllum hexandrum Royle	Podophyllotoxin from rhizome derivatives – anticancerous
Pogostemon heyneanus Benth.	Pogopyrone B from leaves is anticancerous
Roylea cinerea (D. Don) Baillon	Aerial parts – precalyon shows antitumor activity
Rhus succedanea L.	Leaves – anticancerous
Saraca asoca Roxb. (de Wilde)	Stem bark is anticancerous
Satureja hotensis L.	Labiatic acid from plant is anticancerous
Solanum indicum L.	Plant is anticancerous
Teramnus labialis (L.f) Spreng.	Seed oil is anticancerous
Tithonia tagetiflora Des. ex. Juss.	Plant – Lymphocytic leukaemia
Toona ciliata Roem.	Stem bark – anticancerous
Vernonia cinera (L.) Less	Plant is anticancerous

perennial herb with simple glossy leaves. A number of flower color variants are present in the species. The plant contains alkaloids which are medicinally useful. It is native to Madagascar and under commercial cultivation in India.

9.7.5 *Crocusus sativ* (Saffron)

Saffron belongs to the family Iridaceae. The plant has an underground sheathed corm with sheaths closely reticulate and spathes embracing the scape are bivalved. Flowers are violet. Stigma is medicinally important. The species is a native of Europe and cultivated in Kashmir.

9.7.6 *Curcuma longa* (Turmeric)

It is a member of the family Zingiberaceae. The plant is herbaceous in nature. The rootstock is large and bears cylindrical tubers that are yellow or orange inside and are medicinally important. The species is cultivated extensively in the tropics.

9.7.7 *Piper betle* (Betelvine)

Betelvine belongs to the family Piperaceae. It is a dioecious, perennial aromatic

climber with simple, alternate leaves and a jointed stem. The leaves are aromatic and are medicinally important. It is extensively grown in India and used as stimulant. The species is a native of Malaysia.

9.7.8 *Piper longum* (Long pepper)

Long pepper is a member of the family Piperaceae. It is a dioecious, perennial aromatic climber with simple, alternate leaves and jointed stem. The mature fruit and root and basal portions of the stem are medicinally important. The species is native to tropical and subtropical India, Nepal, Bangladesh and Sri Lanka.

9.7.9 *Semecarpus anacardium* (Bhela/marking nut tree)

The plant belongs to the family Anacardiaceae. It is a small to medium sized deciduous tree with rough dark-brown bark. The leaves are simple and large sized and the fruit is used for therapeutical purposes. The species is distributed throughout sub-Himalayan and tropical India, Malaysia and Australia.

9.7.10 *Tinospora cordifolia* (Amrut)

The plant belongs to the family Menispermaceae. It is a dioecious climber bearing aerial roots and with papery bark. The leaves are pedicellate, alternate and polymorphic. The stem contains starch and alkaloids and is mainly used for medicinal purposes.

9.7.11 *Taxus baccata* (Taxus/Yew)

Taxus is a member of the family Coniferae. It is a small or medium sized evergreen tree, stem fluted and branches horizontal. Leaves are long, linear, flattened and narrowed and commonly known as needles. Male flowers are arranged in catkins and female flowers are solitary. The stem bark is used for medicinal purposes. It is distributed in temperate Himalayas, Khasi hills, Tamil Nadu, Europe, Africa and America.

9.7.12 *Vateria indica* (Kundura, Indian Copal tree)

It is a member of the family Dipterocarpaceae. The plant is a resinous tree with whitish bark, leaves entire, penninerved and coriaceous. Young branches and leaves are clothed with hoary, stellate pubescence and flowers are in panicles. The resin and bark are medicinally useful. The species is distributed throughout western India.

9.7.13 *Withania somnifera* (Ashwagandha)

The plant belongs to the family Solanaceae. It is an erect evergreen plant with simple alternate leaves. The plant is widely distributed in tropical and subtropical areas of the world. The roots are considered to be medicinally important.

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10

Herbs, spices and gut health

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10.1 Introduction

Gut health is the most important factor for a healthy life. A large number of people are suffering from gut associated diseases, such as irritable bowel syndrome and inflammatory bowel disease and this leads to loss in man-hours and to consequent economic loss. Conditions such as inflammatory bowel disease are associated with a breakdown in immune tolerance, while exposure to microbial antigens has an important influence on the development of the gut immune system, with likely links to allergies. In the Ayurveda, Unani and Siddha systems of Indian medicine, health of gut is given prime importance. The World Health Organization has published statistics on economic losses due to unhealthy gut especially in developing countries.

Several research projects have been funded by the EU under the 4th and 5th framework programmes on functional and probiotic foods in relation to gut health (Flair-Flow report, 2001). Optimizing microbial balance by modifying the diet (by incorporation of certain foods or food additives) provides an important approach for helping to prevent colitis, colorectal cancer, as well as infectious diseases and to enhance the human immune system. On the other hand, the important role of herbs and spices in our life and their multiple uses as ingredients in food, alcoholic beverages, perfumery, cosmetics, medicine and colouring agents are well established. They are also well evidenced in the nutritional, antioxidant, antimicrobial and pharmaceutical properties of several herbs and spices. Empirical knowledge has led people to use several herbs and spices as medicines and healing agents since ancient times. Nowadays many plant-derived drugs are based on this knowledge from traditional medicine.

In this chapter the effect of various herbs and spices or their constituents on gut health will be reviewed. Their use as digestive stimulants and growth promoters, their antimicrobial activity on certain enteric pathogens, their inflammatory activity on the peptic system as well as their impact on gut immunity will be discussed. There will also be reference to the kind of experiments that examined each activity and information on the possible active constituents and their mechanisms of action.

10.2 Herbs and spices as digestive stimulants

A digestive stimulant action has been attributed to many herbs and spices listed in Table 10.1. Many of these are employed in medicinal preparations for use against digestive disorders and in traditional medicine as tonic, stomachic, carminative, antispasmodic and diuretic (Shylaja and Peter, 2004). Plant substances such as chirata, gentian, calama, quassia, orange peel and many spices such as mint, garlic, ginger, ajowan, cumin, fennel and coriander are contained in preparations available to correct digestive disorders (Platel and Srinivasan, 2004). Extracts from bitter candy taft, chamomile flower, peppermint leaves, caraway fruit, liquorice root, lemon balm leaves, angelica root, greater celandine herbs and milk thistle fruit are also basic constituents of a commercially available drug with multiple pharmacological properties relevant for gastrointestinal pathophysiology (Saller *et al*., 2002). Pharmacological *in vitro* and *in vivo* studies have shown that they possess antibacterial, antisecretory, cytoprotective and anti-ulcerogenic as well as spasmolytic effects (Saller *et al*., 2002; Khayyal *et al*., 2001). Several herbal medicinal products (included in Table 10.1) have been identified also for use in the relief of symptoms of non-ulcer dyspepsia (Thompson Coon and Ernst, 2002). Human studies have shown that red pepper powder is significantly more effective in decreasing the symptom intensity of patients with functional dyspepsia (Bortolotti *et al*., 2002).

Table 10.1 Herbs, plant substances and spices with digestive stimulatory action and recommended for the treatment of dyspepsia

Ajowan[6]	Coriander[6,10]	Mistletoe[10]
Allspice[9]	Cloves[10]	Mustard[3]
Angelica root[4,5,7,10]	Coriander[10]	Onion[8]
Anise[10]	Cumin[6]	Orange peel[6,10]
Artichoke[10]	Dandelion[10]	Oregano[9]
Banana[10]	Devil's claw[10]	Oregon grape[10]
Basil, sweet[9]	Dill[9,10]	Paprika[8]
Bay leaves[9]	Elecampane[10]	Parsley[9]
Bitter candy taft[4,5,7]	Fennel[6,9]	Peach[10]
Black pepper[3,8]	Fenugreek[1,2,10]	Peppermint leaves[4,5,7,9,10]
Blessed thistle[10]	Galangal[10]	Quassia[6]
Bogbean[10]	Garlic[6]	Radish[10]
Boldo[10]	Gentian[6,10]	Red pepper (capsaicin)[1,2,3]
Calama[6]	Ginger[1,2,6,10]	Rosemary[9,10]
Caraway fruit[4,5,7,9,10]	Horsetail[10]	Sage[9,10]
Cardamon[10]	Haronga[10]	Sandy everlasting[10]
Capsicum[3]	Horehound[10]	Spearmint[9]
Celandine herbs[4,5,7,10]	Juniper[10]	St John's wort[10]
Celery[9]	Leek[9]	Star anise[10]
Centuary[10]	Lemon balm leaves[4,5,7]	Tarragon[9]
Chamomile flower[4,5,7]	Liquorice root[4,5,7]	Thyme[9,10]
Chicory[10]	Liu-Jun-Zi-Tang[10]	Turmeric (curcumin)[1,2,0]
Chirata[6]	Marjoram[9]	Wormwood[10]
Chive[9]	Meadowsweet[10]	Yarrow[10]
Cinchona[10]	Milk thistle fruit[4,5,7,10]	
Cinnamon[8,10]	Mint[6]	

1: Bhat *et al*. (1984); 2: Bhat *et al*. (1985); 3: Glatzel (1968); 4: Hohenester *et al*. (2004); 5: Khayyal *et al*. (2001); 6: Platel and Srinivasan (2004); 7: Saller *et al*. (2002); 8: Sanchez-Palomera (1951); 9: Shylaja and Peter (2004); 10: Thompson Coon and Ernst (2002).

10.2.1 Experimental assays

The digestive stimulant action of spices has been examined in animal and human studies. In animal studies, the effect of spices on bile secretion has been examined in the laboratory using experimental rats. In these animal models, bile was systematically collected following the spice treatment and the influence of spices was examined as a result of both continued intake through the diet for a period of time and as a one-time exposure orally (Platel and Srinivasan, 2004). In human studies, patients with functional dyspepsia or other gastric disorders assessed their symptom intensity during and after a treatment period of receiving a certain spice (Thompson Coon and Ernst, 2002; Bortolotti *et al*., 2002; Lee *et al*., 2004).

10.2.2 Mechanisms of action – active compounds

Many of the herbs and spices such as red pepper, ginger, gentian, capsicum, black pepper and mustard, act as digestive stimulants and help in digestion by enhancing the secretion of saliva and the activity of salivary amylase in humans, thus stimulating gastric secretions (Glatzel, 1968; Blumenthal, 1988). Others have reported that paprika, black pepper and cinnamon increased the acid secretion while mustard, celery, nutmeg and sage did not have any such effect (Sanchez-Palomera, 1951). Among all the spices, onion has been reported to have a favourable influence on most digestive enzymes of both the pancreas and small intestine. It has been noted also that the stimulatory influence of the component spices of the spice mixes on digestive enzymes of the pancreas and small intestine is not additive (Platel *et al*., 2002). Curcumin, capsaicin (the active principles of turmeric, red pepper) ginger and fenugreek, onion, mint, cumin, fennel and ajowan, also stimulate bile acid production by the liver and its secretion into bile (Bhat *et al*., 1984, 1985; Platel and Srinivasan 2001a; Sambaiah and Srinivasan, 1991; Srinivasan, 2005).

The analgesic properties of capsaicin have been known for more than a century. Capsaicin (the red pepper is used in functional dyspepsia) can impair selectively the activity of nociceptive C-type fibres carrying pain sensations to the central nervous system (Lynn, 1990; Holzer, 1991).

Many herbal extracts used in medicine alter gastric motility in a dose-dependent and region-specific manner not only in the stomach but in all segments of the gastrointestinal tract (Hohenester *et al*., 2004). The improvement in gastric motility is crucial for the pathogenesis of dyspeptic symptoms and may be specifically useful in patients suffering from dysmotility-like functional dyspepsia. Other herbs referred to that improve the symptoms of non-ulcer dyspepsia, such as turmeric, greater celandine, peppermint, caraway, have a direct antispasmolytic action on smooth muscle or inhibit smooth muscle contraction (Forster *et al*., 1980; Hills and Aaronson, 1991).

Extensive animal studies have revealed that generally the mechanism of digestive stimulant action of most spices is mediated through stimulation of bile secretion with an enhanced bile acid concentration (ingredients essential for fat digestion and adsorption). This activity is usually followed by an appropriate stimulation of the activities of digestive enzymes of pancreas and small intestinal mucosa – lipase, amylase and proteases, disaccharidases, alkaline phosphatase, which play a crucial role in digestion (Sharathchandra *et al*., 1995; Platel and Srinivasan, 1996, 2001a,b; Srinivasan, 2005). Concomitant with such a stimulation of either bile secretion or activity of digestive enzymes by spices, leading to an accelerating digestion, a reduction

in food transit time in the gastrointestinal tract has also been observed (Platel and Srinivasan, 2001b). Indeed, Platel and Srinivasan, (2004) record that all spices except fenugreek and mustard shortened the food transit time. This reduction was more prominent for ginger, ajowan, cumin, piperine, coriander and asafetida. They found that this reduction in food transit time could probably be attributed to acceleration in the overall digestive process as a result of increased availability of digestive enzymes and of bile acids that facilitate fat digestion. The reduction in the whole gut transit time caused by dietary spices probably reflects a short, post-absorptive colonic phase, which is the longest phase of food transit, rather than that of mouth to caecum transit phase. A reduction in colonic transit time reduces the risk and incidence of colon cancer. Thus by reducing food transit time, spices may play a role in the prevention of colon cancer besides combating constipation.

10.3 The effects of herbs and spices on enteric bacterial pathogens

Many herbs and spices are well established as antimicrobials (Wilkins and Board, 1989; Nychas and Tassou, 2000; Tassou *et al*., 2004). They possess a wide spectrum of activity against bacteria, fungi and mycobacteria with Gram (+) being more sensitive than Gram (–). This chapter will focus on the antimicrobial activity of herbs and spices against pathogenic bacteria related to the gastrointestinal system such as *Helicobacter pylori, Clostridium perfringens, Escherichia coli* O157:H7*, Salmonella enterica, Yersinia enterocolitica, Vibrio parahaemolyticus* (Table 10.2). Consumption of living organisms from virulent strains usually causes food-borne gastrointestinal infections. The symptoms of gastroenteritis vary depending on the virulence of the strain and the number of infective bacteria or the production of toxin. Bacteria adhere to and commonly penetrate through the epithelium of intestines. Essential oils from plants have been used traditionally for the prevention and therapy of enteric tract infections, especially common diarrhoea.

Helicobacter pylori infection has been associated with upper gastrointestinal diseases, such as chronic gastritis, peptic ulcer and gastric cancer (Warren, 1983; Marshall and Warren, 1984; Parsonnet *et al*., 1991). The antimicrobial activity of certain herbs and spices against *H. pylori* has been well investigated (Table 10.2). Indeed, the antibacterial effect of crude garlic extracts against *H. pylori* has been demonstrated (Sivam *et al*., 1997; Ohta *et al*., 1999). Other essential oils bactericidal to *H. pylori* in *in vitro* and *in vivo* studies were the oils of cypress, juniper, tea tree, lemongrass, lemon verbena, basil, peppermint, marjoram sweet, eucalyptus, ravensara, lavender, lemon, rosemary (Ohno *et al*., 2003). The essential oil of the Japanese herb wasabi (*Wasabia japonica*, used as a spice in traditional Japanese foods such as sashimi and sushi) has strong antimicrobial effects against *H. pylori* (Shin *et al.,* 2004). Thyme and cinnamon extract also inhibited *Helicobacter pylori* at the concentration range of common antibiotics (Tabak *et al*., 1996, 1999). Hydrolyzable tannins from various medicinal plants showed promising antibacterial activity against it (Funatogawa *et al*., 2004) as well as polymeric phenolics of soybean extracts (McCue *et al*., 2004). Katsuhiro *et al*. (1999) reported that the minimum inhibitory concentration (MIC) of epigallocatechin gallate of green tea against *H. pylori* was 32 μg/ml and MBC was 128 μg/ml. This catechin showed the strongest activity of the six tea catechins tested *in vitro* and in animal studies that was pH dependent (Mabe *et al*., 1999).

Table 10.2 Herbs, plant substances and spices with antimicrobial activity on intestinal pathogens

	Organism	Reference
Garlic, lemon grass, lemon verbena, cypress, juniper, tea tree, lemongrass, lemon verbena, basil, peppermint, marjoram sweet, eucalyptus, ravensara, lavender, lemon, rosemary, soyabean, green tea, thyme, cinnamon	*H. pylori*	Sivam *et al*., (1997); Ohta *et al*., (1999); Ohno *et al*., (2003); Funatogawa *et al*., (2004); McCue *et al*., (2004); Katsuhiro *et al*. (1999); Mabe *et al*. (1999); Tabak *et al*., (1996, 1999)
cinnamon extract, papaya	*H. pylori E. coli, Pseud. aeruginosa, Enteroc. faecalis, Staph. aureus, Staph. epidermis, Kl. pneumoniae, Salmonella* sp., *V. parahaemolyticus, B. subtilis, Ent. cloacae, Salm. typhi, Proteus vulgaris*	Osato *et al.,* (1993) Chang *et al*. (2001)
Olive, mint, carob, mastic gum	*Bacillus cereus, Staph. aureus, Salm. Enteritidis, L. monocytogenes*	Nychas *et al.,* (1990); Tassou, (1993); Tassou *et al*., (1991, 2000); Tassou and Nychas, (1994, 1995a)
Wasabi	*H. pylori, E. coli, Salm. typhimurium, Pseud. aeruginosa Staph. aureus, B. cereus*	Tabak *et al*. (1999); Inoue *et al.* (1983), Shin *et al*. (2004), Masuda *et al*. (2004)
Black pepper, clove, geranium, nutmeg, oregano, thyme, allspice, basil, rosemary, marjoram	*Ent. faecalis, E. coli, Salm. pullorum, Staph. aureus, Y. enterocolitica, Salm. enterica var typhimurium, Cl. perfringens, Cl. sporogenes, E. coli* O157: H7*, Shigella flexneri, Shigella sonnei*	Dorman and Deans, (2000); Briozzo *et al*. (1988); Helander *et al*. (1998); Kim *et al*. (1995b); Paster *et al*. (1990), Dorman and Deans (2000); Juven *et al*. (1994), Cosentino *et al*. (1999); Skandamis *et al*., (2001, 2002); Bagamboula *et al*., (2003)
Curcuma longa, Artemisia princeps var. *orientalis*	*Cl. septicum, Cl. novyi, Cl. sporogenes, Cl. perfringens, Staph. aureus, Bacteroides fragilis*	Lutomski *et al*., (1974); Cho *et al*., (2003)
Acacia catechu, Holarrhena antidysenterica, Peltophorum pterocarpum, Psidium guajana, Punica granatum, Querqus infectoria, Uncaria gambir, Walsura robusta	*E. coli* O157:H7	Voravuthikunchai *et al*. (2004); Prashanth *et al*. (2001); Nimri *et al*. (1999)
Satureja montana	*E. coli, Plesiomonas shigelloides, Shigella flexneri, Salm. enterica ser. typhimurium, Y. enterocolitica, V. parahaemolyticus*	Skocibusic and Bezic (2003)
Panax ginseng, Thea sinensis	*Cl. perfringens, Cl. difficile Clostridium spp.*	Ahn *et al*. (1990a), (1991)

Cinnamon oil and its constituents (cinnamaledehyde and eugenol) have shown antimicrobial activity against *Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis, Staphylococcus aureus, Staphylococcus epidermis, Klebsiella pneumoniae, Salmonella* sp., and *Vibrio parahaemolyticus* (Chang *et al*., 2001). The volatile oils of black pepper, clove, geranium, nutmeg, oregano and thyme – all of them containing carvacrol – were effective against *Enterococcus faecalis, Escherichia coli, Salm. pullorum, Staph. aureus, Yersinia enterocolitica* with the essential oil of thyme being the strongest inhibitor (Dorman and Deans, 2000). Mint oil was bactericidal on *Staph. auerus, Salm. enteritidis* and *L. monocytogenes* (Tassou *et al*., 1995, 2000). Helander *et al*. (1998) have shown that carvacrol and thymol inhibited *E. coli* O157:H7 and *Salm*. enterica serovar *typhimurium* at MIC 3mM and 1mM respectively while Kim *et al*. (1995a, b) found that 1.5% carvacrol was necessary to kill the pathogen.

Clove oil with its active principle eugenol inactivates *Cl. perfingens* and other bacteria (Briozzo *et al*., 1988). Antibacterial effects have been reported for oregano, black pepper, clove, thyme and the essential oil components thymol, carvacrol and eugenol against *Cl. sporogenes* (Paster *et al*., 1990; Dorman and Deans, 2000) and other bacteria such as *E. coli, Staph. aureus* and *Salm.* enterica ser. *typhimurium* (Juven *et al*., 1994; Cosentino *et al*., 1999). The alcohol extract and the essential oil from *Curcuma longa* inhibit the growth of *Cl. septicum, Cl. novyi* and *Cl. sporogenes* (Lutomski *et al*., 1974). However, all these tests were performed *in vitro* with only a limited number of tests performed in animals (Losa and Kohler, 2001).

Olive extract and its active compound oleuropein has also been proved to be antimicrobial against pathogens such as *Bacillus cereus, Staph. aureus, Salm. enteritidis, L. monocytogenes* (Nychas *et al.,* 1990; Tassou *et al.,* 1991, 2000; Tranter *et al.,* 1993; Tassou and Nychas, 1994, 1995a).

Plant extracts of *Acacia catechu, Holarrhena antidysenterica, Peltophorum pterocarpum, Psidium guajava, Punica granatum, Querqus infectoria, Uncaria gambir* and *Walsura robusta* demonstrated antibacterial activity against six strains of *E. coli* O157:H7 with *Querqus infectoria* being the most active (Voravuthikunchai *et al*., 2004). The antibacterial effect of *Satureja montana* L. (Lamiaceae) aromatic plant and spice exhibited on important enteric bacterial pathogens, diarrhoeagenic *E. coli, Plesiomonas shigelloides, Shigella flexneri, Salm. Enterica* serovar *typhimurium, Yersinia enterocolitica* and *Vibrio parahaemolyticus* (Skocibusic and Bezic, 2003). Maximum activity was observed against *Shigella flexneri* and *E. coli. Shigella flexneri* is an important enteropathogen which causes a distinctive and complex disease, bacillary dysentery, caused by invasion of the epithelial cells. The antimicrobial properties of *Shigella flexneri* and *Shigella sonnei* are also possessed by cloves, thyme, oregano, allspice, basil, rosemary and marjoram (Bagamboula *et al.,* 2003).

Extracts of ginseng (*Panax ginseng*) roots and green tea (*Thea sinensis*) leaves have been shown not only to enhance the growth of bifidobacteria but also to selectively inhibit various clostridia (Ahn *et al*., 1990a, 1991). Recent *in vivo* investigations using human volunteers have shown that intake of ginseng extract or green-tea derived polyphenols favourably affected faecal microbiota (Ahn *et al*. 1990b; Okubo *et al*., 1992).

10.3.1 Experimental assays

The experimental assays for testing the antimicrobial activity described in the literature include:

- The paper disc agar diffusion method with measurement of the radius or diameter of the zone of inhibition of bacterial growth around paper discs impregnated with (or wells containing) an antimicrobial compound on agar media.
- Broth or Agar dilution assays with measurement of the inhibition of bacterial growth in broth or agar medium.
- Agar or broth microdilution method and agar dilution method for MIC and MBC.
- Measurement of changes in optical density or impedance in a liquid growth medium containing the antimicrobial compound.

In vivo studies using animal models were conducted for the assessment of the inhibitory activity of plant essential oils against *H. pylori.*

10.3.2 Mechanisms of action – active compounds

The antimicrobial activity of herbs and spices is attributed mainly to their phenolic constituents and/or essential oil fraction (Table 10.3). Phenolics and polyphenols could be simple phenolic acids, quinones, flavones, tannins and coumarins (Cowan, 1999). The phenolic compounds carvacrol and thymol present in the essential oil from oregano and thyme exhibit considerable antimicrobial and antifungicidal activity (Basilico and Basilico, 1999). Carvacrol, occuring in the volatile oils of black pepper, clove, geranium, nutmeg, oregano and thyme has been found to be the component with the widest spectrum of activity (Dorman and Deans, 2000). Phenols such as thymol and carvacrol and their methyl ethers are also the main components of the essential oil of *Satureja montana* (Skocibusic and Bezic, 2003).

Mainly responsible for the bactericidal action of the Japanese herb wasabi is the component allyl isothiocyanate. This has been shown to inhibit *Vibrio parahaemolyticus* (Hasegawa *et al*., 1999; Shin *et al*., 2004). Katsuhiro *et al*. (1999), reported catechins as active compounds in teas with the epigallocatechin gallate of green tea being the catechin with the strongest activity against *H. pylori.* Epigallocatechin gallate and

Table 10.3 Herbs, plants and spices and their active constituents

Herb/spice	Active compound	Herb/spice	Active compound
Allspice	eugenol, methyl eugenol	Olive	oleuropein
Berries	ellagitannins, anthocyanins, hydroxycinnamic acids, flavonols, lignans	Oregano	thymol, carvacrol
Caraway	carvone	Pepper	monoterpenes
Cinnamon	cinnamaldehyde, eugenol	Thyme	thymol, carvacrol, menthol, menthone
Clove	eugenol, eugenol acetate	Wasabi	allyl isothiocyanate
Garlic	diallyl disulphate, diallyl trisulphide, allyl propyl disulphide	*Artemisia princeps* var. *orientalis*	seco-tanapartholides A and B
Green tea	epigallocatechin gallate, gallocatechin gallate	*Punica granatum*	tannins
Mint	α,β-pinene, limonene, 1,8-cineole	*Satureja montana*	thymol, carvacrol

Data from: Cho *et al*., (2003); Hasegawa *et al*., (1999); Nychas *et al*., (2003); Puupponen-Pimia *et al*., (2001, 2005); Shin *et al*., (2004); Skocibusic and Bezic, (2003); Sugita-Konishi *et al*., (1999).

gallocatechin gallate in green tea catechins inhibited extracellular release of verocytotoxin from *E. coli* O157:H7 (Sugita-Konishi *et al*., 1999). Moreover Ahn *et al*. (1991), testing the polyphenols of *Thea sinensis* against *Cl. perfringens* and *Cl. difficile,* found that the gallate moiety of polyphenols seems to be required for growth inhibiting activity.

Phenolic extracts of berries containing ellagitannins, anthocyanins, hydroxycinnamic acids and flavonols, lignans were inhibitory to intestinal Gram (–) pathogens *Salmonella, E. coli, Staphylococcus aureus* (Puupponen-Pimia *et al*., 2001, 2005). Ellagitannins (esters of hexahydroxydiphenic acid, which is a dimeric derivative of gallic acid and a polyol, glucose or quinic acid) were shown to be strong inhibitory compounds on *St. aureus*. Phenolic compounds were only partially responsible for the growth inhibition of *Salmonella* and most of the antimicrobial effects probably originate from other compounds such as organic acids, citric, malic, benzoic acid (Puupponen-Pimia *et al*., 2005). Wen *et al*., (2003) reported that phenolic acids such as hydroxycinnamic acids, exhibited antibacterial activity against several strains of *L. monocytogenes*. Tannins have been reported in general to be bacteriostatic and/or bactericidal for many disease-associated bacteria (Toda *et al*., 1989; Scalbert, 1991; Hussein *et al.,* 1997; Chung *et al*., 1993; Cowan, 1999; Djipa *et al.,* 2000). Chung *et al.,* (1993) demonstrated also that inhibitory effects against a variety of foodborne bacteria, such as *St. aureus, S. enteritidis, S. paratyphi and E. coli* were associated with the ester linkage between gallic acid and polyols. *Punica granatum* also possesses high amount of tannins.

The seco-tanapartholides A and B, active constituents of the *Artemisia princeps* var. *orientalis*, produced a clear inhibitory effect on human intestinal bacteria *C. perfringens*, *Bacteroides fragilis* and *Staph. aureus* without any adverse effects on lactic-acid producing bacteria (Cho *et al*., 2003).

The antibacterial activities of catechins were predominantly related to the gallic acid moiety and the number of hydroxyl groups. It has also been reported that catechins damage the membrane lipid layer (Ikigai *et al*., 1993). Catechins probably damage the membrane of *H. pylori* and epigallocatechin gallate inhibits the urease activity and motility of *H. pylori* which may contribute to its antibacterial activity *in vivo* (Mabe *et al*., 1999). Catechins act bactericidally at high pH while essential oils may show antibacterial activity in the stomach because they are more effective at lower pH (Ohno *et al*., 2003).

Different mechanisms of action proposed to explain tannin antimicrobial activity including inhibition of extracellular microbial enzymes, deprivation of the substrates required for microbial growth or direct action on microbial metabolism through inhibition of oxidative phosphorylation. Complexation of metal ions by tannins could also be a possible mechanism (Scalbert, 1991).

The antibacterial properties of cranberry may be associated with inhibition of *E. coli* adherence to mucosal surfaces by cranberry juice (Schmidt and Sobota, 1988). It has been suggested that proanthocyanidins (condensed tannins) are responsible for this anti-adhesion property (Howell, 2002; Howell *et al*., 1998). Studies with mice fed with cranberry proanthocyanidins showed that properties of the urine may be altered by the proanthocyanidins in such a way that adhesion is inhibited (Howell *et al*., 2001). Another hypothesis is that metabolites of proanthocyanidins could act on the colonic bacterial receptors making them incapable of binding to the uroepithelium and proliferate (Harmand and Blanquet, 1978). Burger *et al*. (2002) reported that a high molecular weight constituent of cranberry juice inhibited adhesion of *Helicobacter*

pylori to immobilized human mucus, erythrocytes and cultured gastric epithelial cells. They suggested that cranberry juice may also inhibit adhesion of bacteria to the stomach *in vivo*, and may be useful for the prevention of stomach ulcers caused by *H. pylori*.

Gram positive bacteria are more susceptible to essential oils than gram negatives (Dabbah *et al*., 1970; Farag *et al*., 1989; Shelef, 1983; Tassou and Nychas, 1995b,c; Smith-Palmer *et al*., 1998). The tolerance of Gram negative bacteria to oils from spices has been ascribed to the presence of a hydrophilic outer membrane that blocked the penetration of hydrophobic essential oils to the target cell membrane (Mann *et al*., 2000).

Generally, essential oils of herbs and spices damage the structural and functional properties of membranes and this is reflected in 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). Thymol and carvacrol, active components of many essential oils, disrupt the membrane integrity, which further affects pH homeostasis and equilibrium of inorganic ions (Helander *et al.*, 1998; Lambert *et al*., 2001). Disruption of membrane causes leakage of ions, ATP, nucleic acids, and amino acids (Tranter *et al*., 1993; Cox *et al*., 1998; Ultee *et al.*, 1999; Tassou *et al.,* 2000). Nutrient uptake, nucleic acid synthesis and ATPase activity may also be affected, leading to further damage of the cell (Denyer and Hugo, 1991).

10.4 Herbs and spices as growth promoters in animal studies

The combination of the properties described above of herbs and spices (effects on digestibility and antimicrobial activity) has found application also in the feeding of animals such as pigs, chickens, sows as growth promoters and antibiotic replacements. Indeed, herbs, spices and various plant extracts have received increased attention as possible antibiotic growth promoter replacements (Table 10.4). Plant extract supplementation (essential oil extract from oregano, cinnamon, pepper and extract from sage, thyme, rosemary) has been shown to improve apparent whole-tract and ileal digestibility of the feeds for broilers (Hernandez *et al*., 2004). The above plant extracts fed to broilers showed little growth promoter effect and live performance levels similar to an antibiotic growth promoter (Hernandez *et al*., 2004). Jamroz and Kamel (2002) observed improvements of 8.1% in daily gain and 7.7% in feed

Table 10.4 Herbs, spices and their constituents used as growth promoters and antibiotic replacements in the feeding of animals

Oregano (carvacrol)[3,5,6,7,9]
Cinnamon (cinnamaldehyde)[5,6,7,9]
Pepper (capsaicin, capsicum oleoresin)[5,6,7]
Sage[5]
Thyme[5,9]
Rosemary (rosmarinic acid, flavones, monoterpenes)[4,5]
blends of essential oils[1,2,8]

1: Alcicek *et al*. (2003), 2: Alcicek *et al*. (2004); 3: Botsoglou *et al*. (2002); 4: Debersac *et al.* (2001); 5: Hernandez *et al*. (2004); 6: Jamroz and Kamel (2002); 7: Manzanilla *et al*. (2004); 8: Mitsch *et al*. (2004); 9: Namkung *et al*. (2004).

conversion ratios in 17-day-old poults fed a diet supplemented with a plant extract containing capsaicin, cinnamaldehyde and carvacrol at 300 ppm. Water-soluble extract from rosemary, containing rosmarinic acid, flavones and monoterpenes, enhanced hepatic metabolism and increased relative liver weight in rats (Debersac *et al.*, 2001).

An essential oil combination derived from herbs growing wild in Turkey, was found to have a beneficial effect on body weight, feed intake, feed conversion ratio and carcass yield when used as a feed additive of broiler chickens (Alcicek *et al*., 2003, 2004). The incorporation of carvacrol, cinnamaldehyde and capsicum oleoresin promotes changes in the digestive function and microbial ecology (Manzanilla *et al*., 2004) while herbal extract containing cinnamon, thyme and oregano extract reduced the proliferation of coliform bacteria of weaned pigs (Namkung *et al*., 2004). It has been reported also that blends of essential oil components can control *Clostridium perfringens* (causative agent of necrotic enteritis) colonization and proliferation in the gut of broilers (Mitsch *et al*., 2004).

On the other hand there are some contradictory results about the effectiveness of certain herbs and extracts as growth promoters. It has been reported by Botsoglou *et al*., (2002) that oregano oil exerted no growth-promoting effect when administered at 50 or 100 mg/kg of feed. Others have found that an essential oil mixture and thymol and cinnamaldehyde did not stimulate growth performance in broiler chickens. They attributed this to the composition of the basal diet (highly digestible) and/or the environmental conditions (Lee *et al*., 2003a). Dietary thymol and its isomer carvacrol did not affect growth performance and did not show hypocholesterolemic activity when used as alternatives to antibiotic feed additives in broiler chickens (Lee *et al*., 2003b).

10.4.1 Experimental assays

The experimental assay usually includes modification of the feeding programme of broilers for some days by supplementation of their basal diet with essential oil extract or their constituents. For feed intake, the feed:gain ratio per pen is measured throughout the experiment (Hernandez *et al.*, 2004). At the end of the experiment, the weights of the proventriculus, gizzard, small and large intestines without content, pancreas and liver without gall bladder are measured individually. Diet, excreta and ileal digesta are analyzed for nitrogen, dry matter and acid insoluble ash. Diet and excreta are analyzed for lipid and diets and ileal digesta are analyzed for starch. The effects of additives on performance, digestibility and organ size are analyzed statistically (Hernandez *et al.*, 2004).

10.4.2 Mechanisms of action – active compounds

Plant extracts contain different molecules that have intrinsic bioactivities on animal physiology and metabolism. The mechanisms by which these products influence the gut microflora and growth performance of animals are not elucidated. As antibiotics, plant extracts could control and limit the growth and colonization of numerous pathogenic and nonpathogenic species of bacteria in the gut as described in the previous part of this chapter. Their possible mechanisms of action are also discussed above. There is evidence to suggest that herbs, spices and various plant extracts have appetite- and digestion-stimulating properties, as is also discussed in this chapter

(Kamel, 2001). Their effects as growth promoters in animals may be due to the greater efficiency in the utilization of feed, resulting in enhanced growth. Essential oils have been shown to increase digestive enzyme activities of the pancreas and intestinal mucosa (pancreatic trypsin and alpha-amylase), thus leading to an increase in the growth performance of broiler chickens (Jang *et al.,* 2004).

10.5 Anti-inflammatory activity

Various plant extracts have been known to possess anti-inflammatory properties since ancient times (Table 10.5). Aloe vera, for example, has been applied topically by ancient and modern cultures throughout the world for its anti-inflammatory properties for the treatment of a range of inflammatory digestive and skin diseases including inflammatory bowel disease (Langmead *et al*., 2002). Aloe vera gel is the mucilaginous aqueous extract of the leaf pulp of *Aloe barbadensis*. It contains over 70 biologically active compounds and has been claimed to have anti-inflammatory, antioxidant, immune boosting, anti-cancer, healing, anti-ageing and anti-diabetic properties (Grindlay and Reynolds, 1986).

Slippery elm bark from the slippery elm, or red elm tree native to North America, is also claimed to have 'soothing' properties in inflammation of the gastrointestinal tract. It is popular among inflammatory bowel disease patients in the UK (Langmead *et al*., 2000). Fenugreek is an aromatic herb that has a beneficial effect on inflamed intestines. Mexican yam is a tropical perennial whose starch-rich tuberized root is a food staple and used for the treatment of menstrual irregularities as well as joint and gut inflammation. Devil's claw (*Harpagophytum procumbens*) is a flowering plant

Table 10.5 Herbs, plant substances and spices with anti-inflammatory, anticancer or protective properties

Aloe vera (*Aloe barbadensis*)	Gingo biloba
Angelica root	*Franseria artemisioides*
Baccharis rubricaulis	Lemon balm leaves
B. genistelloides	Licorice root
Basil	*Mammea americana*
Bitter candytuft	Mexican yam
Black pepper (piperine)	Milk thistle fruit
Caraway fruit	Papaya (*Carica papaya* L.)
Celandine herbs	Peppermint leaves
Chamomile flower	*Phoradendron crassifolium,*
Clove (eugenol)	Red pepper (capsaicin)
Coriander (linalool)	Saffron (*Crocus sativus*)
Cumin (cuminaldehyde)	*Satureja hortensis* L.
Curcumin	Slippery elm bark
Devil's claw	Tormentil
Fenugreek	Turmeric
Ganoderma (G) *lucidum*	Wei tong ning
Ginger (zingerone)	

Based on Abdullaev, 2002; Abdel-Salam *et al*., (1995); Abdel-Salam *et al*., (2004); Aruna and Sivaramakrishnan, (1992); Bin-Hafeez *et al*., (2003); Cheng *et al*., (1982, 1985); Gonzales *et al*., (2000); Ha, (2003); Hajhashemi *et al.,* (2000); Langmead and Rampton, (2001); Langmead *et al*., (2002); Madisch *et al*., (2004); Mozsick *et al*., (1997, 1999); Osato *et al*., (1993); Sharma *et al*., (2005); Srinivasan, (2005); Szolcsanyi and Bartho, (1981); Toma *et al*., (2005); Yeoh *et al*., (1995).

native to southern Africa with anti-inflammatory properties while tormentil (*Potentilla tormentilla*) is a member of the rose family and is said to be effective in the treatment of diarrhoea and bowel inflammation. Wei tong ning is a Chinese herb used for treatment of patients with peptic ulceration and other intestinal inflammatory disorders (Langmead *et al*., 2002). Gingo biloba extract has a remarkable anti-inflammatory effect in rats (Abdel-Salam *et al*., 2004). Commercial preparations also containing bitter candytuft, chamomile flower, peppermint leaves, caraway fruit, licorice root, lemon balm leaves, celandine herbs, angelica root and milk thistle fruit are effective in alleviating irritable bowel syndrome symptoms (Madisch *et al*., 2004).

The anti-inflammatory activity of herbs and spices in most cases is attributed to the antioxidant properties of their components. Measuring the lipid peroxidation, curcumin, capsaicin and eugenol were found to be more effective antioxidants, while piperine (black pepper), zingerone (ginger), linalool (coriander) and cuminaldehyde (cumin) were only marginally inhibitory to lipid peroxidation (Reddy and Lokesh, 1992). Indeed curcumin, the polyphenol of dietary spice turmeric, posseses diverse anti-inflammatory properties due to its antioxidant capacity at neutral and acidic pH (Sharma *et al.,* 2005).

Other herbal treatments investigated for efficacy in peptic ulcer disease are capsaicin/chilli and mastic. The pungent ingredient of chilli, capsaicin is thought to have effects on substance P release and has been tested for its efficacy in peptic ulcer patients. Another ingredient of curry, *Curcuma domestica* val, was tested for its efficacy in dyspepsia while mastic, the resin of the mastic or lentisc tree, was effective for ulcer healing. The most researched herbal treatment for liver diseases is milk thistle. Its active constituents are collectively known as silymarin (Langmead and Rampton, 2001).

10.5.1 Experimental assays

The experimental assay for assessing anti-oxidant activity is conducted *in vitro* in two cell-free, radical-generating systems and by the chemiluminescence of incubated colorectal mucosal biopsies (or mucosal biopsy assay systems). Eicosanoid production by biopsies and interleukin-8 release by CaCo2 epithelial cells in the presence of the extract is measured by enzyme-linked immunosorbent assay (Langmead *et al.*, 2004). Studies include also *in vivo* clinical trials (Madisch *et al*., 2004) in irritable bowel syndrome patients. The antioxidant properties of several spice principles were investigated in rats by measuring the lipid peroxidation induced both *in vivo* and *in vitro* (Joe and Lokesh, 1994; Reddy and Lokesh, 1992, 1994a,b,c,d).

10.5.2 Mechanisms of action – active compounds

Although the pathogenesis of inflammatory bowel disease has not been clearly elucidated, the over-production by the involved colorectal mucosa of reactive oxygen metabolites (Grisham, 1994; Simmonds and Rampton, 1993), eicosanoids (Rampton and Hawkey, 1984) and the chemo-attractant chemokine, interleukin-8 (Gibson and Rosella, 1995; Daig *et al.*, 1996; Keshavarzian *et al.*, 1999), is likely to play a contributory role. The anti-inflammatory activity of herbs and spices in most cases is attributed to the antioxidant properties of their phenolic constituents. Prevention of the activity of radicals after their generation and release can occur as a result of scavenging by antioxidants. *In vitro* studies (Langmead *et al*., 2004) have attributed

the anti-inflammatory activity of aloe vera to its antioxidant properties and inhibitory effects on colorectal prostaglandin E2 and interleukin-8 production. The activity of devil's claw and tormentil is also attributed to flavonoids and tannins respectively which are proven free-radical scavengers while fenugreek and Mexican yam contain steroidal saponins which might be able to influence the local inflammatory response (Vennat *et al*., 1994; Bos *et al.*, 1996; Langmead *et al*., 2002). The effect of commercial preparations of bitter candytuft, chamomile flower, peppermint leaves, caraway fruit, liquorice root, lemon balm leaves, celandine herbs, angelica root and milk thistle fruit may be potentially mediated via their influence on gastrointestinal motility (Okpanyi *et al*., 1993) possibly via 5-hydroxytryptamine (5-HT) pathways (Simmen *et al*., 2003; Madisch *et al*., 2004). Protection of target tissues from radical attack from the lumen of the intestine could also be a result of enhancement of physico-chemical barriers, for example, by increased mucus production (Langmead *et al*., 2002).

The antioxidant effect on lipid peroxidation of several compounds such as curcumin, capsaicin, eugenol, piperine, zingerone, linalool and cuminaldehyde is exerted by quenching oxygen free radicals and by enhancing the activity of endogenous antioxidant enzymes – superoxide dismutase, catalase glutathione peroxidase and glutathione transferase (Srinivasan, 2005).

10.6 Effect on gut immunity

Additionally protective effects have been attributed to many herbs and spices and strengthening of the gut immune system (Table 10.5). Red pepper seems to display a protective effect on the gastric mucosa (Yeoh *et al*., 1995). Small doses of their active compound capsaicin have beneficial (protective) effects against different noxious agents in the stomach in animal models (Szolcsanyi and Bartho, 1981; Langmead and Rampton, 2001). Abdel-Salam *et al*., (1995) indicated that small doses of capsaicin inhibit gastric acid secretion and prevent the gastric mucosal damage produced by different acid- and non-acid-dependent gastric mucosal damaging agents (Mozsik *et al*., 1997). Results on humans showed that small doses of capsaicin inhibit gastric basal acid output via stimulation of the inhibition of capsaicin sensitive afferent nerves (Mozsik *et al*., 1999).

Ganoderma (G) *lucidum*, a traditional Chinese herbal medicine that is popular as a food supplement in Asia is believed to enhance the immune system (Ha, 2003) and to promote longevity (Shiao *et al*., 1994). Antitumour activities are the most notably stimulatory effect on animals subjected to either oral administration (Cheng *et al*., 1985), subcutaneous (Cheng *et al*., 1982), or intraperitoneal (i.p.) injection (Song *et al*., 1985) of the hot water extracts of *G. lucidum*. *G. lucidum* may have potential immuno-modulating effects in patients with advanced colorectal cancer (Chen *et al.,* 2005). Immunomodulatory activity has possessed also been recorded for fenugreek (*Trigonella foenum graecum* L.) on mice. Plant extract elicited a significant increase in phagocytic index and phagocytic capacity of macrophages (Bin-Hafeez *et al*., 2003).

The anticarcinogenic properties of basil and cumin were tested on the induction of squamous cell carcinomas in the stomach of Swiss mice and on induction of hepatomas in Wistar rats and it was found that they significantly decreased the incidence of both neoplasia and hepatomas (Aruna and Sivaramakrishnan, 1992).

Cytoprotective effects on gastric mucosa were shown by extracts of Bolivian plants. The highest cytoprotective activity was exerted by the aqueous extract of *Phoradendron crassifolium, Franseria artemisioides*, the hexane extract of *Baccharis rubricaulis* and the dichloromethane extract of *F. artemisioides.* Other interesting results were obtained with the extracts of *B. genistelloides* (Gonzales *et al*., 2000). *Mammea americana* L. (Guttiferae) fruit, which is very common in the diet of the northern South American population and used as a tonic and against stomach ache, has been shown to possess excellent antisecretory and/or gastroprotective effect in all gastric ulcer models in mice (Toma *et al*., 2005).

Other herbs and spices with protective and antimutagenic effects are cumin and black pepper (Nalini *et al*., 1998), curcumin (Nagabhushan *et al*., 1987; Langmead and Rampton, 2001; Sharma *et al.,* 2005), diallylsulphide of garlic, (Ip *et al*., 1992), ginger, which is used as a remedy for nausea and vomiting and liquorice and mastic, which has long being recognized as an ulcer-healing agent. Turmeric (*Curcuma longa*) has been found to protect DNA against lipid peroxide induced damage (Shalini and Srinivas, 1987) and against fuel smoke condensate induced damage (Shalini and Srinivas, 1990). Curcumin from turmeric was found to exhibit anti-inflammatory, anti-oxidant and chemopreventive properties (Gao *et al.,* 2004). Saffron (the dark red stigmata of *Crocus sativus* L. flowers), which is used as a spice and food colorant, and its main constituents, the carotenoids, possess tumoricidal and chemopreventive properties against cancer *in vitro* and *in vivo* (Salomi *et al.,* 1991a,b; Nair *et al.,* 1994; Tarantilis *et al.,* 1994; Abdoullaev *et al.,* 2000; Abdoullaev, 2002).

10.6.1 Experimental assays

Cytoprotective activity was determined by the method described by Robert *et al.*, (1979) with rats through the ethanol-induced ulcer model. The number of erosions per stomach was assessed according to the score method described by Marhuenda *et al*., (1993) and Gonzales *et al*. (2000). Various different methods have been used to demonstrate the tumoricidal and anticancer properties of saffron (Salomi *et al.,* 1990; Nair *et al.,* 1991; Abdoullaev and Frenkel, 1992; el Daly, 1998).

10.6.2 Mechanisms of action – active compounds

Different mechanisms of action have been recorded for the effect of certain herbs and substances on the gut immune system. It has been reported that cumin and black pepper may protect the colon by decreasing the activity of β-glucuronidase and mucinase that may liberate drugs and toxins that can be harmful to the coloncytes (Nalini *et al*., 1998). Diallylsulphide (DAS), a major garlic component, has also been shown to have anti-cancer effects (Ip *et al*., 1992). The anti-cancer properties of curcumin are attributed to the inhibition of several cell signalling pathways at multiple levels, to the effects on cellular enzymes such as cyclooxygenase and glutathione S-transferases, to immuno-modulation and effects on angiogenesis and the cell's ability to affect gene transcription and to induce apoptosis in preclinical models (Sharma *et al.,* 2005). Others have shown that mitogen, interleucin-2 or alloantigen induced proliferation of splenic lymphocytes and development of cytotoxic T lymphocytes is significantly suppressed by curcumin (Gao *et al.,* 2004).

Saffron contains three main pharmacologically active compounds: (i) saffron-coloured compounds are crocins, which are unusual water-soluble carotenoids (mono

and diglycosyl esters of a polyene dicarboxylic acid, named crocetin). The digentiobiosyl ester of crocetins α-crocin is the major component of saffron. (b) Picrocrocin is the main substance responsible of the bitter taste in saffron. (c) Safranal is the volatile oil responsible of the characteristic saffron odour and aroma (Abdoullaev, 1993; Rios *et al.,* 1996). Many mechanisms of action have been proposed for the antitumour and anticarcinogenic activity of saffron and its components (Abdoullaev, 2002). Some of the mechanisms involve inhibitory effects on cellular DNA and RNA synthesis, inhibitory effects on free radical chain reactions acting as free-radical scavengers or it has been proposed that the antitumour activity is mediated via lectins or via apoptosis. All the extracts of Bolivian plants that possessed cytoprotective effects contained saponins, flavonoids and tannins; coumarins appeared in some of them (*B. genistelloides* and *S. boliviana*). Several references report that polyphenolic compounds (mainly flavonoids and tannins) have gastroprotective activity (Martin *et al*., 1988; Rainova and Nakov, 1988; Alarcon de la Lastra *et al*., 1992, 1994; Montilva *et al*., 1992, 1993), and some of them present anti-inflammatory activity (Galvez *et al*., 1997; Rao *et al*., 1997).

A crude aqueous extract of *G. lucidum* was effective in enhancing the recovery of leucocyte counts, splenic blastogenic responses and splenic CD4 and CD8 T cells subsets in mice subjected to γ-irradiation (Cheng *et al.,* 1995). The percentage of natural killer cells in blood mononuclear cells increased in human subjects orally administered hot-water extracts from the fruiting body of *G. lucidum* (Cheng *et al*., 1985). The cytotoxic activity of splenic natural killer cells increased in normal and tumour-bearing mice subjected to i.p. injection with an alcohol-insoluble fraction of *G. lucidum* extracts (Won *et al*., 1989). An inhibitory effect of *G. lucidum* on immunity has also been reported. Mice injected intraperitoneally with a protein isolated from *G. lucidum* mycelium exhibited low systemic antibody production against the hepatitis B surface antigen (Kino *et al*., 1991). In addition, methanolic extracts of *G. lucidum* reduced the phytohemaglutinin and 12-O-tetradecanoylphorbol 13-acetate induced cell proliferation in human peripheral blood mononuclear cells exposed to the extracts *in vitro* (Kim *et al*., 1997). Thus, both stimulatory and inhibitory activities of *G. lucidum* on immunity are reported in diverse systems. On the other hand *G. lucidum* mycelium appears to depress mucosal IgA responses in mice when taken by the oral route (Ha, 2003).

10.7 Adverse effects

Many herb and spice extracts are used widely in the food, health and personal care industries and are classified as GRAS substances or are permitted food additives (Kabara, 1991). Herbal remedies are the single most used type of complementary and alternative medicine (Moody *et al*., 1998; Hilsden *et al*., 1998; Langmead *et al*., 2000). Usage is particularly common in patients with irritable bowel syndrome and inflammatory bowel disease (Rawsthorne *et al*., 1999; Smart *et al*., 1986; Moser *et al*., 1996). This may be related to the chronic and refractory nature of these disorders as well as physiological factors (Hilsden *et al*., 1998; Langmead *et al*., 2000; Moser *et al*., 1996). However, the use of a herbal remedy for several thousand years does not guarantee either its efficacy or safety. Contrary to the widespread popular view that because it is natural it is safe, herbal therapy probably carries more risks and produces more serious side-effects than any other form of alternative therapy (Vickers and

Zollman, 1999; Langmead and Rampton, 2001). Interactions between herbs and drugs may have toxic or important pharmacological effects (Penn, 1983; D'Arcy, 1991, 1993; Ernst, 1998; Miller, 1998). Some herbal treatments may interact with drugs used in the treatment of digestive disease, causing toxicity (Table 10.6). For example, liquorice can enhance aldosterone-like effects of prednisolone leading to hypokalaemia and fluid retention (Langmead and Rampton, 2001). St John's wort enhances the activity of cytochrome P450 enzymes thereby increasing the degradation of drugs including cyclosporin (Ernst, 1999; Mai *et al.*, 2000; Obach, 2000). Devil's claw and garlic increase prothrombin time in patients on warfarin, while tamarind increases the bioavailability of aspirin; both effects may lead to gastrointestinal bleeding. It has also been reported that the active principle of *Capsicum annum*, capsaicin, may be mutagenic, carcinogenic and a tumour promoting agent (Nagabhushan and Bhide 1985).

10.8 Future trends

The use of herbal preparations by the general population is still largely unsupported by either efficacy or safety data from clinical trials. The future of using plant and spice extracts as medicines for various diseases associated with the gut or generally, lies on the careful selection and evaluation of their efficacy at low concentrations and in combinations of different components, avoiding any adverse effects on human health. More work needs to be done in this area including human clinical studies in order to establish safe limits for use. Moreover, herbal preparations used for medicinal purposes should require licensing by an independent national body in order to improve their quality and safety and to ensure that claims of efficacy are validated by randomized controlled trials.

On the other hand, there is a major concern nowadays for the emergence and spread of antibiotic-resistant bacteria. This concern has widened to include all microorganisms exposed to antimicrobial agents, including the so-called 'natural' compounds. However, there is relatively little information on the resistance mechanisms

Table 10.6 Herbs and spices used in gastrointestinal diseases that may cause side-effects or interactions with drugs

Herbs/spices	Side effects or interactions
Aloes	Diarrhoea, abdominal cramps
Anise	Nausea, vomiting
Chilli/capsaicin	Cough
Devil's claw	Reduced absorption of iron
Fennel	Nausea, vomiting
Garlic	Potentiation leading to GI bleeding when used with warfarin
Ginseng	Potentiation leading to GI bleeding when used with warfarin
Gingko	Reduced absorption of iron
Ginger	Reduced absorption of iron
Gentian	Nausea, vomiting
Liquorice	Antagonist to spironolactone, hypokalaemia with prednisolone
Parsley	Nausea, vomiting

Data modified from Langmead and Rampton, (2001).

of microorganisms against plant-derived antimicrobial compounds. It has been stated in this chapter that Gram positive bacteria are more sensitive than Gram negatives to the antimicrobial compounds in spices. However, variation in the rate or extent of inhibition is also evident among the Gram-negative bacteria. For example *E. coli* was less resistant than *Pseudomonas fluorescens* or *Serratia marcescens* to essential oils from sage, rosemary, cumin, caraway, clove and thyme (Farag *et al*., 1989). Mutants of *E. coli* and sub-populations of *Staph. aureus* resistant to pine and tea-tree oil, respectively, have also been reported (Moken *et al.,* 1997; Nelson, 2000).

10.9 Sources of further information

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11

Volatiles from herbs and spices

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11.1 Introduction

Plants contain an enormous range of isoprenoid compounds with a wide variety of structures and functions. The majority of isoprenoids are synthesized as secondary metabolites that are uniquely plant products (Bramley 1997). Isoprenoids form an integral part of the volatiles from spices and herbs. Volatile oils are chemically complex mixtures, often containing in excess of 100 individual components. Most oils have one to several major components, which impart the characteristic odour/taste, but the many minor constituents also play their part in producing the final product.

Volatile oils, which are used for culinary, pharmaceutical, and perfumery purposes, are composed of two classes of compound, terpenes and phenyl propenes. Of these, the terpenes are by far the more abundant but phenyl propenes are usually the major flavour/odour factors. The high levels of some of these compounds in turpentine oil gave rise to the alternative generic name 'terpenoid'. Terpenoids are the ingredients of perfumes, soaps, flavourings and food colourants. Terpenes constitute a major group, which contain more than 1000 monoterpenes and 3000 sesquiterpene structures (Waterman 1993).

The development of chromatographic and spectroscopic techniques has led to general understanding of structure, biosynthesis and properties of terpenoids. Terpenoids are built up of isoprene (C_5) units and the nomenclature of the main classes reflects the number of isoprenoid units present (Bramley 1997).

11.2 Classification of volatiles

11.2.1 Terpenes

Terpenes found in volatile oils can be subdivided into monoterpenes, which have a 10-carbon skeleton and sesquiterpenes, which have a 15-carbon skeleton. Diterpenes (20-carbon units) do occur in some oils (e.g. ginger). The feature that binds all these

compounds together is the presence of a 5-carbon building block, which is referred to as the isoprene unit. Table 11.1 illustrates the classes of isoprenoids found in plants. Compositional changes occur in essential oils due to the (i) effect of extrinsic conditions (ii) effect of interspecific and infrastructure differences (iii) effect of ontogeny (iv) effect of processing parameters and (v) effect of adulteration (Chikuenshu and Lawrence 1997).

Monoterpenes
In monoterpenes, it is usually possible to detect the presence of two of these isoprene units and in sesquiterpenes, three. Figure 11.1 depicts the structure of an isoprene unit. Monoterepenes can be divided into three sub groups (i) acyclic, no ring systems (ii) monocyclic, one ring and (iii) bicyclic, two rings (Fig. 11.2). Further proliferation occurs through addition (oxidation) or removal (reduction) of double bonds, and by addition of oxygen to form alcohols (-OH), ketones (-CO), aldehydes (-CHO) and esters (-OCO-).

Sesquiterpenes
Sesquiterpenes, because they possess five more carbons than the monoterpenes, have far greater potential for structural and stereo chemical diversity. Sesquiterpenes form the largest class of terpenoids and are found in plants, liverworts, mosses, fungi and algae. They commonly occur with the monoterpenoids in essential oils. They are less volatile and have less direct organoleptic properties, than monoterpenes. They are an essential part of most volatile oils, subtly influencing odour (Waterman 1993, Bramley 1997).

11.2.2 Phenylpropenes

The skeleton of phenylpropenes invariably consists of a 6-carbon aromatic ring with 3-carbon side chain attached. The side chain always contains a double bond but only

Table 11.1 Main classes of isoprenoids found in plants

Carbon atoms	Name	Parent isoprenoid	Sub-class
10	Monoterpenoids	GPP	Iridoids
15	Sesquiterpenoids	FPP	Abscissic acid, sesquiterpene lactones
20	Diterpenoids	GGPP	Gibberellins
25	Sesterpenoids	GFPP	None
30	Triterpenoids	Squalene	Phytosterols, saponins, cardenolides
40	Tetraterpenoids	Phytoene	None
740	Polyprenols, rubbers	GGPP+ (C_5) n	None

GPP – Geranyl pyrophosphate, FPP – Farnesyl pyrophosphate, GGPP – Geranyl geranyl pyrophosphate, GFPP – Geranyl farnesyl pyrophosphate.

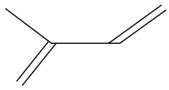


Fig. 11.1 An isoprene unit.

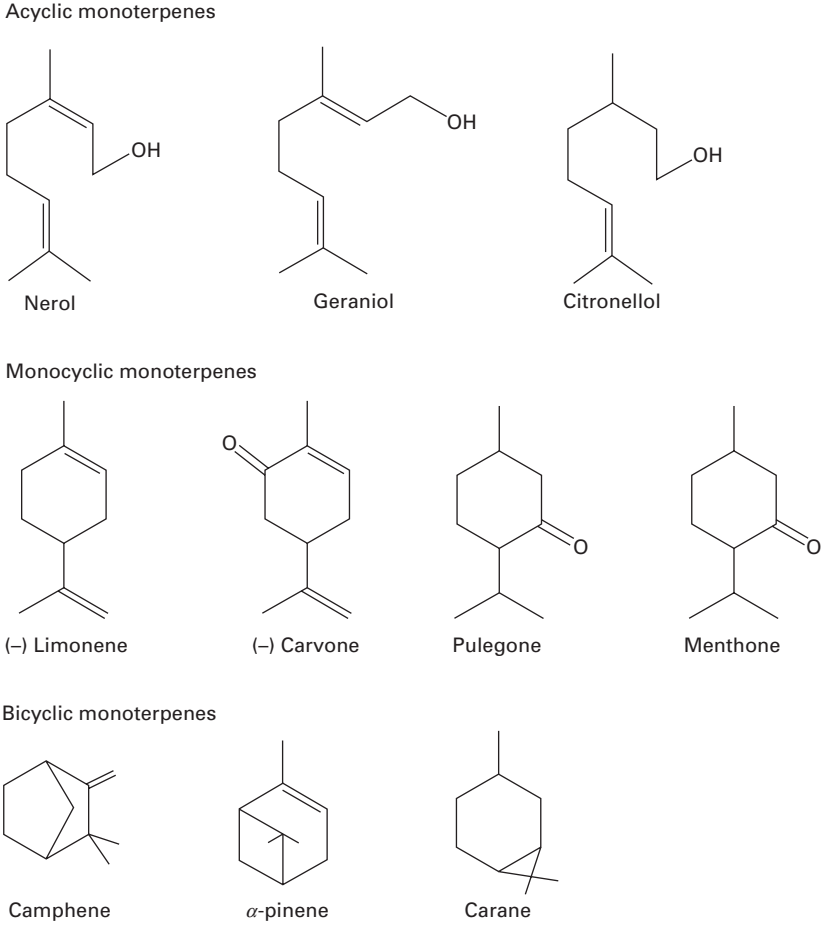


Fig. 11.2 Structures of selected monoterpenes.

occasionally an oxygen functional group (e.g. cinnamaldehyde in cinnamon oil). The aromatic ring may be substituted with up to four oxygens, which are then further modified themselves by the addition of a methylenedioxy ring, as in safrole.

11.3 Biosynthesis of the components of volatile oils

Chemicals produced by plants that are characterized by a limited distribution, and an absence of obvious value in the physiology of the producer plant, are known as secondary metabolites. The array of secondary metabolites, which of course includes volatile oils, is enormous. The terpenes constitute a major group, with more than 1000 monoterpene and perhaps 3000 sesquiterpene structures known. By contrast, the number of phenylpropenes is small, with probably less than 50 being known (Waterman 1993).

Despite the vast numbers and structural diversity of secondary metabolites, almost all arise from one of the three biosynthetic pathways, or from a combination of two or more of these pathways. These are known as the acetate, mevalonate (based on

mevalonic acid) and shikimate (based on shikimic acid) pathways. The terpenes are wholly mevalonate derived whereas the phenylpropenes originate from shikimic acid. Figure 11.3 illustrates the sequence of formation of methyl chavicol from phenylalanine by the shikimic acid pathway. Figure 11.4(a)–(d) illustrates the general biosynthetic pathway from mevalonic acid to sesquiterpenes.

11.3.1 Biosynthesis of monoterpenes and sesquiterpenes

Mevalonic acid is a chemical intermediate containing six carbons that is formed in the plant by the combination of three molecules of acetate, which have, in turn, been derived from acetyl coenzyme A. This is a universal process in all higher plants and produces compounds vital to the life processes. The biosynthesis of mono- and sesquiterpenes from mevalonic acid involves three steps: (i) conversion of mevalonic acid to isopentenyl pyrophosphate (IPP) and 3,3-dimethyl allyl pyrophosphate (DMAPP), (ii) combination of IPP and DMAPP to give geranyl pyrophosphate (GPP) and (iii) combination of GPP with IPP to give farnesyl pyrophosphate (FPP). IPP is the initial product formed from mevalonic acid and it is then converted into DMAPP by the enzyme isopentenyl pyrophosphate isomerase (Gershenzon and Croteau 1990, Waterman 1993).

One molecule of IPP and one molecule of DMAPP combine under the influence of geranyl pyrophosphate synthase to give geranyl phyrophosphate (GPP), the first recognizable monoterpene. This process is then continued by the addition of another

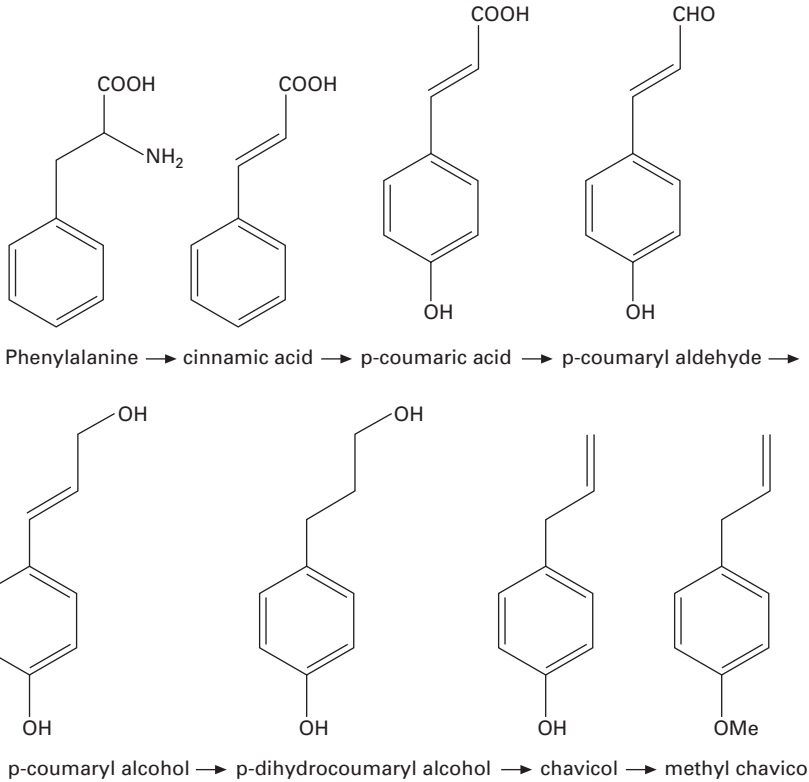


Fig. 11.3 Formation of methyl chavicol from phenyl alanine by shikimic acid pathway.

IPP to GPP through the mediation of a further synthase enzyme, resulting in the production of the first 15-carbon unit, farnesyl pyrophosphate (FPP).

11.3.2 Biosynthesis of phenylpropenes

Shikimic acid is formed from glucose in plants, and is the biogenic precursor of the amino acids L-phenylalanine, L-tyrosine and L-tryptophan. Pathways from shikimic acid generate anthranilates (e.g. in mandarin oil *Citrus reticulata*), cinnamates (e.g. in Peru balsam oil *Myroxylon pereirae*) and other phenylpropanoids, and from this point on to other metabolites such as lignans and flavononoids. In particular, phenyl propanoids (basically compounds with a 3-carbon chain attached to a benzene ring) are formed from trans or (E)-cinnamic acid via the elimination of ammonia from L-phenylalanine. Common phenylpropanoids in essential oils include methyl chavicol, methyl eugenol, eugenol, methyl cinnamate, vanillin and anethole. The shikimic acid pathway produces the amino acid phenylalanine which by the action of phenyl alanine ammonia lyase is converted to trans-cinnamic acid (Bramley 1997, Waterman 1993).

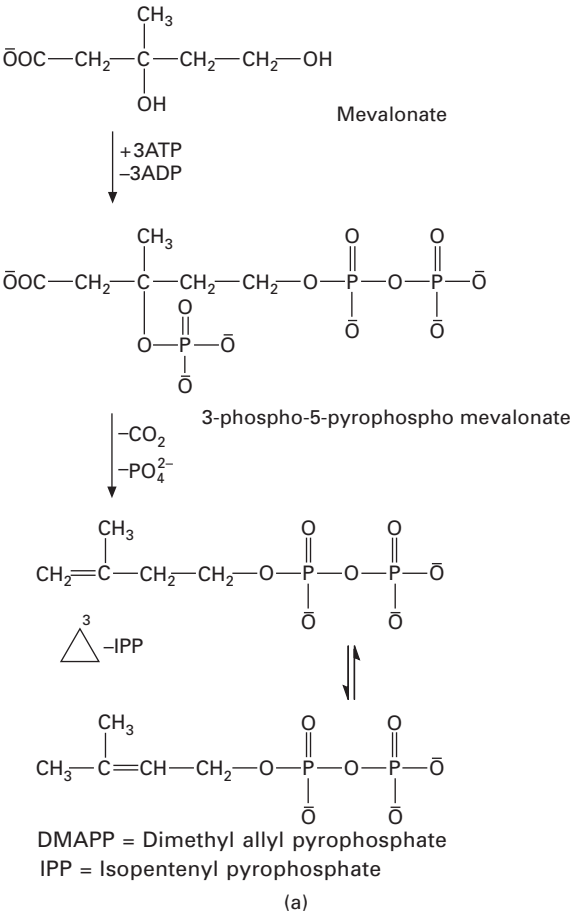
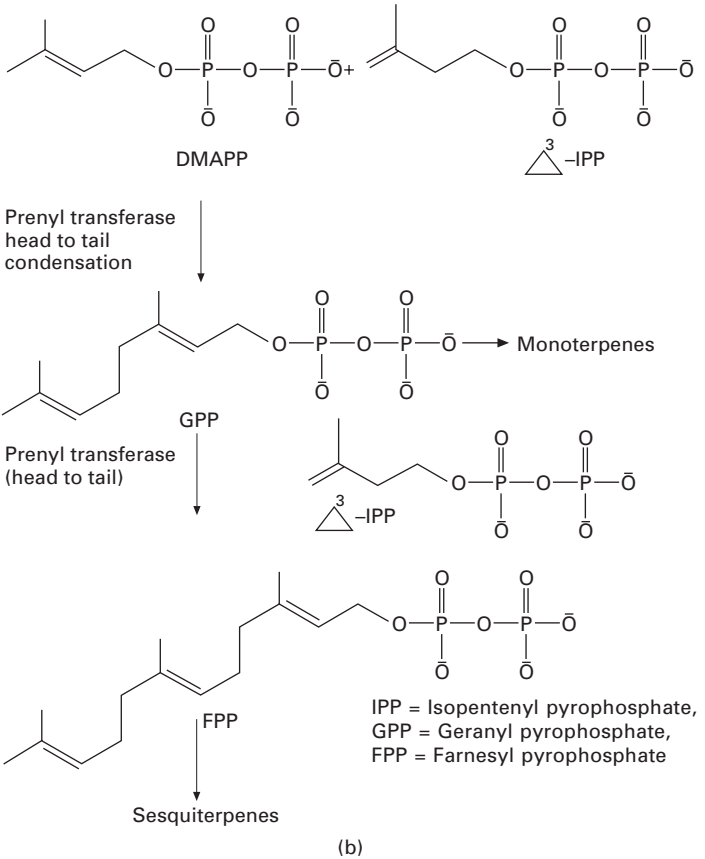
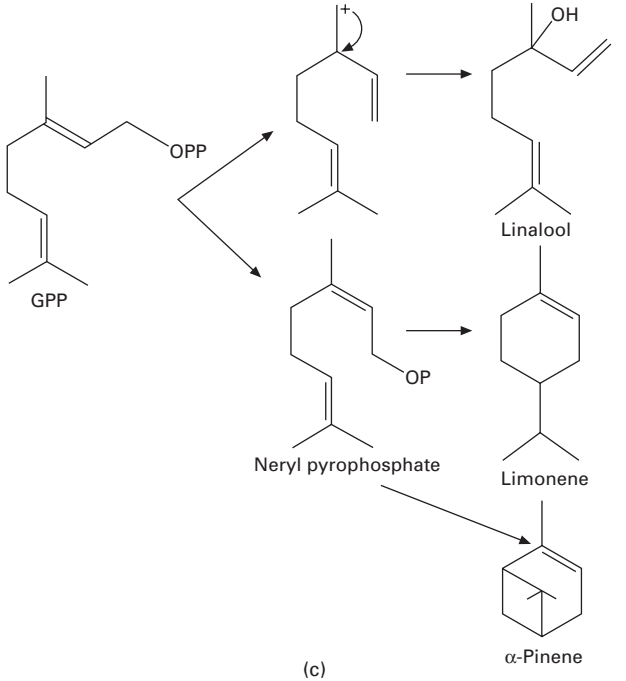


Fig. 11.4 (a) Conversion of mevalonate into activated isoprene units (Source: Nelson and Cox (2001)); (b) formation of GPP and FPP from DMAPP (Source: Nelson and Cox (2001)); (c) formation of monoterpenes from GPP (Source: Waterman (1993)); (d) formation of sesquiterpenes from EPP (Source: Waterman (1993)).

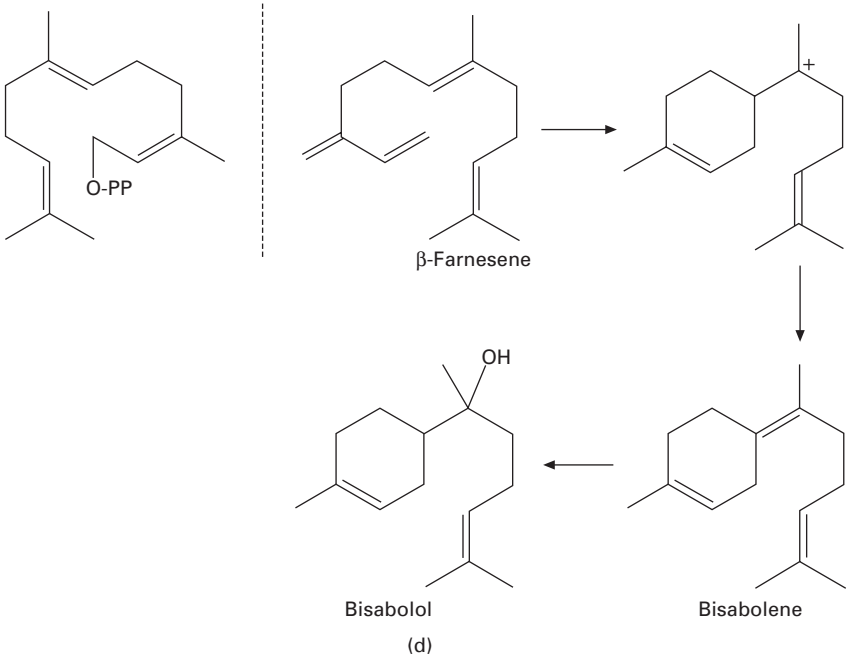


(b)



(c)

Fig. 11.4 continued



(d)

Fig. 11.4 continued

11.4 Volatiles and plant sources

The spices and herbs discussed here consist of black pepper, cardamom, ginger, turmeric, cinnamon, cassia, clove, nutmeg, cumin, coriander, fennel, fenugreek, ajowan, asafoetida, basil, mint, spearmint and rosemary. The chief chemical constituents of these spices and herbs are listed in Table 11.2.

11.4.1 Major volatiles in herbs and spices

The common volatiles found in spices and herbs are as follows:

Monoterpene hydrocarbons

Camphene, δ-3-carene, p-cymene, limonene, myrcene, cis-ocimene, α-phellandrene, β-phellandrene, α-pinene, β-pinene, sabinene, α-terpinene, γ-terpinene, terpinolene, α-thujene.

Oxygenated monoterpenes

Borneol, camphor, carvacrol, cis-carveol, trans-carveol, carvone, carventanacetone, 1,8-cineole, cryptone, p-cymen-8-ol, p-cymen-8-methylether, dihydrocarveol, dyhidrocarvone, linalool, cis-menthadien-2-ol, 3,8,(9)-p-menthadien-1-ol, 1(7)-p-menthadien-6-ol, 1(7)-p-menthadien-4-ol, 1,8(9)-p-menthadien-5-ol, 1,8(9)-p-menthadien-4-ol, cis-p-2-menthen-1-ol, myrtenal, myrtenol, methyl carvacrol, trans-pinocarveol, pinocamphene, cis-sabinene hydrate, trans-sabinene hydrate, 1-terpinen-4-ol, 1-terpinen-5-ol, α-terpineol, 1,1,1,4-trimethyl cyclo-hepta-2, 4-dien-6-ol, phellandral, piperitone, citronellal, nerol, geraniol, isopinocamphone, methyl citronillate, methyl geranate, α-terpinyl acetate, terpinolene epoxide and trans-limonene epoxide.

Table 11.2 Principal constituents of certain spices and herbs

Spice	Average volatile oil (%)	Total volatile oil ml/100 g (range)	Average NVEE (%)	Principal volatile constituents
Basil (Sweet) -	0.4	–	3.6	Methyl chavicol, linalool, methyl cinnamate, cineole, eugenol
Cardamom	4.0	2.0–8.0	–	α-Terpinyl acetate, 2-terpineol, limonene, cineole, borneol, linalyl acetate, linalool, α-terpineol
Cassia	2.5	0.5–5.0	4.0	Cinnamic acid, benzaldehyde, methyl salicylaldehyde, cinnamic aldehyde, cinnamyl acetate
Cinnamon	0.75	0.5–2.0	5.0	Cinnamic aldehyde, eugenol, caryophyllene.
Clove	16.0	12.0–20.0	7.0	Eugenol, eugenol acetate, caryophyllene
Coriander	0.3	0.0–0.1	16.0	d-linalool, d-2-pinene, dl-α-pinene, geraniol
Cumin	2.5	2.5–4.5	20.0	Cuminaldehyde, p-cymene, dihydro cuminaldehyde
Dill	3.0	2.0–4.0	17.0	Carvone, d-limonene, phellandrene
Fennel	3.0	3.0–4.0	15.0	Anethole, fenchone, d-α-pinene
Fenugreek	Trace	0.02	7.0	δ-cadinene, α-cadinol, γ-eudesmol
Ginger	2.0	1.0–3.0	5.0	D-camphene, zingiberene α- and β-phellandrene
Mace	12.0	7.0–14.0	23.0	d-α-pinene, d-camphene, myristicin, elemicin
Nutmeg	6.5	7.15	28.0	Myristicin, geraniol, d-camphene, dipentene, pinenes, safrole, p-cymene
Pepper (black)	2.5	1.0–3.0	5.5	α-pinene, β-pinene 1-α-phellandrene β-caryophyllene, limonene
Peppermint	–	0.4–1.0	–	1-menthol, l-limonene, l-menthone, α-pinene, phellandrene, d-menthone.
Spearmint	–	–	–	l-carvone, l-limonene 1-phellandrene
Turmeric	–	1.3–5.5	7.0	Zingiberene, borneol, d-sabinene, tumerone, ar-turmerone, d-α-phellandrene, cineole

Sesquiterpene hydrocarbons

β-Caryophyllene, α-cis-bergamotene, α-trans-bergamotene, β-bisabolene, δ-cadinene, γ-cadinene, calamenene, α-copaene, α-cubebene, β-cubebene, ar-curcumene, β-elemene, δ-elemene, β-farnesene, α-guaiene, α-humulene, γ-humulene, isocaryophyllene, γ-muurolene, α-santalene, α-selinene, β-selinene, ledene, sesquisabenene and zingiberene (Purseglove *et al.* 1981a).

Oxygenated sesquiterpenes

Oxygenated sesquiterpenes identified are 5, 10 (15) cadinen-4-ol, caryophylla-3-(12),7(15)-dien-4-β-ol, caryophylla-2,7(15)-dien-4-β-ol, caryophylla-2-7(15)-dien-4-β-ol, caryophyllene alcohol, caryophyllene ketone, caryophyllene oxide, epoxydihydrocaryophyllene, cis-nerolidol, 4,10,10-trimethyl-7-methylene bicyclo-(2.0)decane-4-caboxaldehyde, γ-eudesmol, elemol, cubebol, α-bisabolol, β-bisabolol, virideflorol, cubebol, epi-cubenol, turmerone, ar-turmerone and turmerol.

Miscellaneous compounds

Eugenol, methyleugenol, benzaldehyde, trans-anethole, myristicin, safrole, piperonal, m-methyl acetophenone, p-methyl acetophenone, n-butyrophenone, methyl heptanone, pinol, methyl heptanate, methyl octanoate, 2-undecanone, n-nonane, n-tridecane and aromatic acids such as benzoic acid, phenyl acetic acid, cinnamic acid, piperonic acid, butyric acid, 3-methyl butyric acid, hexanoic acid and 2-methyl pentanoic acid. The structures of the selected compounds are depicted in Fig. 11.5(a) and 5(b) (Purseglove *et al.* 1981a).

11.4.2 Volatile oil constituents

The details of volatiles from individual spices and herbs are discussed below.

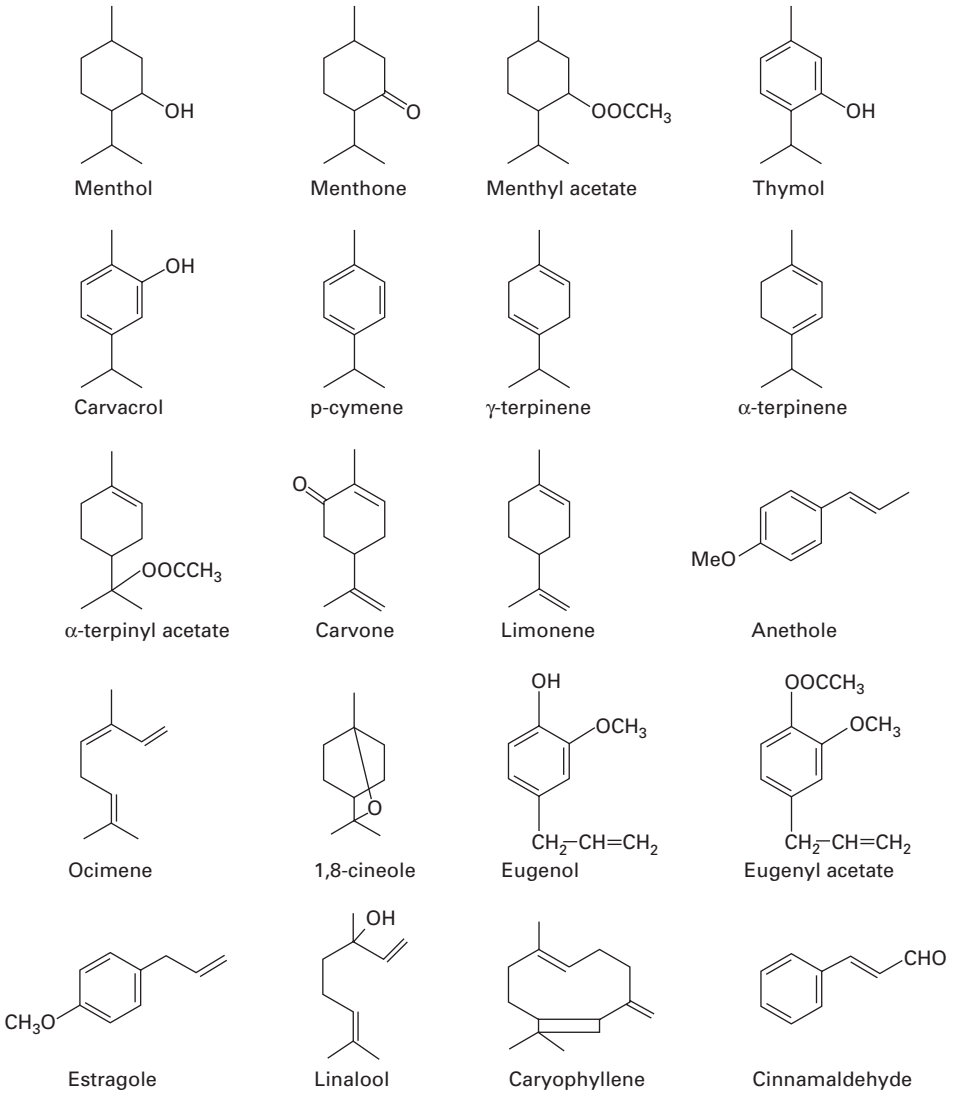


Fig. 11.5 Major volatiles from spices and herbs

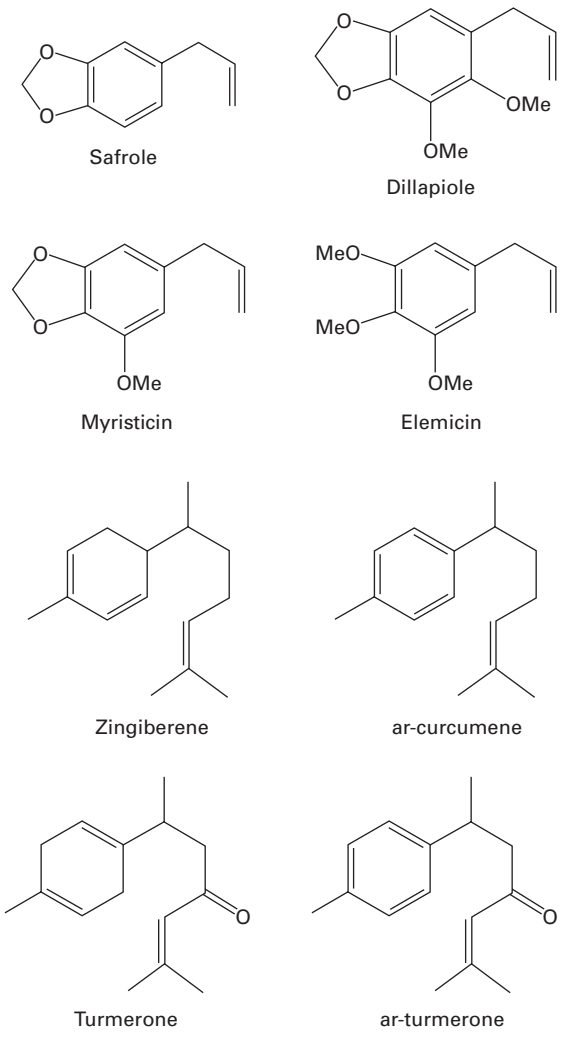


Fig. 11.5 continued

Ajowan

Ajowan or bishop's weed is cultivated for its fruits, which are commonly used as a spice and medicine. It is used for its characteristic smell and pungent taste in pickles, certain biscuits, confectionery and beverages. Nagalakshmi *et al*. (2000) determined the physicochemical characteristics of ajowan volatile oil. GC-MS profile of ajowan seed volatile oil indicated the composition as follows: α-pinene (1.48%), β-pinene (5.45%), β-myrcene (1.40%), α-terpinene (0.09%), p-cymene (19.47%), limonene (0.48), γ-terpinene (30.97%), p-cymene (0.06%), menth-2-en-1-ol (0.13%), linalool (0.07%), terpinene-4-ol (0.12%), α-terpineol (0.12%) and thymol (39.36%). They have also reported the variability in the constituents from seeds of different locations.

Asafoetida

The spice asafoetida is the dried latex (gum oleoresin) exuded from the living

underground rhizome or tap root of several species of Ferula (three of which grow in India), which is a perennial herb (1 to 1.5 m high). It is greyish-white when fresh, darkening with age to yellow, red and eventually brown. It is sold in blocks or pieces as a gum and more frequently as a fine yellow powder, sometimes crystalline or granulated. Studies conducted in Pakistan on fresh mature seed oils of *Ferula foetida* Regel indicated presence of α-pinene (1.69–2.36%), camphene (0.9–1.04%), myrcene (2.0–2.5%), limonene (0.60–0.72%), longifolene (1.60–5.9%), caryophyllene (3.8–5.0%), β-selinene (15.2–17.2%), eugenol (4.68–5.00%), bornyl acetate (2.25–4.5%), fenchone (1.5–2.4%), linalool (0.05–0.06%), geraniol (0.05–0.08%), isoborneol (0–0.4%), borneol (0–0.15%) and guaicol (0.57–0.9%). The oil was also found to contain a mixture of sesquiterpene alcohols (0–39.32%) and a mixture of coumarins (7.5–7.8%) (Ashraf and Bhatty 1979).

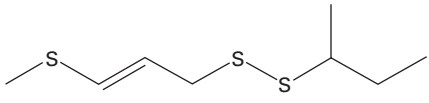
The major constituents of asafoetida are the resin (40–64%), gum (25%) and essential oil (10–17%) (Abraham *et al*. 1979). The aroma of asafoetida is attributed mainly to secondary butyl propenyl disulphide. Using MS, NMR, IR and UV spectra these were further characterized as 1-methyl propyl-(1-propenyl) disulphide (secondary butyl-(1-propenyl)-disulphide), 1-methyl thiopropyl-(1-propenyl) disulphide and 1-methyl propyl-(3) methyl-thio-(2-propenyl) disulphide (sec.butyl-(3)-methylthioallyl-disulphide): the composition of these in asafoetida oil is 36–84%, 9–31% and 0–52%, respectively (Abraham *et al.* 1979, Lawrence 1981) (Fig. 11.6).

Pakistan sample of asafoetida contained 1-(methylthio)-propyl–(E)-1-propenyl disulphide (37.93%), 1-(methylthio)-propyl-Z-1-propenyl disulphide (18.46%), 2-butyl–(E)-1-propenyl disulphide (11.17%), dibutyl trisulphide (1.82%), isobutanol (7.65%), methyl–(E)-1-propenyl disulphide (1.69%) as major compounds (Noleau *et al*. 1991). Essential oils extracted from asafoetida gums contained more than 150 compounds of which 25 compounds, including 13 sulphur-containing compounds, were common to both leek and asafoetida (Noleau *et al.* 1991).

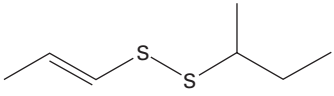
The oil from Iran was constituted by α-pinene (2.1%), sabinene(1.0%), β-pinene(5.0%), myrcene (1.0%), α-phellandrene (2.4%), β-phellandrene (2.5%), Z-β-ocimene (11.5%), E-β-ocimene (9.0%), 2-butyl-1-propyldisulphide (0.6%), 2-butyl-Z-1-propenyldisulphide (3.9%), 2-butyl-E-1-propenyldisulphide (58.9%), di-1-methylpropyl disulphide (0.3%) and di-1-methyl-propenyl disulphide (1.2%) (Sefidkon *et al*. 1998).

Basil

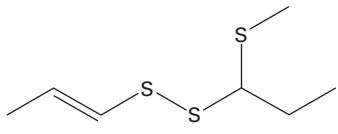
The chemical composition of volatile oils obtained from two forms of sweet basil



1-methyl propyl (3-methylthio-2-propenyl) disulphide



1-methyl propyl-(1-propenyl) disulphide



1-methylthiopropyl-(1-propenyl) disulphide

Fig. 11.6 Volatiles from asafoetida.

(purple basil and green basil) by GC-MS indicated about 35 compounds. Major compounds are eucalyptol and linalool. Estragole was not found in purple form while a low percentage was found in the green basil (Wjerdak 2001). The main constituents identified in basil condensate are linalool and methyl chavicol (Machale *et al.* 1997). Linalool and 1,8 – cineole comprised more than 50% of total yield of sweet basil oil. Volatiles in fresh leaves was about 50-fold higher than those found in air dried leaves (Loughrin 2003). Sanda *et al.* (1998) described the chemical composition of *Ocimum* species growing in Togo. Linalool, estragole and α-bergamotene are the major compounds (Table 11.3).

Black pepper

Gopalakrishnan *et al.* (1993) studied four genotypes of pepper using GC-MS. The oil of these cultivars possessed α-pinene in the range of 5.07–6.18%, β-pinene 9.16–11.68%, sabinene 8.5–17.16%, limonene 21.06–22.71% and β-caryophyllene 21.52–27.70%. Zachariah (1995) studied 42 black pepper accessions and reported 3.8–16.6% pinene, 2.2–33% sabinene, 1.6–31.8% myrcene, 3.6–21.2% limonene, 0.2–1.8% linalool and 11.8–41.8% β-caryophyllene.

Orav *et al*. (2004) determined the essential oil composition of black, green and white pepper using GC/mass spectrometry. Most abundant compounds in pepper oils were (E)-β-caryophyllene (1.4–70.4%), limonene (2.9–38.4%), β-pinene (0.7–25.6%), Δ-3-carene (1.7–19.0%), sabinene (0–12.2%), α-pinene (0.3–10.4%), eugenol (0.1–41.0%) terpinene-4-ol (0–13.2%) hedycaryol (0–9.1%), β-eudesmol (0–9.7%) and caryophyllene oxide (0.1–7.2%). Green pepper oil (dried by sublimation method) had a higher content of monoterpenes (84.2%) than air-dried green pepper corns (26.8%). The oil from ground black pepper contained more monoterpenes and less

Table 11.3 Percentage composition of *Ocimum basilicum* volatiles

Compound	% Composition
α-Thujene	0.4
Myrcene	0.4
Limonene + 1,8-cineole	0.4
(E)-β-Ocimeme	0.7
γ-Terpinene	0.2
Terpinolene	1.4
β-Elemene	1.2
β-Caryophyllene	0.2
(E)-α-Bergamotene	7.6
α-Caryophyllene	0.4
Germacrene D	0.8
β-Selinene	0.6
Bicyclogermacrene	0.4
γ-Muurolene	0.8
Cadinene	1.4
Cadinol	0.4
p-Cymene	0.5
Estragole	22.2
(Z)-Sabinene hydrate	0.9
Linalool	41.2
Camphor	0.3
Terpin-4-ol	2.3

Source: Sanda *et al.* 1998.

sesquiterpenes and oxygenated terpenoids as compared to green and white pepper oils. Sumathykutty *et al.* (1999) identified elemol as the most abundant component of black pepper leaf oil. Murthy *et al.* (1999) reported that pepper powder with an average particle size of 0.7 mm is essential to release the maximum concentration of monoterpenes and sesquiterpenes.

Jagella and Grosch (1999a), by adopting dilution and concentration experiments as well as enantioselective analysis of optically active monoterpenes, indicated (±) linalool, (+)-α-phellandrene, (–)- limonene, myrcene, (–)-α pinene, 3-methyl butanal and methyl propanal as the most potent odorants of black pepper. Storage studies conducted by Jagella and Grosch (1999b) using ground black pepper revealed that losses of α-pinene, limonene and 3-methyl butanal were mainly responsible for deficits in the pepper-like, citrus-like, terpene-like and malty notes after 30 days at room temperature. The musty/mouldy off flavour of a sample of black pepper was caused by a mixture consisting of 2,3-diethyl-5-methyl pyrazine and 2-isopropyl-3-methoxy pyrazine. The key odorants of white pepper as identified by Jagella and Grosch (1999c) are limonene, linalool, α-pinene, 1,8-cineole, piperonal, butyric acid, 3-methyl butyric acid, methyl propanal and 2- and 3-methyl butanal. Narayanan (2000) described the percentage composition of the volatile constituents in four black pepper varieties Panniyur-1, 2, 3 and 4 (Table 11.4).

Cardamom

The active constituent of cardamom is the aromatic volatile oil. The freshly dried unsplit capsules filled with seeds are the best material for distillation of volatile oil. Oils from freshly separated seeds or from whole capsules are almost identical as the husk practically does not yield any oil (Govindarajan *et al.* 1982). Zachariah (2002) described the chemical composition of cardamom oil from different samples (Table 11.5). Govindarajan *et al.* (1982) described the trace components in cardamom oil (Table 11.6). Gopalakrishnan (1994) conducted studies on the storage quality of CO_2-extracted cardamom oil. The class of components that underwent quantitative reduction was the terpene hydrocarbons in the oil, whereas the other components showed varying responses at low and ambient temperatures of storage

Cassia

Cinnamomum cassia yields bark and leaf oils that are economically important. The bark of *cassia* is coarser and thicker with a more intense aroma than the true cinnamon, *C. verum* (Bercht. and Presl.). The bark is used for flavouring food and beverages and also in pharmaceutical preparations and perfumery. The volatile oils from leaf and bark and the oleoresin from bark are used in soaps, perfumes, spice essences and beverages. The major component of the oil from cassia bark and leaf is cinnamaldehyde.

The *Cinnamomum cassia* Blume bark oil from Nigeria contained mainly cinnamaldehyde, with some eugenol while the leaf oil contained high levels of benzyl benzoate (Lockwood 1979). Cinnamon plants with purple leaf flushes had 29% more bark oil (1.84%) as compared to those with green flushes (1.43%), whereas bark oleoresin (8.41% and 7.90% in purple and green respectively) and leaf oil (1.68% and 1.73% in purple and green respectively) contents were on a par in both the types (Krishnamoorthy *et al.* 1988).

Headspace composition of cinnamon and cassia quills of different origin showed that the cinnamaldehyde and benzaldehyde contents were in the ranges 2.3–86.2% and 0.5–40.5%, respectively (Vernin *et al.* 1994). Jayatilaka *et al.* (1995) examined

Table 11.4 Chemical constituents of four black pepper varieties

No.	Compound	Percentage composition			
		1	2	3	4
1	α-Thujene	0.73	1.26	1.59	0.91
2	α-Pinene	5.28	6.18	5.07	5.32
3	Camphene	0.14	0.18	0.14	0.13
4	Sabinene	8.50	13.54	17.16	1.94
5	β-Pinene	11.08	10.88	9.16	6.40
6	Myrcene	2.23	2.30	2.20	8.40
7	α-Phellandrene	0.68	0.20	–	2.32
8	δ-3-Careme	2.82	0.18	–	1.03
9	α-Terpinene	–	–	0.39	1.13
10	p-Cymene	–	0.18	0.07	9.70
11	(Z)-β-Ocimene + β-phellandrene	–	0.15	0.23	0.37
12	Limonene	21.06	21.26	22.71	16.74
13	(E)-β-Ocimene	0.18	2.84	0.30	0.17
14	γ-Terpinene	0.01	0.49	–	0.03
15	*Trans*-sabinene hydrate	0.14	–	0.30	0.19
16	Terpinolene	0.10	0.20	0.22	0.08
17	*Trans*-linalool oxide	0.03	0.18	–	0.08
18	Linalool	0.22	0.22	0.46	0.28
19	*Cis*-p-menth-2-en-1-ol- + *cis*-p-menth-2,8-diene-1-ol	0.04	0.04	0.05	0.02
20	*Trans*-p-menth-2-en-1-ol	0.01	0.01	0.01	0.01
21	Citronellal	0.02	0.03	0.03	0.01
22	p-Menth-8-en-1-ol	0.03	t	–	t
23	Borneol	t	t	t	t
24	Terpinen-4-ol	0.19	0.32	0.52	0.18
25	α-Terpineol	0.10	0.17	0.12	0.07
26	Dihydrocarveol	0.01	–	0.02	0.02
27	p-Menth-8-en-2-ol	–	0.01	0.02	0.02
28	*Trans*-carveol	0.01	0.01	–	0.02
29	*Cis*-carveol + carvone	0.01	0.03	0.03	0.03
30	Piperitone	0.04	t	0.03	t
31	Carvone oxide	0.01	0.01	–	0.01
32	Myrtenol	0.20	0.04	0.11	0.04
33	α-Terpinyl acetate	0.86	1.22	1.33	1.05
34	Neryl acetate	0.20	0.07	0.05	0.13
35	Geranyl acetate	0.12	0.01	0.07	0.11
36	α-Cubebene/δ-elemene	3.25	0.26	0.16	2.56
37	α-Copaene	0.82	0.49	0.44	0.71
38	β-Elemene	0.09	0.09	0.06	0.05
39	β-Caryophyllene	21.59	27.70	23.2	21.19
40	*Trans*-α-berga motene	0.31	–	–	0.28
41	α-Humulene	0.21	0.20	–	0.29
42	(E)-β-Farnesene	0.08	0.22	0.11	0.13
43	α-Amorphene	1.51	1.53	0.03	1.28
44	α-Guaiene	0.11	0.07	1.54	0.10
45	Clovene	0.14	0.07	0.07	0.13
46	Germacrene-D	0.04	0.03	0.04	0.26
47	ar-curcumene	0.26	0.12	0.04	0.29
48	β-Selinene	0.64	0.87	1.37	0.63
49	α-Selinene	0.07	0.12	0.48	0.14
50	δ-Muurolene	0.73	0.93	0.16	0.58
51	(E,E)-2-Farnesene	0.72	–	0.47	0.72

Table 11.4 Continued

No.	Compound	Percentage composition 1	2	3	4
52	β-Biabolene +2-bisabolene	4.25	2.15	3.10	0.49
53	δ-Guaiene	0.82	0.17	0.09	1.85
54	Cuparene	1.38	0.09	0.14	0.04
55	δ-Cadinene	0.12	–	0.07	0.13
56	(2)-Nerolidol	0.20	0.05	0.11	0.05
57	Elemol	0.11	0.06	0.07	0.08
58	(E)-Nerolidol	0.12	0.04	0.07	0.03
59	Caryophyllene alcohol	0.07	0.02	0.04	0.02
60	Caryophyllene oxide	0.90	0.35	0.38	0.25
61	Cedrol	0.07	–	0.05	0.05
62	α-Cadinol	1.51	0.29	0.12	1.27
63	α-Cadinol	0.26	0.12	0.15	0.25
64	β-Bisabolol	0.20	0.09	0.17	0.14

Source: Narayanan 2000.
t = trace (<0.01%)
1 = Panniyur-1 2 = Panniyur-2 3 = Panniyur-3 4 = Panniyur-4.

Table 11.5 Percentage composition of cardamom volatile from different sources

Component	Var. Malabar (Ceylon)	Var. Malabar (Gautemala)	Var. Mysore	Sri Lanka (Wild)
α-Pinene	1.10	0.71	1.40	13.00
Camphene	0.02	0.03	0.04	0.13
Sabinene	2.50	3.40	3.10	4.90
β-Pinene	0.20	0.34	0.26	4.90
Myrcene + terpinene	1.80	1.50	1.10	2.50
α-Phellandrene	<0.01	<0.01	<0.01	0.42
D-Limonene	0.02	0.12	0.14	2.10
1,8-Cineole	31.0	23.4	44.0	3.30
γ-Terpinene	0.12	0.34	0.10	22.2
Linalool	2.10	4.50	3.00	3.70
Citronellal	<0.01	0.04	0.06	0.13
4-Terpineol	0.14	0.28	0.87	15.3
α-Terpineol	1.40	1.90	1.50	0.86
Citronellol	<0.01	0.04	<0.01	0.01
Nerol	0.02	0.04	0.06	0.78
Linalyl acetate	3.30	6.30	3.10	0.31
Geraniol	0.27	0.38	0.25	0.34
α-Terpinyl acetate	52.5	50.7	37.0	0.14
Geranyl acetate	0.08	0.13	0.15	1.50
Trans-nerolidol	0.09	0.83	0.07	0.44
Cis-nerolidol	0.23	1.60	0.28	0.37

Source: Zachariah 2002.

the composition of bark oil from 25 samples of *Cinnamomum cassia* and the major components identified were (E)-cinnamaldehyde (92.0–98.0%), (Z)-cinnamaldehyde (0.8–2.7%), β-caryophyllene (0.4–3.6%), coumarin (0.1–1.6%) and α-ylangene (0.1–2.7%). Analysis of the chinese cassia oil by HPLC method and supercritical CO_2 extraction indicated cinnamaldehyde content as 68.2–71.9% and 73.9–74.4%,

Table 11.6 Trace components in cardamom volatile oil

Hydrocarbons	Alcohols and phenols
α-Thujene	3-Methyl butanol
Camphene	*p*-Menth-3-en-l-ol
α-Terpinene	Perillyl alcohol
cis-Ocimene	Cuminyl alcohol
trans-Ocimene	*p*-Cresol

Source: Govindarajan *et al*. 1982.

respectively (Ehlers *et al*. 1995, Lawrence 2001). Evaluation for chemical constituents in open pollinated seedling progenies of *C. cassia* accessions from Calicut (India) showed that these contained 1.2–4.95% bark oil, 6.0–10.5% bark oleoresin and 0.4–1.65% leaf oil. The major component of both the oils, namely, cinnamaldehyde, varied from 40.7–86.0% and 61.9–91.5% respectively in leaf and bark oils (Krishnamoorthy *et al*. 1999). The leaf oil of cassia from China contained 74.1% cinnamaldehyde, 10.5% 2-methoxy cinnamaldehyde and 6.6% cinnamyl acetate as major components whereas the Australian cassia recorded 77.2% cinnamaldehyde, 15.3% coumarin and 3.6% cinnamyl acetate as chief constituents (Dao 1999). Composition of leaf and bark oil of *Cinnamomum cassia* from Yunnan Province is indicated in Table 11.7 (Li *et al*. 1998).

Cinnamon

Cinnamomum verum (Syn. *C. zeylanicum*) yields mainly leaf and bark oils, that are used in perfumery and flavouring. The major component of the leaf oil is eugenol while that of bark oil is cinnamaldehyde. Senanayake *et al*. (1978) identified 32 components in cinnamon oil, of which eugenol (70.1%) and cinnamaldehdyde (75.0%) were the major compounds in leaf and bark respectively. The oil from its root bark contained camphor (56.2%) and 1,8-cineole (11.7%) as chief components. The cinnamon varieties Navashree and Nithyasree, recorded 2.7–2.8% bark oil, 10% bark oleoresin and 3% leaf oil contents (Krishnamoorthy *et al.* 1996). Two types of *Cinnamomum zeylanicum* leaf oils exist, the main constituent of one being eugenol and that of the other benzyl benzoate. Nath *et al*. (1996) reported a variety of *C. verum* growing in Brahmaputra valley (India) with benzyl benzoate as a major constituent in both leaf and bark oils. The essential oil of the leaves of *C. zeylanicum* from Cameroon contained eugenol (85.2%), (E)-cinnamaldehyde (4.9%), linalool (2.8%) and β-caryophyllene (1.8%) (Jirovetz *et al*. 1998).

A chemotype of *Cinnamomum zeylanicum* with 85.7% linalool in leaf oil was reported from Calicut (South India) by Jirovetz *et al*. (2001) (Table 11.8). Cinnamon leaf oils of Indian origin contained 81.43–84.5% eugenol (Mallavarappu *et al.* 1995) (Table 11.9). Syamasundar *et al*. (2000) reported variation in the composition of unripe and ripe fruits of cinnamon. The oil from unripe fruits was dominated by δ-cadinene (19.15%), α-pinene (11.47%), β-pinene (10.51%), E-cinnamyl acetate (7.11%) and γ-cadinene (8.05%) whereas the ripe fruits contained γ-cadinene (23.48%), α-pinene (11.52%), E-cinnamyl acetate (8.62%) and α-muurolene (8.22%) as chief components. The fruit oil from South India was dominated by α-pinene (11.2%), β-pinene (9.2%), β-caryophyllene (11.0%), α-muurolene (6.1%), δ-cadinene (20.2%) and α-muurolol (9.8%) (Mallavarapu and Ramesh 2000) (Table 11.10). Volatile oil from cinnamon flowers was dominated by (E)-cinnamyl acetate (41.98%), trans-α-

Table 11.7 Comparative percentages, composition of the leaf and bark oils of *Cinnamomum cassia*

Compound	Leaf oil (%)	Bark oil (%)
α-Pinene	0.05–0.36	0.10–0.25
Camphene	0.04–0.05	0.05–0.10
β-Pinene	0.04–0.15	0.14–0.22
Myrcene	0.02–0.03	t–0.10
α-Phellandrene	0.01–0.03	t–0.13
Limonene	0.13–0.24	0.14–0.29
1,8-Cineole	0.05–0.08	0.06–1.07
δ-3-Carene	0.03–0.05	t–0.07
p-Cymene	0.11–0.19	0.04–0.18
Camphor	0.07–0.15	0–0.08
Benzaldehyde	1.42–1.48	0.50–1.10
Linalool	0.11–0.23	0.08–0.16
Terpinolene	t	0–0.04
β-Caryophyllene	0.16–0.20	t–0.27
α-Humulene	t–0.03	0-0.15
β-Elemene	–	t–0.06
Isoborneol	0–0.20	0–0.27
Borneol	0.15–0.41	0.06–1.27
α-Terpineol	t–0.10	0.07–2.05
Geraniol	t	0.08–0.31
Carvone	0.57–0.64	0–0.34
2-Methoxybenzaldehyde	0.08	0–0.12
Safrole	–	t–0.20
γ-Elemene	0–t	0–0.41
δ-Cadinene	t	t–0.13
β-Cadinene	–	t–0.10
Hydrocinnamaldehyde	0.88–0.89	0–0.24
Phenylacetaldehyde	0.07–0.16	t–0.27
Methyl eugenol	0.14–0.15	t–0.05
(E)-Cinnamaldehyde	64.10–68.30	80.40–88.50
α-Copaene	0.41–0.49	0.23–0.68
Vanillin	t	t–0.10
Salicylaldehyde	0.05–0.42	0.04–0.85
2-Phenethyl alcohol	0.11–0.27	t–0.16
Benzyl alcohol	t–0.05	–
Acetophenone	t–0.1	0–0.6
Eugenol	0.04–0.06	0.03–1.08
(Z)-Isoeugenol	0.14–0.28	0.12–0.66
(E)-Cinnamyl acetate	4.50–12.50	0.60–5.10
γ-Muurolene	t	t–0.50
Anisaldehyde	0.58–1.02	t
2-Phenethyl acetate	t–1.55	–
β-Bisabolene	t–0.06	t–0.18
β-Bisabolol	t	t–0.35
α-Muurolol	0–0.08	0–0.24
Coumarin	0.03–0.08	t–0.45
(E)-Cinnamic acid	0.80–2.48	0.12–3.10
(E)-2-Methoxycinnamaldehyde	8.40–10.50	t–2.50
Hydrocinnamic acid	0.18–0.51	0.024
4-Hydroxy-2-phenethyl alcohol	0–0.12	0–0.10
Caryophyllene oxide	0.15–0.17	0–0.10
Patchoulene	0.06–0.07	0–0.04
Octanoic acid	t	0–t

Table 11.7 Continued

Compound	Leaf oil (%)	Bark oil (%)
3-Phenylpropyl acetate	0.21–0.43	0.05–0.22
Nonanoic acid	t–0.10	0–t
Guaicol	t	0–0.08
(E)-Cinnamyl alcohol	0.15	0.05–0.13
(E)-Ethyl cinnamate	0.11–0.27	t–0.14
Benzyl benzoate	0.07–0.15	t–0.38
Methyl alaninate	t–0.05	–
Guaicyl cinnamate	t	t
Decanoic acid	t	0–t
Undecanoic acid	0–0.05	0–0.11
Dodecanoic acid	t–0.04	0–t
Benzoic acid	0.07–0.11	0.07–0.10
Salicylic acid	t–0.10	0.10–0.20

Source: Li *et al.* 1998.
Note: T = trace.

Table 11.8 Composition of oil from *Cinnamomum zeylanicum* leaves from Calicut, India

(E)-2-Hexenol (0.1%)	Borneol (0.1%)
(Z)-3-Hexenol (0.1%)	Terpinen-4-ol (0.3%)
1-Hexen-3-ol (0.1%)	α-Terpineol (1.1%)
Hexanol (0.1%)	Dihydrocarveol (t)
α-Pinene (t)	Linalyl aetate (0.1%)
(Z)-3-Hexenyl acetate (0.1%)	(E)-Cinnamaldehyde (1.7%)
(E)-2-Hexenyl acetate (0.1%)	Safrole (t)
p-Cymene (t)	(E)-Cinnamyl alcohol (0.1%)
β-Phellandrene (t)	Eugenol (3.1%)
(E)-β-Ocimene (t)	(E)-Cinnamyl acetate (0.9%)
1,8-Cineole (0.1%)	β-Caryophyllene (2.4%)
Limonene (0.2%)	α-Humulene (0.2%)
Cis-Linalool oxide* (0.1%)	Eugenyl acetate (0.1%)
Terpinolene (0.1%)	Caryophyllene oxide (0.1%)
Trans-Linalool oxide* (0.1%)	Spathulenol (0.2%)
Linalool (85.7%)	
Nonanol (0.3%)	

Source: Jirovetz *et al.* 2001.
Note: * furanoid form; t = trace (<0.01%).

bergamotene (7.97%), caryophyllene oxide (7.29%) and α-cadinol (6.35%) (Jayaprakasha *et al.* 2000).

Clove

Clove essential oils are extracted from *Eugenia caryophyllata* (*Syzygium aromaticum, Eugenia aromatica, E. caryophyllus*) from the Myrtaceae family. Clove oil is extracted from the leaves, stem and buds. However, only the clove bud oil is used in aromatherapy, since it contains less eugenol. Phenolic reactivity was seen almost throughout the bud, with a greater concentration in the outer glandular region of the hypanthium than in the inner aerenchymatous spongy tissue (Mangalakumari and Mathew 1985). Dried leaves of clove grown in Little Andaman (India), on hydrodistillation, gave

Table 11.9 Volatiles from *Cinnamomum verum* leaves

Compound	% Composition
	Leaf
α-Thujene	0.04–0.06
α-Pinene	0.38–0.49
Camphene	0.17–0.18
Sabinene	t
α-Pinene	0.16–0.18
Myrcene	0.09–0.13
n-Octanal	t
α-Phellandrene	0.50–1.03
Δ-3-Carene	0.05
α-Terpinene	0.03
p-Cymene	0.16–0.28
1,8-Cineole	0.23–0.38
β-Phellandrene	t
(z)-β-Ocimene	t
(E)-β-Ocimene	0.05
γ-Terpinene	0.05
cis-Linalool oxide (furanoid)	t
trans-Linalool oxide (furanoid)	t
	0.05–0.11
Terpinolene	1.57–3.70
Linalool	t
2-Phenylethanol	0.10
Camphor	0.39
Citronellal	0.12
Borneol	0.04–0.05
Terpinen-4-ol	0.10–0.14
α-Terpineol	0.19
Methylchavicol	t
(Z)-Cinnamaldehyde	0.26
Nerol	t
Cuminaldehyde	0.03
Piperitone	0.63–1.51
(E)-Cinnamaldehyde	0.52
Linalyl acetate	0.19
Safrole	t
(E)-Cinnamyl alcohol	t
2-Phenylethyl propionate	81.43–84.50
Eugenol	t
(E)-Methyl cinnamate	t
(Z)-Cinnamyl acetate	0.25–0.28
β-Elemene	t
(Z,E)-α-Farnesene	0.73
(E)-Cinnamyl acetate	2.49
β-Caryophyllene	0.47–2.25
(Z)-Methyl isoeugenol	t
α-Humulene	0.12–0.46
(E)-Methylisoeugenol	t
β-Selinene	t
Eugenyl acetate	0.14–2.85
γ-Cadinene	

Source: Mallavarapu *et al.* (1995).
Note: t = trace (<0.01%).

Table 11.10 Composition of *Cinnamomum zeylanicum* fruit oil

(E)-2-Hexenol (t)	(E)-Cinnamyl acetate (0.4%)
Tricyclene	β-Caryophyllene (11.0%)
α-Pinene (11.2%)	(E)-β-Farnesene (0.8%)
Camphene (0.6%)	α-Humulene (2.2%)
β-Pinene (9.2%)	γ-Muurolene (0.2%)
Myrcene (1.6%)	Germacrene D (0.2%)
α-Phellandrene (0.7%)	α-Muurolene (6.1%)
α-Terpinene (0.2%)	δ-Cadinene (7.1%)
p-Cymene (0.1)	δ-Cadinene (13.1%)
Limonene (2.8%)	*Cis*-Calaminnene (2.2)
1,8-Cineole (0.1%)	α-Cadinene (1.2%)
(Z)-β-Ocimene (0.1%)	Elemol (1.9%)
(E)-β-Ocimene (0.2%)	(E)-Nerolidol (0.1%)
γ-Terpinene (0.1%)	Isocaryophyllene oxide (0.2%)
Tepinolene (0.5%)	Spathulenol (0.8%)
Linalool (0.2%)	Caryophyllene oxide (0.4%)
α-Fenehyl alcohol (0.5%)	Globulol (0.4%)
Isoborneol (t)	Humulene epoxide 1 (0.5%)
Borneol (0.5%)	Humulene epoxide 11 (0.6%)
Terpinen-4-ol (0.1%)	1-Epi-cubenol(0.1%)
α-Terpineol (0.5%)	T-Cadinol (0.2%)
Nerol (t)	Cubenol (0.9%)
Geraniol (t)	α-Muurolol (9.8%)
Isobornyl acetate (0.1%)	Selin-11-en-4a-ol (0.1%)
(Z)-Cinnamyl acetate (0.1%)	α-Cadinol (3.1%)
α-Copaene (2.1%)	4-Hydroxy-3,4-dihydrocalacorene* (0.2%)
β-Elemene (0.4%)	4-Hydroxy-3,4-dihydrocalacorene* (0.1%)

Source: Mallavarapu and Ramesh, 2000.
Notes: * correct isomer not identified; t = trace (<0.1%).

4.8% oil. The major compound was eugenol (94.4%), followed by β-caryophyllene (2.9%) (Raina *et al.* 2001) (Table 11.11).

The chemical composition of bud and leaf oils of *S. aromaticum* from Cuba indicated 36 and 31 volatile compounds, respectively. The major components of the bud oil were eugenol (69.8%), β-caryophyllene (13.0%) and eugenyl acetate (16.1%), whereas the leaf oil contained eugenol (78.1%) and β-caryophyllene (20.5%) as the main constituents (Pino *et al.* 2001). During leaf growth (between days 2 (initial leaf stage) to 41 (yellow leaves) days), the content of caryophyllene in the essential oil of leaves decreased from 6.3% to 0.2% and the content of eugenol acetate decreased from 51.2% to 1.5% but the eugenol content increased from 38.3% to 95.2% (Gopalakrishnan and Narayanan 1988).

In the clove bud and stem essential oils from Madagascar four components predominated: eugenol (73.5–79.7% in bud and 76.4–84.8% in stem oils); β-caryophyllene (7.3–12.4% in both oils); α-humulene (1.0–1.4% in both oils); and eugenyl acetate (4.5–10.7% and 1.5–8.0%, respectively) (Gaydou and Randriamiharisoa 1987). The neutral fraction of the bud oil from Madagascar contained β-caryophyllene (75.64%), α-humulene (14.12%) and δ-cadinene (2.34%) as the major components (Muchala and Crouzet 1985). Gopalakrishnan and Narayanan (1988) reported that the eugenol content in leaves increased from 38.3% to 95.2%, with maturity, while the contents of eugenyl acetate (51.2% to 1.5%) and caryophyllene (6.3% to 0.2%) decreased. The clove bud and stem oils from Madagascar were dominated by eugenol, eugenyl acetate and β-caryophyllene (Gaydou and Randriamiharisoa 1987).

Table 11.11 Percentage composition of clove oil

Components	Percentage
(E)-β-Ocimene	0.03
Linalool	0.08
Terpinen-4-ol	0.03
Nerol	0.79
Eugenol	94.4
α-Copaene	0.04
β-Caryophyllene	2.91
α-Humulene	0.36
(E.E)-α-Farnesene	0.06
γ-Cadinene	0.18
(E)-Nerolidol	0.03
β-Caryophyllene oxide	0.67
Humulene oxode II	0.07
l-Cadinol	0.07
Cadalene	0.18
Hexadecyl acetate	0.09

Source: Raina *et al*. (2001).

Gopalakrishnan *et al*. (1984) characterized six sesquiterpenes namely, α-cubebene (1.3%), α-copaene (0.4%), α-humulene (9.1%), β-caryophyllene (64.5%), γ-cadinene (2.6%) and δ-cadinene (2.6%) in the hydrocarbon fraction of the freshly distilled Indian clove bud oil. Clove oil from the Malagasy republic was dominated by eugenol (72–73%), eugenyl acetate (6.3–7.8%) and caryophellene (15.7%) (Lawrence and Reynolds 1985). The essential oil content ranged from 12.9–18.5% in clove buds and 3.0–7.7% in pedicel. Eugenol content varied from 44–55% in bud oil and 60.0–72.4% in the oil from pedicel (Zachariah *et al*. 2005).

Coriander

Coriander oil is clear, colourless to light yellow liquid. Norwegian seeds contain higher levels of volatile oil (1.4–1.7%) (Purseglove *et al.* 1981b). Indian coriander seeds are poor in oil content (0.1–0.4%) (Agrawal and Sharma 1990). The major component of the essential oil was linalool (67–70%). Kumar *et al*. (1977) observed that small-fruited coriander was characterized by high oil content and preferred for distillation. Large fruited coriander seeds are lower in oil content and are more suited for use as spice.

Leaf oil of coriander is dominated by decanal (10%) and dodecanals (35%). Indian coriander oil is lower in linalool content and higher in linalyl acetate (Rao *et al.* 1925). Coriander seed oil contained 21% linalyl acetate and 42% linalool (Gupta *et al.* 1977). Steam distilled oil contained less linalool (71.9%) compared to CO_2 extract (83.2%) (Hirvi *et al.* 1986). Boelens *et al.* (1989) reported that linalool content (70.4%) was higher by hydrodistillation as against by hydrodiffusion (66.2%) and organoleptic preference was slightly more for the oil obtained by hydrodiffusion over hydrodistillation.

Nitz *et al.* (1992) compared the composition of the distilled oil of coriander with that of the SFE extract and found that the major compounds were linalool (63%), limonene (4%), γ-terpinene (9%), camphor (4%), α-pinene (8%) and geranyl acetate (2%). Diederischen (1996) analyzed 237 accessions of fruit oil and the main constituents

in these varied as follows: α-pinene (6.5–28.9%), γ-terpinene (0.7–35.4%), camphor (0.4–6.3%), linalool (19.8–82.0%), geranyl acetate (1.3–12.4%) and geraniol (0.3–3.3%).

Bandoni *et al*. (1998) compared the composition of coriander seed oil produced by water and steam distillation and found that the oils were quite similar. The chemical composition of the seed essential oil grown in Brazil contained linalool (77.48%), γ-terpinene (4.64%), α-pinene (3.97%), limonene (1.28%), geraniol (0.64%) and 2-decenal (0.16%) as the main components (Figueiredo *et al.* 2004).

Cumin

Cumin seeds yield 2.3–4.8% volatile oil. The oil is yellow amber liquid that tends to darken on ageing. The characteristic odour of cumin is mainly due to the aldehydes present in the seeds namely, cuminaldehyde, p-menth-3-en-7-al and p-menth-1,3-dien-7-al. (Agrawal 2001). Indian cumin oil is reported to be lower in cuminaldehyde content. Turkish cumin seed oil was reported to have cuminaldehyde (19.2%), p-mentha-1,3-dien-7-al (4.2–12.2%), p-mentha-1,4-dien-7-al (24–48%), γ-terpinene (7.0–14.1%), p-cymene (9.1–12.0%) and β-pinene (2.9–8.9%) as major constituents (Baser *et al.* 1992). Shaath and Azzo (1993) reported 25.01% cuminaldehyde in the cumin seed oil of Egyptian origin (Table 11.12). Pande and Goswami (2000) identified 12 constituents contributing to 86.4% of the oil of which the chief components were cuminaldehyde (32.6%), p-cymene (14.7%), p-mentha-1,4-dien-7-al (13.5%) and β-pinene (12.7%).

Dill

Essential oil is extracted from the seeds and leaves of dill. Fresh herb yields 0.19% light yellow oil and seeds yield 1% oil (light yellow). The major component of seed oil is d-carvone while that of leaf oil is α-phellandrene (Guenther 1961a, Pino *et al*. 1995, Kruger and Hammer 1996, Faber *et al*. 1997, Ranade 1998, Vera and Chane-Ming 1998 and Minija and Thoppil 2004).

Ravid *et al*. (1987) isolated optically active S (+)-carvone, the major component of the fruits of dill oil. The importance of S-(+) carvone is that it is used as the starting material for the synthesis of (R, Z)-3-methyl-6-isopropenyl-3,9-decadien-1-yl acetate, a pheromone component of the female California red scale, while R-(–)- carvone is used as a starting material in the preparation of picrotoxinin (Ravid *et al*. 1987).

Table 11.12 Chemical composition of cumin seed oil of Egyptian origin

Compound	Percentage content
α-Thujene (0.28%)	Terpinolene (0.11%)
α-Pinene (0.78%)	Terpinen-4-ol (0.16%)
Camphene (trace)	p-menth-3-en-7-al (3.83%)
Sabinene (0.40%)	α-terpineol (0.05%)
β-Pinene (14.64%)	Cuminaldehyde (25.01%)
Myrcene (0.92%)	p-mentha-1,4,dien-7-al (17.36%)
α-Phellandrene (0.63%)	p-mentha-1,3,dien-7-al (5.84%)
p-Cymene (4.91%)	β-caryophyllene (0.20%)
β-Phellandrene (0.30%)	*Trans*-α-bergamotene (0.31%)
limonene (0.37%)	
γ-Terpinene (19.12%)	

Source: Shaath and Azzo (1993).

Huopalahti *et al.* (1988) compared the composition of dill herb oil obtained by hydrodistillation, solvent extraction and CO_2 extraction by GC-MS and HS-GC (head-space GC). Each method gave different composition for the volatiles of dill herb (Table 11.13). However, the maximum concentration of the most important aroma compound in the dill herb, namely, 3,6-dimethyl-2,3,3a,4,5,7a-hexahydro benzofuran was obtained with head-space GC (38.5% ± 1.2%) analysis. The oil obtained by the hydrodistillation method contained 3,6-dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran (36.7% ± 1.6%) and α-phellandrene (32.1% ± 1.6%) as major components. CO_2 extracted oil contained 3,6-dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran (33.2% ± 3.7%) and neophytadiene (19.9% ± 4.3%). The solvent extracted oil was dominated by 3,6-dimethyl-2,3,3a,4,5,7a-hexahydro benzofuran (27.1% ± 2.2%), α-phellandrene (22.3% ± 3.7%) and neophytadiene (14.0% ± 1.8%).

Pure oil of dill weed should contain a minimum of 5% 3-9-epoxy-p-menthene. In pure dill oil the percentage ratios of α-phellandrene to limonene to β-phellendrene are 20:25:3. The chemical composition of dill oil of Hungarian origin is as follows: α-thujene (0.3%), α-pinene (0.8%), myrcene (0.7%), α-phellandrene (29%), limonene (25%), β-phellandrene (4.2%), p-cymene (1.3%), 1-methyl-4-isopropyl-benzene, α-p-dimethylstyrene (trace), dihydrocarvone (0.3%), isodihydrocarvone (0.2%), and D-carvone (35.2%). Lab distilled oil of *Anethum graveolens* seed from Pakistan indicated the presence of limonene (9.34%), dillapiole (28.28), carvone (52.25%) and dihydrocarvone. (Lawrence 1981). Zawirska-Wojtasiak *et al.* (1998) studied the aroma profile of dill varieties grown in Poland. They found that carvone and limonene amount to 90–96% of total volatiles content. Other compounds of the oil are α-pinene, α-phellendrene, p-cymene, terpinene-4-ol, dihydrocarvone, eugenol and vanillin. They could establish variability in the organoleptic properties between varieties.

Fennel

Fennel seeds yield about 2–2.5% oil on dry weight basis. Fennel seeds have fragrant odour and pleasant aromatic taste. There are two types of fennel – common fennel and sweet fennel. Common fennel (*Foeniculum vulgare* Mill) contains 2.5–6.5% volatile oil. The oil is a colourless to pale yellow liquid with an aromatic, spicy

Table 11.13 Composition of dill volatiles extracted by different methods

No.	Compound	Amounts (%)			
		Solvent extraction	Hydro-distillation	CO_2-extraction	Head-space
1.	α-Pinene	0.5	1.1	0.2	1.4
2.	α-Phellandrene	22.3	32.1	6.8	16.1
3.	Limonene	1.5	2.5	0.7	3.2
4.	β-Phellandrene	3.7	5.6	1.7	5.5
5.	ρ-Cymene	2.1	5.7	1.8	13.5
6.	3,6-Dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran	27.1	36.7	33.2	38.5
7.	Carvone	0.7	0.8	1.0	t
8.	Neophytadiene	14.0	tr	19.9	
9.	Myristicin	3.3	2.9	5.8	4.7
10.	Apiol	3.1	0.6	4.2	

Source: Huopalahti *et al.* (1988).

odour. The major component in the seed oil is anethole. The herb oil of fennel contains α-phellandrene, pinenes, anethole and methyl chavicol. Bitter fennel oil is obtained from *F. vulgare* var. vulgare which is cultivated in Europe. Sweet fennel (*F. vulgare* Mill var. dulce) is mainly cultivated in France and Italy. It is also known as Roman or French oil. The essential oil is yellowish green liquid with characteristic Anise odour.

Naves and Tucakov (1959) reported that Yugoslavian fennel oil contained *trans*-anethole (50–80%), *cis*-anethole (>0.3%), methyl chavicol (3–20%) and fenchone (0.7–2.2%). Indian fennel oil was found to contain 1,8-cineole (1.95%), linalool (7.98%), safrole (3.67%), anisaldehyde (8.72%), anethole (64.88%) and methyl chavicol (1.94%) (Srinivas 1986, Raina *et al.* 2004). The main constituents are anethole (50–60%) and fenchone (10–25%) (Agrawal 2001). Yamini *et al*. (2002) compared the compositions of hydrodistilled and supercritical CO_2 extracted oils from the fennel seeds from Iran with those of France and Spain. Both contained anethole as the major component, but at higher temperatures and pressures higher solubility of anethole was noticed (Table 11.14). The major compounds in the oils from Iran and Spain contained anethole and limonene, but the oil from Iran was richer in E-anethole whereas the Spanish oil contained relatively higher amount of limonene. The oil from France was markedly different from both these oils. The French oil was dominated by limonene with traces of E-anethole.

Fenugreek

Fenugreek has been used in Indian folk medicine as an antipyretic, diuretic and suppurative and for treatment of dropsy, heart disease, chronic cough and spleen and liver enlargement (Bhatti *et al.* 1996). Studies on the effect of roasting on the quality of fenugreek seeds indicated that light roasted seeds (150 °C) were superior to those roasted at 175 °C and 200 °C with respect to their flavour (Sankaracharya *et al.* 1973).

Girardon *et al.* (1989) identified 39 components including n-alkanes, sesquiterpenes and some oxygenated compounds in the volatiles of fenugreek. But 3-hydroxy-4,5-dimethyl-2(5H)-furanone, which was earlier proposed as a flavouring component of fenugreek seeds was not identified in the volatiles by Girardon *et al.* (1989). However, the contribution of n-alkanes to the aroma of fenugreek seeds was considered minimal. According to Girardon *et al*. (1989) elemenes, muurolens and γ- and δ-lactones that are present in small quantities could be of great importance in the aroma of seeds because of their olfactory properties. Compared to volatile oil, solvent extracts of fenugreek gave typical flavour of fenugreek and the characteristic compound was identified as 3-hydroxy-4,5-dimethyl-2(5H)-furanone (Girardon *et al.* 1989).

Fresh aerial parts of fenugreek plant yielded 0.3% light yellow oil. The main constituents of the oil were δ-cadinene (27.6%), α-cadinol (12.1%), γ-eudesmol (11.2%) and α-bisabolol (10.5%). Other constituents were α-muurolene (3.9%) liguloxide (7.9%), cubenol (5.7%), α-muurolol (4.2%) and epi-α-bisabolol (5.7%) (Ahmadiani *et al.* 2004).

Ginger

Ginger is valued primarily for its aroma and in some products for its mild pungency. These characters together contribute to the typical ginger flavour. Ginger oil is prepared by steam distillation and the aroma quality and composition will depend on the raw material and the area of cultivation. Agroclimatic conditions play a great role in the

Table 11.14 Volatiles from fennel

Compound	% Composition
α-Pinene	0.76–2.00
Camphene	0.09
Sabinene	0.15–0.89
β-Pinene	0.36
Myrcene	0.58–2.24
α-Phellandrene	0.8–7.67
ρ-Cymene	0.24
Limonene	2.82–52.4
(z)-β-Ocimene	0.79–1.39
γ-Terpinene	0.09–12.1
Fenchone	0.3–11.00
Terpinolene	0.13
Camphor	0.24–0.62
Linalyl propanoate	0.32
Estragole	0.78–4.45
ρ-Anisaldehyde	0.57–2.8
(Z)-Anethole	0.27
(E)-Anethole	0.4–90.14
Copaene	0.1
Germacrene D	0.25–5.26
δ-3-Carene	0.1–0.3
α-Terpinene	0.1
l-Limonene	2.1–3.7
l-Fenchone	7.0–11.6
Methyl chavicol	3.1–10.8
t-Carveol	0.1
carvone	tr
α-Fenchyl acetate	0.1
Safrole	tr
Trans-anethole	73.2–80.4
α-Copaene	tr
n-Tetradecane	0.2–0.9
Anisketone	0.2–0.9
n-Hexadecane	0.2
Dillapiole	0.1
Apiole	tr

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

concentration of these constituents. Dry ginger oil is characterized by the high proportion of sesquiterpene hydrocarbons, predominantly zingiberene, a small percentage of monoterpene hydrocarbons and oxygenated compounds (Govindarajan 1982).

Nishimura (2001) separated odorants from fresh rhizomes of Japanese ginger using the multidimensional GC system and found that monoterpinoids such as linalool, 4-terpineol, isoborneol, borneol, geranial and neral contribute towards the characteristic odour. Bartley and Jacons (2000) described the ginger volatiles from fresh and dry rhizomes. The oil is extracted using supercritical carbon dioxide (Table 11.15). Vernin and Parkanyi (2005) compared chemical composition of commercial oils from India and China. Zingiberene and ar-curcumene levels are on a par in both types.

Mint

Japanese mint (*Mentha arvensis*) popularly known as menthol mint is a source of natural menthol which is widely used in pharmaceutical and flavour industries. Xue-

Table 11.15 Volatile compounds in supercritical fluid extracts of fresh and dried ginger

Compound	Amount fresh (%)	Amount dry (%)
Octane	0.39	0.07
Hexanal	1.58	0.87
α-Pinene	0.35	1.24
Camphene	1.08	2.89
β-Pinene	0.00	0.11
6-Methyl-5-hepten-2-one	0.00	0.04
β-Myrcene	0.30	0.94
Octanal	0.42	0.24
Octan-2-ol	0.13	0.31
Limonene	0.27	0.31
β-Phellandrene	1.30	4.68
Heptyl acetate	0.00	0.11
Terpinolene	0.00	0.12
Linalool	0.41	0.39
Citronellal	0.02	0.14
Isoborneol	0.00	0.11
Borneol	0.73	0.39
Decane	0.00	0.00
Decanal	0.96	0.91
Citronellol	0.76	0.47
Neral	1.46	2.30
Geraniol	3.11	1.14
Geranial	18.47	3.90
Bornyl acetate	0.00	0.04
2-Undecanone	0.11	0.24
Cirtonellyl acetate	0.47	0.77
α-Camphane	0.00	0.17
Geranyl acetate	3.00	5.87
δ-Elemene	0.43	0.60
β-Elemene	0.00	0.14
γ-Elemene	0.16	0.30
(Z)-β-Farnesene	0.15	0.31
(E)-β-Farnesene	0.14	0.17
α-Guaicene	0.02	0.21
ar-Curcumene	1.54	2.29
Germacrene D	0.74	1.26
Zingiberene	13.44	24.58
(E,E)-γ-Farnesene	7.13	14.19
β-Bisabolene	2.49	3.32
γ-Cadinene	0.22	0.19
β-Sesquiphellandrene	5.85	7.64
Elemol	0.80	0.44
Nerolidol	0.38	0.38
α-Bisabolol	0.21	0.15
Sesquisabinene hydrate	0.30	0.29
Zingiberenol	0.15	0.13
Guaicol	0.22	0.14
Zingerone	7.49	3.42
β-Eudesmol	0.21	0.11
Sesquiterpene alcohol	0.64	0.30
Phenyl curcumene	0.05	0.14
6-Paradol	0.50	0.17
6-Shogaol	6.30	2.35
6-Ginger dione	1.92	1.00

Source: Bartley and Jacons (2000).

Qi Han *et al*. (1998) found variation in oil content and menthol content in micropropagated mint plants compared to control. Some somaclones exceeded controls in oil and menthol contents by 27.77% and 8.16–10.86%, respectively. Kumar and Bhatt (1999) found mint oil effective as a bioinsecticide against *Amritodus atkinsoni* and *Scirtothrips mangiferae.* Saxena and Singh (1998) studied the effects of irrigation, mulch and nitrogen on yield and composition of Japanese mint (*Mentha arvensis* subsp. haplocalyx var. piperascens) oil. They found essential oil from the first harvest was richer in menthol (78.8%) than the oil obtained from second harvest (75.2% menthol).

Croteau (1991) reviewed metabolism of monoterpenes in mint (Mentha) species. The biosynthesis and catabolism of C3- and C6-oxygenated p-menthane monoterpenes, cyclization of geranyl pyrophosphate to their precursor (–)-limonene, the metabolism of limonene, the developmental regulation of monoterpene metabolism and its potential role in the defence mechanisms of *Mentha* species are discussed. Monoterpene biosynthesis tends to occur mainly in young leaves; whereas catabolic activities increase at maturity, in parallel with oil gland senescence. It is concluded that for commercial mint oil production a dynamic balance between biosynthetic and catabolic processes is essential.

Spencer *et al.* (1990) evaluated the production of terpenes by differentiated shoot cultures of *Mentha citrata* transformed with *Agrobacterium tumefaciens* T37. The shoot cultures synthesized a mint oil fraction which contained the major terpenes characteristic of the parent plant in quantities similar to those in intact tissue. Oil glands were observed to be present on the leaves of the transformed culture. In the mint condensate they were 1-menthol, menthone and neomenthol (Machale *et al.* 1997).

Essential oil glandular trichomes are the specialized anatomical and structural characteristic of plants accumulating significant quantities of commercially and pharmaceutically valuable essential oil terpenoids. The developmental dynamics of these structures together with the oil secretory process and mechanisms have a direct bearing on the secondary metabolite production, sequestration, and holding potential of the producer systems. The essential oil gland trichomes of menthol mint leaf have been stereologically analyzed to discern their anatomical archetype *vis-à-vis* volatile oil secretion and sequestration as integrated in the overall leaf ontogeny. Cuticular 'dehiscence' or decapping, leading to collapsing of the peltate trichomes was a notable characteristic of the menthol mint oil glands. Ecophysiological, evolutionary, phytopharming and biotechnological connotations of the novel phenomenon have been hypothesized (Sharma *et al.* 2003).

Ozel and Ozguven (2002) conducted field experiments to determine the effect of different planting dates on the essential oil components of different mint varieties (*Mentha arvensis* var. piperascens, *M. piperita* cultivars Mitcham, Eskisehir, and Prilubskaja). The mint oil components, i.e., α-pinene (0.49–1.00%), β-pinene (1.38–2.12%), 1,8-cineole (eucalyptol) (2.64–10.85%), menthone, menthofuran (28.09–49.52%), menthol (22.55–38.89%), pulegone (0.00–1.32%), menthyl acetate (0.46–6.78%), and β-caryophyllene (0.54–2.84%), were determined. The results indicated that the essential oil components were affected by planting date, mint cultivar, and cutting numbers. The highest menthol ratio was obtained from *M. arvensis* var. piperascens (33.50–38.89%) from second cutting and autumn transplanting. Frerot *et al.* (2002) reported a new p-menthane lactone from *Mentha piperita* L 3,6-dimethyl-4,5,6,7-tetrahydro-benzo(b)-furan-2(3H)-one (Menthofurolactone)

Nutmeg

Dried nutmeg and mace are used as spices and also for extracting oil and oleoresins. Mallavarapu and Ramesh (1998) indicated the nutmeg oil composition as follows: α-thujene (2.2%), α-pinene (13.6%), camphene (0.3%), sabinene (32.1%), β-pinene (12.9%), myrcene (2.2%), δ-3-carene (0.8%), α-phellandrene (0.7%), α-terpinene (2.2%), p-cymene (0.7%), limonene (4.0%), 1,8-cineole + β-phellandrene (2.3%), γ-terpinene (3.9%), trans-sabinene hydrate (0.5%), terpinolene (1.2%), linalool (0.8%), cis-p-menth-2-en-1-ol (0.4%), trans-p-menth-2-en-1-ol (0.3%), terpinen-4-ol (7.2%), α-terpineol (0.8%), safrole (2.8%), eugenol (0.4%), methyl eugenol (1.6%), β-cubebene (0.1%), β-caryophyllene (0.2%), trans-α-bergamotene (0.1%), (E)- methyl isoeugenol (0.2%), germacrene D (0.1%), myristicin (2.6%) and elemicin (2.4%).

Lawrence (2000) compared the oil composition from various sources such as the West Indian nutmeg oils, fresh and dried nutmeg pericarp oil and mace oil using different GC stationary phases. Gopalakrishnan (1992) studied the chemical composition of nutmeg and mace oil. β-pinene and sabinene dominated in both the oils (Table 11.16). Maya *et al.* (2004) reported myristicin as high as 45% in Indian nutmeg oil and 36.6% in Indian mace oil. Mallavarapu and Ramesh (1998) reported nutmeg oil having 76.8% monoterpenes, 12.1% oxygenated monoterpenes and 9.8% phenyl propanoid ether. They also reported mace oil with 51.2% monoterpenes, 30.3% oxygenated monoterpenes and 18.8% phenyl propanoid ether. Their study indicated that in quality, Indian nutmeg oils are intermediate between East Indian and West Indian oils.

Ehlers *et al*. (1998) using HPLC analyzed nutmeg and mace oils produced by supercritical CO_2 extraction and compared it with steam distilled oils and also with oils of East Indian, West Indian and Papuan origin. Myristicin in nutmeg oil of East Indies ranged from 17.5–25.9% and West Indies 2.8–3.7%. Mace oil of whole blades from East Indies contain myristicin 19.1–24.6%, West Indies 4.4–9.1% and that of Papua 1.1–1.4%. Oil yield from raw material was high in the supercritical extraction. Myristicin, the hallucinogenic principle of nutmeg oil, was high in the steam distilled oil. Safrole content in the nutmeg and mace oil of the East Indies ranged from 2.5–3.7% while safrole was very high in the mace oil from Papua (20.5–30.7%). Elemicin was high in the West Indies (3.9–10.1%) and Papua oils (2.1–3.0) compared to East Indian oil (nutmeg: 0.5–1.5%, mace 0.4–0.7%).

Rosemary

Rosemarius officinalis is an aromatic plant, widely used in the pharmaceutical, perfumery and food industries. Steam distillation of the fresh leaves and flowering tops yield 1–2% oil (Boutekedjiret *et al.* 1997). The main constituents of rosemary oil are α-pinene, camphor, cineole, borneol and bornyl acetate. Wide variability occurs in the chemical composition of rosemary oil of different countries (Arnold *et al*. 1997, Dellacassa *et al.* 1999, Fournier *et al.* 1989, Lawrence 1995). Mainly there are two types of rosemary oil in trade, Tunisian and Moroccan, having 1,8 cineole (38–55%) and Spanish with camphor (12.5–22.0%) and cineole (17–25%) (Arnold *et al.* 1997, Mallavarapu 2000). The leaves of rosemary grown in the Kumaon hills of Uttaranchal contained 0.25–0.52% volatile oil on fresh weight basis (Kumar *et al*. 2004). The chief components of oil were α-pinene (14.90%), 1,8-cineole (17.50%), camphor (12.7%), borneol (5.50%) and verbenone (11.00%) (Table 11.17).

Studies conducted to determine the effect of different temperatures during the drying process on the amount and quality of essential oils of rosemary (*Rosmarinus*

Table 11.16 Composition of nutmeg and mace oil

Compound	Composition	
	Nutmeg oil	Mace oil
α-Pinene	14.72	15.24
β-Pinene + Sabinene	62.66	45.52
α-Phellandrene	3.06	3.17
Δ^3-Carene	0.60	0.67
α-Terpinene + P-Cymene	1.08	3.53
1,8-Cineole + Limonene	6.18	6.97
β-Phellandrene	1.08	2.80
γ-Terpinene	0.54	1.83
Linalool + Terpinolene	0.48	0.42
β-Terpineol	0.25	0.32
Borneol (tentative)	0.05	0.16
Terpinen-4-ol	1.85	4.59
α-Terpineol + Piperitol	0.36	0.94
Geraniol	0.02	0.22
Safrole + p-Cymene-8-ol	0.53	0.67
Bornyl acetate	0.07	0.09
Methyl eugenol	0.14	0.22
Eugenol + terpenyl acetate	0.22	0.15
Geranyl acetate + α-Copaene	0.29	0.16
Isoeugenol (*cis*)	0.31	0.45
β-Caryophyllene + isoeugenol (*trans*)	0.07	0.07
α-Humulene	0.02	0.03
δ-Cadinene	0.08	0.15
Myristicin	3.28	5.92
Elemicin	1.38	3.14
Myristic acid	0.01	0.01
Trimyristin	0.06	0.05

Source: Gopalakrishnan (1992).

officinalis) indicated that higher drying temperature decreased the essential oil content (% v/w) from 2.13 (40 °C) to 1.62 (60 °C) and 1.09% (80 °C). Essential oil composition was similar, except for camphor at 40 °C and 60 °C. However, concentrations of alpha-pinene, beta-myrcene and camphor were decreased at 40 °C and 80 °C (Blanco *et al*. 2002).

Tucker and Maciarello (1986) reported α-pinene, camphene, 1,8-cineole, camphor, bornyl acetate and borneol as the major compounds in five varieties of rosemary oil. Rosemary oil from Argentina contained 20 components of which the major ones were α-pinene, myrcene, 1,8-cineole, camphor and β-caryophyllene (Mizaahi *et al*. 1991). Lawrence (1995) reported that rosemary oil from Spain and Portugal contained 30–50% oxygenated monoterpenes where as the oils of Moroccan, Tunisian and Yugoslavian origin contained 70–80% oxygenated monoterpenes.

Rao *et al*. (1998) compared the oil loss in rosemary leaves by convection and microwave drying methods. The loss of volatile oil was less (7.25%) during convection drying while microwave drying led to a loss of 61.45%. The volatile oil of fresh rosemary contained mostly monoterpenes and their derivatives (95–98%). The major components of rosemary leaf oil were camphor (23.9–34.0%) and 1,8-cineole (15.5–29.8%).

Boutekedjiret *et al*. (2003) reported that oil yield from the hydrodistilled herbage

Table 11.17 Volatiles from rosemary

Compound	% Composition
α-Pinene	5.5–26.0
Camphene	1.5–13.0
β-Pinene	2.60
Myrcene	1.50
Δ-3-Carene	2.30
Limonene	2.80
p-Cymene	1.80
1,8-Cineole	9.4–55.0
γ-Terpinene	1.30
Terpinolene	0.50
Linalool	0.5–4.9
Camphor	5.0-26.4
Isoborneol	0.30
Borneol	1.1–5.5
Dihydrocarveol	0.13
Verbenone	0.0–14.1
Linalyl acetate	1.40
Bornyl acetate	1.90
β-Caryophyllene	1.40
α-Humulene	1.20
Methyl isoeugenol	0.50
Trans-β-farnesene	0.40
γ-Muurolene	0.30
δ-Candinene	1.20
β-Sesquiphyllandrene	1.30
Carophyllene oxide	0.60
Humulene epoxide	0.06
α-Bisabolol	0.78
Unidentified	1.93

Source: Kumar *et al.* (2004).

was lower (0.44%) than that of steam distilled herbage (1.2%). The steam distilled oil contained 52.4% 1,8-cineole where as the hydrodistilled oil contained much less (31.9%) cineole. The contents of camphor (19.7%), borneol (12.1%) and α-terpineol (12.8%) were higher in hydrodistilled oil compared to the steam distilled oil.

Spearmint

Tsuneya *et al.* (1998) studied acidic components in Scotch spearmint oil (*Mentha gracilis* Sole) and 46 acidic components (including 35 carboxylic acids and 11 phenols) were identified. Three carboxylic acids peculiar to *M. gracilis* were identified from spectral data: *cis*-2-pentylcyclopropane-1-carboxylic acid, 3-isopropenylpentane-1,5-dioic acid and 3-isopropenyl-6-oxoheptanoic acid.

Platin *et al.* (1994) studied equilibrium distributions of key components of spearmint oil in sub/supercritical carbon dioxide. Effects of temperature (at 35 °C, 45 °C or 55 °C) and pressure (10–110 atm) on the relative distribution coefficients of 12 key components (6 monoterpenes, 3 monoterpenoids and 3 sesquiterpenes) of spearmint oil (essential oil of *Mentha cardiaca* (*M. gracilis*); Scotch spearmint) at equilibrium in dense CO_2 were investigated under conditions ranging from subcritical to supercritical regions. At 35 °C all key components of spearmint oil were equally soluble in dense CO_2 within the 12–102 atm pressure region. Vapour-pressure effects, coupled with

the decrease in solvating power, dominated the effects of polarity and molecular mass of the key components. The quality of essential oils decreased with increasing fraction of monoterpenes, and it is concluded that deterpenation of spearmint oil with dense CO_2 is possible either at 45 °C/27 atm or 55 °C/35 atm, where the monoterpene hydrocarbons tend to concentrate, and can be preferentially recovered.

Ishihara *et al.* (1992) reported new pyridine derivatives and basic components in spearmint oil (*Mentha gentilis* f. cardiaca) and peppermint oil (*Mentha piperita*). A total of 38 nitrogen-containing components including 11 new pyridine derivatives, 2-isopropyl-4-methylpyridine, 4-isopropenyl-2-methylpyridine, 2-ethyl-4-isopropenylpyridine, 2-acetyl-4-isopropylpyridine, 2,4-diisopropenylpyridine, 2-acetyl-4-isopropenylpyridine, 4-acetyl-2-isopropenylpyridine, 5-[(Z)-1-buten-1-yl]-2-propylpyridine, 5-[(E)-1-buten-1-yl]-2-propylpyridine, 3-[(Z)-1-buten-1-yl]-4-propylpyridine and 3-[(E)-1-buten-1-yl]-4-propylpyridine, were identified by comparing their spectroscopic data with those of synthetic samples. Among them, 2-acetyl-4-isopropenylpyridine was a major component with a powerful grassy-sweet and minty odour.

Ringer *et al.* (2005) made a detailed review on monoterpene metabolism, cloning, expression and characterization of (–)-isopiperitenol/(–)-carveol dehydrogenase of peppermint and spearmint. They stated that the isolation of the genes specifying redox enzymes of monoterpene biosynthesis in mint indicates that these genes arose from different ancestors and not by simple duplication and differentiation of a common progenitor, as might have been anticipated based on the common reaction chemistry and structural similarity of the substrate monoterpenes. The full-length spearmint dehydrogenase shares >99% amino acid identity with its peppermint homolog and both dehydrogenases are capable of utilizing (–)-*trans*-isopiperitenol and (–)-*trans*-carveol. These isopiperitenol/carveol dehydrogenases are members of the short-chain dehydrogenase/reductase superfamily and are related to other plant short-chain dehydrogenases/reductases involved in secondary metabolism (lignan biosynthesis), stress responses, and phytosteroid biosynthesis, but they are quite dissimilar (approximately 13% identity) to the monoterpene reductases of mint involved in (–)-menthol biosynthesis.

The undesirable top notes or off-notes found in mint, clary sage, and cedarwood oils could be quantitatively determined using a non-equilibrated solid phase microextraction/gas chromatography/selected ion monitoring/mass spectrometry (SPME/GC/SIM-MS) technique. Using the low threshold components, dimethyl sulfide, 2-methylpropanal, 2-methylbutanal, and 3-methylbutanal, which are associated with the off-notes of these oils, their levels could be quantitatively determined. The highest level of off-notes was found in a sample of Scotch spearmint oil where the levels of the four constituents were, dimethyl sulfide (238 μg g^{-1}), 2-methylpropanal (286 μg g^{-1}), 2-methylbutanal (1048 μg g^{-1}) and 3-methylbutanal (1489 μg g^{-1}). These quantitative results in combination with sensory evaluations could provide for a powerful overall assessment of essential oil quality (Coleman *et al.* 2004).

A study was conducted to identify the fragrance compounds of *Mentha spicata* oil from Cameroon and its solid-phase microextraction (SPME)-headspace by means of gas chromatograph spectroscopy (GC and GC-MS) and olfactoric methods (GC-sniffing technique and olfactory correlations) to determine the importance of each single constituent with their specific odour attributes. The odour impression was very pleasant in spearmint, with green, floral, fruity, and spicy side notes. The composition of the spearmint essential oil and its corresponding SPME-headspace sample was

very similar and differed only in the concentrations of the main compounds, namely, (–)-limonene (essential oil: 6.55%, SPME: 8.31%), 1,8-cineole (4.19%, 7.12%) and *trans*-1-hexen-3-ol (0.66%, 1.72%). In addition to the composition of both samples, the olfactory evaluations certify a high quality of the essential oil and its possible use in food, perfumery, and cosmetic products requiring a fresh-spearmint odour (Jirovetz *et al*. 2002).

The major chemical constituents of the hydrodistilled essential oil and their major isolates from cultivated *M. spicata* was identified by IR, ^{1}H- and ^{13}C-NMR and GC. (S)-(–)-limonene (27.3%) and (S)-(–)-carvone (56.6%) (representing 83.9% of the spearmint oil) and (R)-(+)-limonene (21.4%), dihydrocarvone (5.0%), (R)-(+)-carvone (50.4%) and dillapiole (17.7%), respectively. *In vitro* biological activity evaluation of the isolated oil components revealed that both the optical isomers of carvone were active against a wide spectrum of human pathogenic fungi and bacteria tested. (R)-(+)-Limonene showed comparable bioactivity profile over the (S)-(–)-isomer. The activity of these monoterpene enantiomers was found to be comparable to the bioactivity of the oils in which they occurred (Aggarwal *et al.* 2002).

Thyme

The volatile oil of Egyptian *T. vulgaris* was richer in linalool and terpene hydrocarbons. The oil contained thymol and carvacrol in only moderate concentrations. The highest thymol and carvacrol concentrations were observed during the beginning of flowering (Karawya and Hifnawy 1974). Commercial samples of Ethiopian thyme (*T. schimperi*) contained carvacrol and thymol (Lemordant 1986).

Oszagyan *et al.* (1996) compared the composition of steam distilled and SFE oils. SFE product contained 10–15% thymol and 30–35% carvacrol while steam distilled oil contained 48–50% thymol and 8–10% carvacrol. Cuban thyme oil contained thymol (34.6%), γ-terpinene (17.61%) and p-cymene (17.65%) as major components (Pino *et al.* 1997). Fresh plant material from Bulgarian thyme (*T. vulgaris*) yielded 0.46% essential oil (Stoeva *et al*. 2001).

Studies on the effect of harvest time on yield and oil composition of thyme (*T. mongolicus*) indicated that the best time of harvest for the highest oil yield and high thymol and carvacrol content was during or immediately after the full bloom (Fan-ming and Chen-Jin 2002). Asllani and Toska (2003) evaluated Albanian thyme oils, which were dominated by p-cymene (7.76–43.75%), γ-terpinene (4.20–27.62%), thymol (21.38–60.15%) carvacrol (1.15–3.04%) and β-caryophyllene (1.30–3.07). Thyme (*T. pulegioides*) growing wild in Lithuania contained five chemotypes (i) linalool type, (ii) geranial/geraniol/neral type, (iii) thymol type, (iv) carvacrol/γ-terpinene/p-cymene type and (v) thymol/carvacrol/p-cyme/γ-terpinene type (Loziene *et al.* 2003).

The constituents of essential oils isolated by hydrodistillation of aerial parts of *Satureja hortensis*, used as thyme in Turkey recorded α-terpinene (2.34 and 2.66%), p-cymene (21.82 and 14.64%), γ-terpinene (18.92 and 23.09%) and β-caryopyllene (3.75 and 4.56%), as the main components (Ozcan and Chalchat 2004). Commercial essential oils of thyme from different geographical areas of Italy and France were rich in thymol (22–38%) and its biogenetic precursors, namely, γ-terpinene and p-cymene (Zambonelli *et al.* 2004). The main constituents of the hydro-distilled essential oil from the herb of lemon thyme (*Thymus citriodorus* L.) cultivated in Iran were geraniol (54.4%), geranial (13.9%), neral (10.1%), nerol (5.2%), 3-octanone (3.3%) and borneol (3.2%) (Omidbaigi *et al*. 2005).

Turmeric

Volatile oils are extracted from rhizomes and leaves of turmeric. The chemical composition of volatiles from various parts of turmeric has been investigated extensively. The oil yield and composition show wide variation depending on geographic conditions, variety, agronomic practices, maturity at harvest and post-harvest processing. GC-MS analysis of the oil indicated the presence of as many as 84 components in the oil in varying levels. Volatile oil content in turmeric rhizomes ranged from 1.3–5.5% (Guenther 1961b). The chief constituents of rhizome oil were turmerone, ar-turmerone and turmerol (Govindarajan 1980). The rhizome oil contained limonene, cineole, curcumene, zingiberene, bisabolene, β-phellandrene, ar-turmerone and turmerone (Gopalam and Ratnambal, 1987). The rhizome oil of Indonesian origin was constituted by the following compounds: ar-turmerone (41.4%), turmerone (29.5%), turmerol (10%) and α-atlantone (2.4%) (Zwaving and Bos, 1992).

Nigam and Ahmad (1991) reported 59.7% ar-turmerone in the rhizome oil. The oil from Malaysian rhizomes was dominated by α-turmerone (45.3%), linalool (14.9%) and β-turmerone (13.5%) (Ibrahim *et al.* 1999). Among six turmeric cultivars grown in Maharashtra namely, Rajapuri, Krishna, Mydukur, Salem, Tekurpetta and Armoor, the highest essential oil contents were recorded in mother rhizomes of Mydukur and fingers of Salem (Rakhunde *et al.* 1998). Garg *et al.* (1999) reported that oil content in the rhizomes of 27 accessions from North Indian Plains varied between 0.16% and 1.94% on fresh weight basis. Based on the contents of β-pinene, p-cymene, α-curcumene, β-curcumene, ar-turmerone, α-turmerone and β-turmerone the accessions were classified into two groups: (i) those in which the sum of the seven major terpenes was in the range 58–79%, (ii) those in which the sum was 10–22%. The rhizome oil from Bhutan was constituted by 30–32% α-turmerone, 17–26% ar-turmerone and 15–18% β-turmerone (Sharma *et al.* 1997).

Gopalan *et al*. (2000) noticed that during supercritical carbon dioxide extraction, the solubility of turmeric oil was maximum at 313–333 K and 20–40 MPa and about 60% of the oil was composed of turmerone and ar-turmerone. Fresh rhizome oil from Pakistan was abundant in ar-turmerone (31.1–41.2%) and turmerone (9–11.1%) (Riaz *et al*. 2000). Iron deficiency significantly increased the essential oil and curcumin contents in turmeric rhizomes (Dixit *et al.* 1999).

Chatterjee *et al.* (2000) reported that no detectable differences were observed in the aroma impact compounds of γ-irradiated and commercial volatile oils. The rhizome oil of *C. longa* cv. Roma from North Indian Plains was rich in 1,8-cineole (11.2%), α-turmerone (11.1%), β-caryophyllene (9.8%), ar-turmerone (7.3%) and β-sesquiphellandrene (7.1%) (Raina *et al.* 2002). The rhizome essential oils of *C. longa* cv Roma grown in Indo-Gangetic plains were rich in α- and β-turmerones (40.8%), mycrene (12.6%), 1,8-cineole (7.7%) and p-cymene (3.8%) (Bansal *et al.* 2002). The turmeric oils from Calicut (South India) was dominated by ar-turmerone (31.1%), turmerone (10.0%), curlone (10.6%), ar-curcumene (6.3%), p-cymene (3.0%), β-sesquiphellandrene (2.6%), β-phellandrene (2.4%) and dehydrocurcumene (2.2%). The root oil also contained ar-turmerone (46.8%) as the chief component followed by ar-curcumene (7.0%), dehydrocurcumene (4.3%) and p-cymene (3.3%) (Leela *et al*. 2002). The rhizomes from Reunion Island yielded 1.1% oil, which contained ar-turmerone (21.4%), terpinolene (15.8%), zingiberene (11.8%), ar-turmerol (7.7%), β-turmerone (7.1%), sesquiphellandrene (8.8%) and β-caryophyllene (5.7%) as major compounds (Chane-Ming *et al*. 2002) (Table 11.18).

The essential oil from Cuban rhizomes was reported to contain 47.7% ar-turmerone

Table 11.18 Chemical composition (%) of essential oils of rhizome, leaves and flowers of *Curcuma longa* L. from Reunion Island (HP-5 column)

Compounds	Rhizomes	Leaves	Flowers
Tricyclene	–	0.1	0.1
α-Pinene	0.2	0.7	–
α-Fenchene	–	0.1	0.8
Sabinine	–	0.1	0.1
β-Pinene	–	0.7	0.6
Myrcene	0.3	1.4	2.1
δ-2-Carene	–	0.1	0.2
α-Phellandrene	1.0	2.8	3.6
δ-3-Carene	0.3	1.2	1.7
α-Terpinene	1.4	3.7	4.4
p-Cymene	0.6	0.3	0.4
Limonene	–	–	0.4
1,8-Cineole	2.0	4.6	4.6
(Z)-β-Ocimene	–	0.4	0.8
(E)-β-Ocimene	–	0.7	1.8
γ-Terpinene	–	0.4	0.8
p-Cymenene	0.4	–	–
Terpinolene	15.8	76.8	67.4
Linalool	–	0.7	0.5
p-Mentha-1,3,8-triene	–	0.2	0.3
p-Cymen-7-ol	–	–	0.2
Terpinen-4-ol	0.2	–	–
p-Cymen-8-ol	–	0.2	0.3
α-Terpineol	–	0.3	0.3
2-Undecanone	–	–	0.2
Geranyl acetate	–	–	0.1
Cis-α-bergamotene	0.3	–	–
β-Caryophyllene	5.7	0.1	0.2
α-Humulene	1.4	–	–
(E)-β-Farnesene	0.6	0.1	0.1
ar-Curcumene	4.5	0.1	0.1
Zingiberene	11.8	1.0	1.3
β-Bisabolene	1.9	0.1	0.2
β-Sesquiphellandrene	8.8	0.4	0.5
(E)-γ-Bisabolene	0.7	–	–
(E)-Nerolidol	0.2	0.1	0.2
ar-Turmerol	0.3	–	–
ar-Dehydro-turmerone	0.6	–	–
ar-Turmerone	7.7	–	–
α-Turmerone	21.4	–	–
β-Turmerone	7.1	–	–
(Z)-γ-Atlantone	–	0.1	0.9
Germacrone	–	0.1	0.1
Curcuphenol	0.2	–	–

Source: Chane-Ming *et al.* (2002).

and 16.1% turmerone as major compounds (Pino *et al.* 2003). Turmeric rhizomes from Gorakhpur region (North India) was reported to contain 1.6% oil and ar-turmerone, ar-turmerol, β-bisabolene and zingiberene as chief components (Singh *et al.* 2003). Rhizome oil extracted by the solid phase microextraction method contained ar-curcumene, ar-turmerone, zingiberene, β-sesquiphellandrene, sabinene, 1,8-cineole and 1,4-terpineol as major components (Mata *et al.* 2004). The rhizome oil of *Curcuma*

longa from the lower Himalayan region was rich in α-turmerone (44.1%), β-turmerone (18.5%) and ar-turmerone (5.4%) (Raina *et al*. 2005).

The leaves of turmeric yield 0.37–2.5% volatile oil. The leaf oil from Nigeria contained mainly monoterpenes with 47.7% α-phellandrene and 28.9% terpinolene (Oguntimein *et al*. 1990). The leaf oil from Kerala (South India) was dominated by 56.7% α-phellandrene and 11.8% tepinolene (McCarron *et al.* 1995). The leaf oil of Vietnam origin contained 2.5% oil (dry weight basis) and was dominated by the monoterpenes, α-phellandrene (24.5%), 1,8-cineole (15.9%), p-cymene (13.2%) and β-pinene (8.9%) (Dung *et al*. 1995). The leaf oil from Bhutan was dominated by α-phellandrene (18.2%), 1,8-cineole (14.6%) and p-cymene (13.3%) (Sharma *et al.* 1997). The turmeric leaves from South India yielded 1.3% volatile oil. The oil was dominated by α-phellandrene (32.6%), terpinolene (26.0%), 1,8-cineole (6.5%) and p-cymeme (5.9%) (Leela *et al*. 2002). The leaf petiole and lamina oils of *C. longa* cv. Roma were rich in myrcene (35.9%), 1,8-cineole (12.1%) and p-cymene (12.7%) (Bansal *et al.* 2002). *C. longa* leaf oil from North Indian Plains was mainly constituted by p-cymene (25.4%) 1,8 cineole (18%), *cis*-sabinol (7.4%) and β-pinene (6.3%) (Garg *et al.* 2002).

The leaf oil of *C. longa* cv. Roma contained terpinolene (26.4%) 1,8-cineole (9.5%), α-phellandrene (8%) and terpinene-4-ol (7.4%) as chief constituents (Raina *et al.* 2002). The leaf oil of *C. longa* var Rasmi from Orissa was reported to contain α-phellandrene (38.24%), C-8 aldehyde (20.58%), 1,8-cineole (8.64%), α-pinene (2.88%) and β-pinene (2.36%) as chief constituents (Behura *et al.* 2002). The fresh leaves of Bhutan origin contained 0.37% to 0.42% oil and the main constituents were α-phellandrene (18.2%), 1,8-cineole (14.6%) and p-cymene (13.3%) and terpinolene (11.6%) (Sharma *et al*. 1997). The leaves of turmeric from Reunion Island yielded 0.5% volatile oil. The major constituent in the leaf oil was terpinolene and it differs from the oils of other origins in its high level of terpinolene (76.8%) and its small amount of phellandrene (2.8%) (Chane-Ming *et al*. 2002) (Table 11.18). The leaf oil of turmeric from the lower Himalayan region contained α-phellandrene (53.4%), terpinolene (11.5%) and 1,8-cineole (10.5%) as major constituents (Raina *et al*. 2005).

Freshly harvested flowers of turmeric from South India yielded 0.3% volatile oil. Twenty-five components contributing to 52% of the oil were identified among which p-cymen-8-ol (26%), terpinolene (7.4%) and 1,8-cineole (4.1%) were the major components (Leela *et al.* 2002). The flowers of *C. longa* from Reunion island contained 0.1% volatile oil and the oil was dominated by terpinolene (67.4%), 1,8-cineole (4.6%), α-terpinene (4.4%), α-phellandrene (3.6%) and myrcene (2.1%) (Chane-Ming *et al.* 2002) (Table 11.18).

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Part III

Particular herbs and spices

12

Asafetida

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12.1 Introduction

Asafoetida or Asafetida is the dried latex, oleogum or oleoresin exuded from the taproots of perennial herbs belonging to many species of the genus *Ferula,* of the family Umbeliferacaea (Pruthi 1976). There are about 60 species of the genus *Ferula*, found mainly in three geographical areas, Central Asia, Europe and North Africa. Central Asia is the main source of asafetida and Afghanistan and Iran are the major producers in this region. The trade name, asafetida, is based on the scientific name of one of the most important species, *Ferula asafetida* (Duke 2003). The commercially important species of *Ferula* have been reviewed by Raghavan *et al*. (1974) and are summarized in Table 12.1.

12.1.1 Botany

Commercial asafetida is obtained mainly from *F. asafetida* and also from *F. narthex,* as well as a few other species. While *F. asafetida* is grown extensively in Iran and Afghanistan, *F. narthex* is found in the dry valleys of the Ladakh region in Kashmir, at an altitude of 4000 m. The oleogum of *F. narthex*, though not true asafetida, is used as a substitute for asafetida in India. *F. asafetida* is indigenous to Iran (Andi *et al.* 1997).

The asafetida plant has a perennial fusiform root that is several centimetres in diameter, with a coarse, hairy summit, either simple like a parsnip or with one or more forks. The bark is wrinkled and blackish, and the internal structure is fleshy and white, containing a large amount of thick, milky, fetid, alliaceous juice. The leaves are few in number, radical, and appear in the autumn. They grow to about 45 cm in length during winter and wither by the end of spring. The leaves are shiny, coriaceous like those of lovage, glaucous green, and pinnated with pinnatifid segments whose lobes are oblong and obtuse. The petioles are terete and channelled only at the base. The stem is herbaceous, 2.5 m to 3.25 m long and about 15 cm in circumference at the base, solid, smooth and clothed with membranous sheaths.

Table 12.1 Commercial species of *Ferula*

Species	Where found	Remarks
F. alliacea Boiss.	Iran	Gum is used as an intestinal antiseptic and carminative and also for hysteria and epilepsy
F. asafoetida Linn.	Iran, Afghanistan, Kashmir, Punjab and Africa	Resin is amorphous and reddish brown. It is used for flavouring sauces and curries and also as an expectorant, laxative and antispasmodic
F. communis Linn.	N. Africa	Source of a gum known as 'Ammoniac of Morocco' used for medicinal purposes in Europe
F. foetida (Bunge) Reget	S. Turkey, Iran and Afghanistan	Same as *F. asafetida*
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.	–	Used for medicinal purposes in Syria
F. jaeschkeana Vatke.	Kashmir and Turkey	Source of a gum resin
F. marmarica Asch..and Taub	N. Africa	Source of gum 'amariac of Cyrenaica'
F. narthex Boiss.	West Tibet and Ladakh region of Kashmir	Used as a spice because of the flavour
F. orientalis Linn.	–	Used for medicinal purposes in the Middle East
F. persica Wild	Iran	Gum (sagapenum) is sold as 'tears' or 'masses'
F. rubricaulis Boiss.	–	Used for medicinal purposes in Iran
F. schair Brosz	Turkey	Probably a source of galbanum. Used for medicinal purposes in Europe
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 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

There are two kinds of plant, male and female. The female plant produces inflorescences whereas the male plant does not. Only the female plant produces the oleogum or asafetida (Pruthi 2001). The stalk of the inflorescence is big and leafless. The umbels have 10 to 20 rays and partial ones have 5 or 6 flowers. The flowers are pale yellow, succeeded by a flat, thin, reddish-brown fruit, like that of parsnip but larger and darker, slightly hairy and rough.

The carrot-like taproots attain a diameter of 12 to 15 cm at the crown after four to five years of growth. At this stage, the plant is ready for the commercial extraction of asafetida. The fruits are 0.8 cm long and 0.6 cm broad, with tender hairs. The flowers and fruits generally appear in March–April. The white exudate of the fruit is fragrant, pure and crystalline. The brownish to reddish exudate smells foul. Today there are many varieties of exudates in the market sourced from different species and using different collection procedures. It is reported that some unscrupulous nurserymen in Kerala, India, sell seedlings of *Gardenia gummifera* as asafetida plants as they have an exudation with a similar odour, but this plant does not yield commercial asafetida (George 1995).

Ushak is similar to asafetida and obtained from another genus of the family umbelliferarae, *Doremia ammoniacum* Don. or *D. aureum.* Ammon is a God of ancient Rome, Egypt and Greece. This particular plant was found in all the places

where there was a temple of Ammon. Dioscoides, the Greek herbalist, first described this plant scientifically and named it ammoniacoon. The present botanical name, *Doremia ammoniacum* is derived accordingly. It is available in the bazaars of Mumbai, India, and sold elsewhere as Bombay Sambal. *Dormia ammoniacum* is a shrub and the gum is found on its flowering and fruiting branches.

12.1.2 Forms of asafetida

Asafetida is available commercially in three forms, tears, mass and paste. Tears constitute the purest form of 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 those that gradually become dark or yellowish brown. Mass asafetida is the common commercial form. It comprises tears agglutinated into a more or less uniform mass, often mixed with fragments of root, soil, etc. Paste also contains extraneous matter (Anon. 1991).

12.1.3 Varieties of asafetida

There are many varieties of asafetida, and they come under different classifications and are priced differently. The two major varieties are *Hing* and *Hingra. Hingra* is inferior to *Hing,* which is richer in odour and more desirable. *Hingra* is heterogeneous in colour and consistency. *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* contains woody residues but *Pathani Hing* is comparatively free from wood. *Hadda* is the most expensive variety of *Pathani Hing* and has the strongest odour. *Irani Hing* has two varieties based on the taste, sweet and bitter. Sweet *Irani Hing* is collected from the horizontal cutting of the stem and is brown in colour. The *Irani Hing* obtained from the root is transparent and it is gathered by making injuries on the root. Bitter *Irani Hing* is conventionally produced in Iran (Anon. 1991).

The two most commonly sold and broadly recognized groups of asafetida are the white or pale variety and the dark or black variety. The former is soluble in water while the latter is soluble in oil. The chemical composition of both these types of asafetida is almost the same, because asafetida is basically only an oleogum. But, where the gum portion preponderates, as in *Hing*, it is water-soluble and where the resin portion preponderates, as in *Hingra*, it is oil-soluble. The constituents to which asafetida owes its characteristic ordour reside in the oil. There are two groups of compounds in the oil, one group belongs to the ferulic esters and the other, which is more important, is a volatile oil fraction consisting of different sulphur compounds, some of which are similar to those found in garlic and onion. The major difference in their origin is that *Hingra* is obtained from *F. foetida* while *Hing* is obtained from *F. asafetida* (Anon. 1991).

12.1.4 Area and production

There is no reliable information on the area under asafetida or the amount produced. This is because production and trade are not organized in any of the producing countries. While the area under asafetida may not decrease suddenly, it being a perennial plant, production can go up and down steeply as demand and price determine the amount of oleogum extracted.

12.2 World trade

Data relating to world trade of asafetida are scarce as it has not been reported separately in the International Trade Classification (Harmonious System). Accordingly there is no reliable information available on the export and import of asafetida for different countries. In India, asafetida is popularly used in some vegetarian dishes and for the preparation of indigenous medicines; hence it is regularly imported from Afghanistan and Iran. Imports of asafetida into India during the five years, 2000–5 are given in Table 12.2.

The importation of asafetida into India is erratic, but growth in imports during 2003–4 and 2004–5 was notable compared to the three previous years, the average of which stood at 643.5 MT. There was 40.19% increase in the amount of asafetida imported during 2003–4 over the average for the previous three years. The value of imported asafetida per kg increased considerably over the five-year period from USD 4.47 during 2000–1 to USD 10.97 in 2004–5.

Some of the imported asafetida is processed in India and re-exported. Unprocessed material may also be exported, depending on the profit margin, but separate export figures are not available for unprocessed and processed asafetida. Processed asafetida is mainly compounded asafetida. The quantity and value of asafetida, including compounded asafetida, exported from India during the five-year period, 2000–5 are shown in Table 12.3.

Exports did not follow a steady pattern. The quantities exported during 2003–4 and 2004–5 were almost the same, but the value went up by 64.29% during 2004–5 over the previous year. Compared to the price of USD 10.97 per kg for the imported asafetida in 2004–5, the export price was only USD 4.40 per kg, as compounding made the product much cheaper.

Table 12.2 Importation of asafetida into India

Year	Quantity in MT	Value in million USD
2000–1	658.0	2.94
2001–2	758.3	3.83
2002–3	514.2	3.18
2003–4	902.1	8.36
2004–5	831.9(E)	9.13

(E): estimate
Source: Spices Board, Government of India, Cochin, India.

Table 12.3 Export of asafetida from India

Year	Quantity in MT	Value in million USD
2000–1	371.6	1.03
2001–2	270.8	0.79
2002–3	473.9	1.00
2003–4	735.3	1.96
2004–5	731.6	3.22

Source: Spices Board, Government of India, Cochin, India.

12.3 Chemical constituents

The different oleogum resins are asafetida, galbanum, sambal, sagapenum and ushak. Of these, asafetida is the most important.

Asafetida consists of organic sulphur compounds, volatile oil, gum resin and impurities. Both tears and masses have the same amount of volatile oil. A sample analysis shows volatile oil at 3.5%, resin 46.6%, asaresinol ferulate 16.67%, free ferulic acid 1.33%, ether insoluble resin 1.0% and gum and impurities 31.0%.

A sequeterpinoid coumarin and two other coumarins (asfoetidin and ferocolicin) can be isolated from the root and gum resin respectively. Three new compounds (asadisulphide, asacoumarin and asacoumarin B) have also been obtained from the root. Six new sulphide derivatives (foetisulphide A, foetisulphide B, foetisulphide C and foetisulphide D, foetithiphene A and foetithiphene B) along with known compounds have also been isolated and named from the ethyl acetate soluble fraction of methanol extract prepared from asafetida (Peter 2004).

Galbanum contains 6–9% essential oil similar to turpene, 60–75% sulphurous resin and 19–22% other exudates, including the principal compound, umbelliferon. Galbanum melts at 100 °C and becomes a thick fluid.

Sagapenum contains 50–54% resin, 31–32% gum and 3–11% essential oil. It can be softened in the palm of the hand by body heat. While the fragrance is fine and not as strong as that of asafetida, the taste is not acceptable.

Sambal is available as bits, which are light in weight and dark in colour outside and yellowish white inside. It has a highly pungent taste and a fibrous appearance. The odour resembles musk or *Kasturi*. It is often adulterated in India, the main market, with roots of *jatamamsi* (*Nardostactys* sp.) or tagar (*Valleriana celtica*).

Ushak is dark coloured on the outside as long as it remains on the plant, *Doremia ammoniacum,* but the inside is milky white to yellow. When cold, it hardens and breaks easily, but on slight warmth it becomes soft and flexible. If mixed with water, it becomes a milky emulsion. It has a pungent taste and the fragrance of frankincense. Ushak contains 20% different exudates, 70% resin and 4% essential oil, moisture and ash.

12.4 Extraction

Before flowering in spring, the female plant puts forth sprouts and foliage from the taproot. After about a month, the green foliage turns yellow. It is at this stage that the taproot of the female plant is tapped for asafetida. The process is described below.

1. Soil and stones surrounding the yellow foliage are removed and the base of the foliage, including the top of the taproot, is exposed.
2. Foliage is pulled out, leaving the base on the upper part of the taproot as a brush-like mass.
3. The brush-like mass is covered with loose earth and gravel. The taproot is 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. This

is then scraped, making an area up to 6.5 cm^2. When scraping is complete, the taproot is shaded using a construction of twigs and stones.

5. Two to three days after scraping, the first flow of sap is collected from the top of the taproot. After this, a slightly deeper cut is made, about 0.5 cm from the top and sap is collected again. A third cut may be made to induce further flow of sap. The process of cutting and collecting sap is continued for 10 to 15 cycles until the flow of sap stops.
6. After each cutting, the taproot is covered with twigs and stones to prevent soil or gravel falling onto the cut surface, and to maintain cool conditions under which the taproot can mature.
7. The sap is stored in pits dug in the soil. The pit size may vary but it is typically 1.8 m long, 1.8m wide and 2.4 m deep. The sides of the pit are plastered with mud and the top covered with stalks of male asafetida plants, leaving an opening of about 0.3 m diameter, through which the daily collection of sap can be poured into the pit.

The asafetida collected in the pit is generally very thick and sticky, and can be moulded into any shape by hand. It continues to mature during storage in the pit (Pruthi 2001), and it is this resin made into tears or mass that is marketed as asafetida. It varies in colour from white to greyish or reddish. White asafetida is packed first in cloth bags and then in jute bags. Dark red asafetida is generally packed in goat- or sheep-skin, where it matures further. It has a powerful foul odour, and bitter and acrid taste due to sulphur compounds, and is called 'Devil's Dung'. Interestingly it is also known as 'Food of the Gods'. All parts of *F. foetida* have the strong asafetida smell.

Some Pathans in Afghanistan collect the resin from wild plants by cutting the above-ground stems. They also chop and boil roots and stems in water and collect the resin by evaporating the water, but the quality of such resin is inferior. The average yield of oleogum is roughly 40 g per plant, but certain plants may yield as much as 900 g (Krishnamurthy 1994).

Galbanum is a gum resin exuded from the lower stems of another species, *F. galbaniflua* Boiss and Buinse, a stout perennial herb of North Western Asia. The gum occurs in the form of distinct irregular tears or masses, is yellow to brown in colour and has a powerful and tenacious aroma.

Sagapenum is similar to asafetida, 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 as broken fragments.

Sambal is the oleoresin gum of *F. sumbal*. It is produced in Iran and the main market is again India.

12.5 Processing

The main processed products from asafetida are oil of asafetida and compounded asafetida. The oil does not have much commercial value. The flavouring and pharmaceutical industries use mainly alcoholic tinctures of the gum resin (Anon. 1991). Oil of asafetida is extracted by steam distillation of the gum resin and yield varies from 3.3 to 3.7%. The chief component of the oil is secondary butylpropenyl

disulphide, along with pinene, terpenine, trisulphide and other compounds. The disagreeable odour of the oil is due to disulphide (Tiwari and Ankur 2004).

Compounded asafetida is a ready-to-use preparation designed in particular for making Indian curries because natural asafetida is very strong and is not used directly in cooking. It is composed of asafetida from one or more origins (Irani or Pathani or both) and gum arabic, with edible starch or edible cereal flour. The blending formula varies from manufacturer to manufacturer and is a trade secret.

12.6 Quality issues

Asafetida is one of the most adulterated agricultural products in the world. It is not strange to find clay, sand, stone or sometimes gypsum added to increase the weight. Other adulterants used 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 the same genus, are supplied to buyers who are not thoroughly familiar with the product and may not recognize the substitution. As a result, the pure material seldom reaches the buyer.

According to the Prevention of Food Adulteration Act 1954 of the Government of India, *Hing,* which is the superior-quality asafetida, 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% alcohol) not exceeding 12% as estimated by the U.S.P. 1936 method and starch not more than 1% by weight. The inferior 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 exceeding 50% as estimated by the U.S.P. 1936 method and starch not more than 1% by weight (Anon. 2003).

Compounded asafetida is adulterated during processing with materials such as chalk and other oleogums like galbanum, ammoniacum and colophony (Raghavan *et al*., 1974). Officially, compounded asafetida should not contain colophony resin, galbanum resin, ammoniaccum resin or any other foreign resin, coal tar dyes or mineral pigment. The total ash content of compounded asafetida 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 alcohol) as estimated by the U.S.P. 1936 Method not more than 5% by weight (Anon., 2003).

12.7 Main uses

The most important uses of asafetida are as a flavouring and in traditional medicines. Both uses are common in India, but in China asafetida is used only for certain medicinal preparations. In Iran and Afghanistan, where most of the production comes from, it is used in some foods and medicines. In other Asian countries asafetida is used in local medicines on a small scale.

As a flavouring, asafetida can be used either directly in curries or added after it has been fried in oil or steeped in water. It is used extensively in India to flavour curries, soups, sauces and pickles, most often in conjunction with onion and garlic. Some Brahmin communities and Jains in India who do not eat garlic or onion, use asafetida

as a substitute (Andi *et al*. 1997). Vegetarian foods of South India that use asafetida include sambar, rasam and some lentil preparations. It is also used to season some fish dishes and in making certain types of pappadam. In Iran, asafetida is rubbed onto warmed plates prior to serving meat dishes, and the large cabbage-like tops of asafetida plants are eaten raw (Anon., 1991).

While asafetida itself has many medicinal uses, other parts of the plant also have some therapeutic properties. The leaves have anthelmintic, carminative and diaphoretic properties, the stem is good as a brain and liver tonic and the root is antipyretic. The gum resin is antispasmodic, anthelmintic, aphrodisiac, diuretic, expectorant, mildly laxative and a nerve tonic. It is a useful remedy for asthma, bronchitis, croup, flatulence, colic pain and for spasmodic movement of the bowels and infantile convulsions (Duke, 2003). It is also an important ingredient in medicinal preparations prescribed for controlling diarrhoea, flatulence, habitual abortion, indigestion and liver problems. Applied externally, it is good against ringworm (Chatterjee and Pakrashi, 1995), goitre and swelling of joints.

Asafetida is reported as an effective carminative against intestinal flatulence and gas formation. It is an antispasmodic drug widely administered by Hakims in India for hysteria and also for nervous disorders among women and children, especially neurological diseases such as facial paralysis and other types of paralysis, including epilepsy, convulsions and tremors. It also has anti-malarial properties.

Asafetida helps to dissolve abscesses and acts as a purgative, promotes menstruation, destroys worms and heals wounds. It is one of the ingredients used in ointments for wounds, lesions and ulcers. After dissolving in vinegar, it is applied on skin afflictions such as black spots, freckles and disfigurements. It can also be used for curing hard growth of piles. Asafetida acts an expectorant in chronic bronchitis and is administered with honey as an electuary in chronic cough and asthma. Asafetida kills germs in phlegm and is therefore taken to eliminate the foul smell associated with phlegm. It also lowers the viscosity of phlegm, promoting its expulsion. Modern medicine has observed that asafetida is expelled from the body through the kidneys and the skin. It stimulates these organs to encourage urination and sweating. It also has applications in a few veterinary preparations (Krishnamurthy 1994).

Asafetida, galbanum and ushak have many common medicinal properties. Galbanum is a stimulative and reduces provocations of *kapha* and *vata*. It heals abscesses and convulsions. Like asafetida, it promotes healing of wounds and ulcers. Galbanum is also used to strengthen the uterus after delivery. It is employed in neurological afflictions, such as facial paralysis, nervous tremors, epilepsy, fits in children and coma. It is beneficial for the common cold, stops a running nose and is effective against indigestion and stomach pain. It is reported that galbanum has some deleterious effects on testicular functions.

Ushak is good against abscesses and swellings. It is a purgative, promotes menstruation and cleans up worm-infected wounds. It is used for eliminating hard growth of piles and also in skin afflictions, such as black spots, freckles and disfigurements (Krishnamurthy 1994). According to traditional Chinese medicine, asafetida enters the liver, spleen and stomach. It stimulates the intestinal, respiratory and nervous systems. It is used in weak digestion, intestinal parasites, flatulence, asthma, whooping cough and chronic bronchitis. It is also administered for neurological problems associated with hysterical and epileptic affections, and in the case of cholera.

A traditional practice in some European countries was to tie a small piece of asafetida around the necks of children to ward off diseases. During the days of the

American wild west, asafetida was mixed with other strong spices to treat alcoholism. Saleem *et al*. (2001) found that asafetida inhibits early events of carcinogenesis. They reported that asafetida demonstrated antioxidant and anticarcinogenic properties in mice. Studies on several spices by Unnikrishnan and Kuttan (1990) have shown that oral extract of asafetida can increase the lifespan of mice by 52.9%. However, the effectiveness of asafetida in medicinal preparations is disputed in modern medicine. The precise mode of action of asafetida in the human body is yet to be clearly understood and little research has been done in this area.

Asafetida is still very much a wild crop. The asafetida-producing countries have not studied the economics of production or the income it generates for the owners of the plants. Since trade is not organized or controlled, traders enjoy the major share of the profit. People are not generally aware of the price at which traders supply it to other countries. There is scope to increase the productivity of asafetida by selecting and growing high-yielding varieties and improving agronomic practices. Yield depends greatly on the size the taproot attains after three to four years of growth. No serious pests or diseases have been reported in the asafetida crop. Harvest and post-harvest technology need to be improved to increase the amount of sap collected and to ensure that better quality asafetida is produced.

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13

Capers and caperberries

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13.1 Introduction: brief description

The caper bush (*Capparis spinosa* L., Capparidaceae) is a winter-deciduous species widespread in Mediterranean Europe, Africa, Asia and Australia. Its young flower buds, known as capers, are greatly favoured for food seasoning and different parts of the plant are used in the manufacture of medicines and cosmetics (Sozzi, 2001; Rivera *et al*., 2003). 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 semiarid 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 circum-Mediterranean countries and neighbouring regions: Turkey, Morocco, southeastern 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 indigenous to this region 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 and only naturalized in the Mediterranean basin (Pugnaire, 1989).

The caper bush is a perennial shrub 30 to 50 cm tall. Its roots can be six to ten metres long (Reche Mármol, 1967; González Soler, 1973; Luna Lorente and Pérez Vicente, 1985; Bounous and Barone, 1989). The root system may account 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 from two to three metres long. Stipular pale yellowish spines are often hooked and divaricate but sometimes weakly developed or absent. Leaves are alternate, two to five centimetres 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, five to seven centimetres across, axillary and solitary, with purplish sepals and white petals. Stamens are numerous, with purplish filaments. The gynophore is 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 three to four millimetres 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).

Caper bush is the most important member of the Capparidaceae economy-wise. *Capparis* and relatives have been proposed to form a basal paraphyletic complex within Brassicaceae (Zomlefer, 1994; Judd *et al*., 1999) 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*.

13.2 Chemical composition

A considerable amount 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, 1999b; Inocencio *et al*., 2000). Capers and caperberries are a good source of K, Ca, S, Mg, and P (Özcan, 2005) (Table 13.1). High salt brine treatments greatly affect their chemical composition. Protein and fibre, as well as mineral (Mg, K, Mn) and vitamin (thiamine, riboflavin,

Table 13.1 Proximate composition of raw *Capparis spinosa* fruit and flower bud

	Fruits (caperberries)	Flower buds (capers)
Constituent		
Water (%)	79.6[A] ; 82.7[B]	78.4[C] ; 76.8 to 80.3[D]
Protein (%)	4.6[A] ; 3.34[B]	6.31[C] ; 4.59 to 6.79[D]
Lipid (fat) (%)	3.6[A]	0.47[C] ; 1.51 to 1.77[D]
Carbohydrate (%)	3.2[A]	–
Fibre (%)	7.2[A]	2.0[C] ; 4.5 to 5.9[D]
Ash (%)	1.8[A]	1.7[C] ; 1.33 to 1.84[D]
Rutin (%)	–	0.28[C]
Minerals		
Calcium (mg/100 g)	28[A]	183[C] ; 49 to 134[D]
Iron (mg/100 g)	0.9[A] ; 0.54[B]	1.37[C] ; 0.9 to 2.1[D]
Magnesium (mg/100 g)	39[A]	57[C] ; 46.9 to 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 to 26.4[D]
Potassium (mg/100 g)	383[A] ; 326.9[B]	504.9[C] ; 502.4 to 598.3[D]
Sodium (mg/100 g)	18[A] ; 12.1[B]	5.9[C] ; 19 to 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]

([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).

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 to 63.5% of total fatty acids in flower buds (Nosti Vega and Castro Ramos, 1987; Rodrigo *et al*., 1992) and 73% in fruit (Özcan, 1999b). The oil content of the seeds ranges from 27.3 to 37.6% in *C. spinosa* and from 14.6 to 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 13.1): 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, 1976b; 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 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 to 7.85 mg/g) (Inocencio *et al*., 2000). A serving of capers (ten grams) 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 (Kjœr, 1963; Kjœr 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*., 1999). In fact, the conclusion that Capparidaceae and Brassicaceae should remain together, based on the presence of glucosinolates, was drawn 45 years ago (Hegnauer, 1961; Kjœr, 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).

13.3 Cultivation and production

13.3.1 Environmental requirements

The caper bush requires a semiarid 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. A rainy spring and a hot dry summer with intense daylight are considered advantageous (Barbera, 1991). Harvest should last at least three months for profitability. 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 in the form of stump, as it happens in the foothills of the Alps. Caper plants have been found even 1000 m above sea-level though they are 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 one per cent organic matter (Ahmed, 1986; Kala and Mathur, 2002). It grows on bare rocks, crevices, cracks and sand dunes in Pakistan (Ahmed and Qadir, 1976), in dry calcareous escarpments of the Adriatic region (Lovric, 1993), in dry coastal ecosystems of 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), though 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 are optimum (Gorini, 1981) though pH values from 6.1 to 8.5 are tolerated (Duke and Terrel, 1974; Duke and Hurst, 1975; Ahmed, 1986). Caper bush is usually not considered to be a halophyte but it was detected in the loamy solonchacks of Bahrain coastal lowlands, where the conductivity may reach 54 dS/m (Abbas and El-Oqlah, 1992).

Aerosols from sea-water-fed cooling towers proved to produce 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 multilayered 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. This correlates with greater stomata opening which leads

to a higher transpiration rate and leaf temperature below air temperature. Additionally net photosynthetic rate is conserved at important levels under high irradiance and temperature without showing symptoms of photooxidative damage. 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). Fertilization of cultivated bushes probably leads to a luxury consumption of some nutrients, a typical response of wild plants from infertile environments.

13.3.2 Reproductive biology

Caper bush is a perennial plant with a relatively short juvenile period. The biotype Mallorquina can yield one 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). It blossoms for approximately 16 h, from ca. 18:00 h to ca. 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). *Capparis 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 semiarid ecosystems (Eisikowitch *et al*., 1986). Flower rewards in genus *Capparis* is affected by the location and year (Petanidou *et al*., 1996) and differ 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 hard-coated seeds. Wasps are attracted by mature caperberry scent and also act as dispersal agents (Li Vigni and Melati, 1999).

13.3.3 Propagation

Caper bush yields a large amount of seeds per generative shoot, although those seeds have a low germination rate either under semidesert or optimal cultivation conditions. 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, l988; Bond, l990). 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 two to three 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). 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, some of them with distinct thickening; its tegmen consists of an outer fibrous, lignified layer four to ten-cell 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 three to four days after partial removal of the lignified seed coats (Sozzi and Chiesa, 1995), while GA_3-treated seeds germinate within 20 to 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 two 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. Careful moisture control and the use of well-drained containers are essential to ensure proper wetting as well as aeration. Sprouted seeds are obtained and planted after 25 to 50 days. In Spain, nursery preparation begins in February using calcareous soils with loam to clay-loam textures and irrigation. After proper cultivating, seeds (1.5–2 g/m) are planted about 1.5 cm deep, in 30 or 40 cm-

apart rows. Most caper nurseries use furrow irrigation on a two-week basis. Yields of 45 to 50 seedlings per metre may be obtained after 30 days. Transplants may also be produced under protected conditions using floating row covers. Some nurseries use pots or polyethylene bags where plants remain until outdoor transplanting.

Use of stem cuttings avoids high variability in terms of production and quality. Nevertheless, plants grown from cuttings are more susceptible to drought during the first years after planting. 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 one-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 to 50 cm and diameter of the cuttings may range from 1 to 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). Using semi-hardwood cuttings, collected and planted during August and September, low survival rates (under 30%) have been achieved. Softwood cuttings are prepared in April from 25- to 30-day shoots. Each cutting should contain at least two nodes and be six to ten centimetres long. Basal or subterminal cuttings are more successful than terminal ones. Then, cuttings are planted in a greenhouse under a mist system with bottom heat; 150 to 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.

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).

13.3.4 Orchard establishment

Caper plantings over 25 to 30 years old are still productive. Thus, physical properties of the soil (texture and depth) are particularly important. Caper bush can develop an extensive root system and grows best on deep, non-stratified, medium-textured, loamy soils. Mouldboard plowing 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 to 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 to 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. In the Aeolian Archipelago, transplanting is carried out in January or February, but in zones of the Iberian Peninsula with prolonged winter, it takes place during February through early March, after the last frosts. In Argentina, transplanting is generally made in July through August. Transplanting is carried out by hand. 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. Field beds should be well prepared and watered. Containerized plants are used only where lack of irrigation is the chief factor limiting transplanting success.

13.3.5 Pruning

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 one-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 to 40 days after budding. A strong plant may have as many as six stems, strategically distributed to obtain an open canopy with uniform light penetration throughout. Summer pruning also involves heading back a few of the new shoots to induce flower bud formation.

13.3.6 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 surface band applied. 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 is useful to diagnose mineral deficiencies (Sozzi, 2001). Phosphate and potassium fertilizers are generally applied every two to three 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.

13.3.7 Irrigation

Caper bush is cultivated mostly in poor non-irrigated lands. Though it tolerates water stress well, water is the most limiting production factor. Irrigation is 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 hydric resources (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), though 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).

13.3.8 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 (1984–85) 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 life cycle 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*, *Crematogaster 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).

13.3.9 Main cultivars

The commercial product known as 'capers' 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 that should represent the current scope in caper bush improvement programs are: (i) high productivity (long stems, short internodes and high node fertility); (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 postharvest 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' (a cultivar within *C. orientalis*), and 'Nocella' (Barbera *et al*., 1991; Fici and Gianguzzi, 1997). Both are highly productive and yield high quality capers (almost spherical shape, 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 'Ciavulara' (Barbera *et al*., 1991; Fici and Gianguzzi, 1997), 'Testa di lucertola' (Barbera *et al*., 1991), 'Spinoso of Pantelleria' (Barbera *et al*., 1991; Fici and Gianguzzi, 1997) and 'Spinoso of Salina' (a cultivar

within *C. sicula* subsp. *sicula*; Barbera *et al*., 1991; Fici and Gianguzzi, 1997). 'Ciavulara' is less productive and its buds tend to open precociously; capers are flatter and flake easily during postharvest 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 postharvest curing. Another Italian biotype is 'Tondino' (Caccetta, 1985), grown in Pantelleria and Salina.

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 can be considered a promising biotype due to the quality and quantity of its produce.

13.3.10 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. Caper production and trade have become highly competitive. 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. Caper commercial exchange involves over 60 countries. Turkey is the leading caper-exporting country. The United States was one of the most important caper consumers during the 1990s.

Harvest is the costliest operation of caper production. It may represent 2/3 of the total labour in the crop management process as it is done manually. Harvest is difficult and 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 three-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 three-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.

13.4 Uses in food processing

13.4.1 Postharvest technology

Different physico-mechanic 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, postharvest conditioning is generally performed by local traders, cooperatives or producer associations. After removing rests of 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 size-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 recollection 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 (ca 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. Thus, capers may be classified as fully brined vegetables (Ranken, 1988) which may be regarded as a stable product during storage.

High salt-containing brines 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 pre-treated 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 a 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 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 13.2). Finally, capers are prepared in a variety of ways and packed as a finished product.

Pasteurization (80 °C, 15 min) of the final product attains favourable consumer acceptance. It is used to prevent the development of human 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 to 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 grants a more intensive 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 to 1 kg. Five-kilogram flasks and sachets are usually sold to restaurants and coffee-shops.

Traditionally, caperberries are fermented by dipping in water for four to seven 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

Table 13.2 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	5,500	7,000
7–8	Surfine	4,000	4,000
8–9	Capucine	3,250	4,000
9–10	Capote	2,600	2,000
10–11	Capote	2,200	2,000
11–12	Fine	1,900	1,300
12–13	Fine	1,600	1,300
13–14	Grosse	–	800

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 HPLC determination could be used to control the levels of those preservatives in caperberries (Montaño *et al*., 1995).

13.4.2 Food use

Consumption of capers and caperberries has a long history. Direct evidence of the consumption of *Capparis* spp. from 18,000 to 17,000 years ago was obtained by archaeological excavations from an Old World Palaeolithic site (Wadi Kubbaniya, west of 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 400 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 of capers (Redgrove, 1933; Vivancos Guerao, 1948; Seidemann, 1970; Mitchell and Rook, 1979; Stobart, 1980; Bond, 1990).

13.5 Functional 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, antiescorbutic, 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 *Capparis 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 administrated *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 antiallergic 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, 1994b). 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, shampoo, 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.

13.6 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. Besides, caper isothiocyanates are well-known as cancer preventive agents and different caper extracts have hypoglycaemic properties and protective effects against hepatotoxic substances. Moreover, 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 in caper consumption. 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 postharvest processing and storage; and (v) efficient marketing systems and strategies. Caper yields are much higher in irrigated plantings, with NPK 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. Finally, marketing research remains an area of great importance. Marketing of capers without pre-arranged 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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14

Carambola

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14.1 Introduction

A curious and attractive fruit, the carambola, *Averrhoa carambola* L, belongs to the family Oxalidaceae. The fruit with five corners, commonly called the Star Fruit, is very crisp and juicy with a refreshing taste. Fruits are yellow to green, depending on the variety. Yellow fruit tend to be more acid in flavour, and the green ones sweeter (Fig. 14.1).

Carambola is known by a number of regional names in addition to the popular Spanish *appelation* which belies its Far Eastern origin. In the Orient, it is usually called *balimbing, belimbing,* or *belimbing manis* ('sweet belimbing'), to distinguish it from the related species, *A. bilimbi* commonly known as *bilimbi* or *belimbing asam,* L. In Ceylon and India carambola is called *kamaranga, kamruk,* or *kamrakh.* In Vietnam, it is called *khe, khe ta*; in Cambodia, *spu*; in Laos, *nak fuang, carambolier;*

Fig. 14.1 Fruits of carambola – 'the star fruit'.

in Thailand, *ma fueang.* Malayans may refer to it as *belimbing batu, belimbing besi, belimbing pessegi, belimbing sayur, belimbing saji, kambola, caramba,* or as 'star fruit'. Australians use the descriptive term, five corner; in Guam, it is *bilimbines;* to the Chinese, it is *yang-táo.* Early English travelers called it Chinese or Coromandel gooseberry or cucumber tree. In Guyana, it is five fingers; in the Dominican Republic, it is *vinagrillo;* in Haiti, *zibline;* in some of the French Antilles, *cornichon;* in El Salvador, *pepino de la India;* in Surinam, *blimbing legi* or *fransman-birambi;* Costa Rica, *tiriguro;* in Brazil, *camerunga* or *caramboleiro,* or *limas de Cayena;* in Mexico, *carambolera* or *caramboler* or *árbol de pepino;* in Trinidad *coolie tamarind* and in Venezuela it is called *tamarindo chino* or *tamarindo dulce.*

14.2 Description

Carambola is a small tree with attractive foliage, produces large quantities of fruit and is ideal for the home orchard. It is slow-growing, short-trunked with a much-branched, bushy, broad, rounded crown and reaches to 6–10 m in height. Its deciduous leaves are irritable to touch, spirally arranged, are alternate, imparipinnate, 15–20 cm long, with 5–11 nearly opposite leaflets. Leaflets ovate or ovate-oblong acuminate, entire, base oblique, short and stout petioled, 3–9 cm long; soft, medium-green, and smooth on the upper surface, finely hairy and whitish on the underside. The leaflets are sensitive to light and more or less inclined to fold together at night or when the tree is shaken or abruptly shocked.

Small clusters of red-stalked, lilac, purple-streaked, downy fragrant flowers, about 6 mm wide, are borne in clusters in axils of leaves (short axillary racemes) on young branches, or on older branches without leaves on the twigs. There are several flushes of bloom throughout the year. The showy ovoid, oblong or ellipsoid, longitudinally (five) (rarely four or six) angled fruits, 6–15 cm long and up to 9 cm wide, have thin, waxy cuticle, orange-yellow skin and juicy, crisp, yellow translucent juicy flesh when fully ripe (Fig. 14.2). Slices cut in cross-section have the form of a star (Fig. 14.1). The fruit is a berry and has a more or less pronounced oxalic acid odor and the flavor ranges from very sour to mildly sweetish. The so-called 'sweet' types rarely contain more than 4% sugar. There may be up to 12 flat, thin, brown seeds 6–12 mm long or none at all. Seeds lose viability in a few days after removal from fruit. Seeds number 8–10, are arillate, and compressed yellow to light brown in colour. Ovule and seed development have been studied (Govil and Kaur, 1989). Chattopadhyay and Ghosh (1994) have elaborated the changes in mineral composition of inflorescence and developing carambola fruit. Salakpetch *et al.* (1990) have suggested that the flowering of carambola is influenced by cultivar and water stress than by diurnal temperature variation and photoperiod after conducting day/night temperature, photoperiod and soil water stress experiments with cultivars Fwang Tung and Thai Knight.

14.3 Origin and distribution

Averrhoa carambola L. belongs to the family Averrhoaceae. *Averrhoa* is a genus of tropical trees. Carambola is believed to have originated in the Moluccas islands

(a)

(b)

Fig. 14.2 (a) and (b) Fruiting branches of carambola.

of Indonesia and Sri Lanka but it has been cultivated in southeast Asia and Malaysia for many centuries. It is commonly grown in the provinces of Fukien, Kuangtung and Kuangsi in southern China, in Taiwan and India. It is popular in the Philippines, Queensland, Australia, and in some of the South Pacific islands, particularly Tahiti, New Guinea, Guam, Hawaii and Netherlands. A sweet-fruited variety is grown in China, Taiwan, Thailand, etc., and most of the trees in India are sour-fruited types.

The carambola is also grown in South Florida primarily as an ornamental tree that produces fruit of unique flavor and relatively high ascorbic acid content. Smaller amounts of carambola are grown for the fresh fruit market and for export to Europe. Commercial acceptance has been limited because the fruit is susceptible to shipping damage and requires storage below 70 °F to maintain optimum quality during shipment. Carambola has relatively high levels of oxalic acid content that is comparable to that of spinach and rhubarb.

14.4 Cultivars and varieties

There are two distinct classes of carambola. One is a smaller, very sour type, richly flavored, with more oxalic acid. The other is a larger, 'sweet' type, mild-flavored, rather bland, with less oxalic acid. Several cultivars were known – like 'Mih Tao', 'Dah Pon' and 'Tean Ma' from Taiwan, Fwang 'Tung' from Thailand and 'Newcomb', 'Thayer' and 'Arkin' from Florida. Some cultivars and seedlings bear flowers with short styles, others only flowers with long styles, a factor which affects self- and cross-pollination. Several carambola varieties are sold in California. There are a number of excellent carambola varieties available in Florida, including the following:

Arkin: Arkin is a leading commercial cultivar with uniform 4–5 inches-long fruit. Bright yellow to yellow-orange skin and flesh. Very sweet, juicy, firm flesh with few seeds. Keeps and ships well. Tree partially self-fertile.

Fwang Tung: fruit 5–8 inches long. Pale yellow skin and flesh. Very sweet and juicy, firm flesh with few seeds. Beautiful star shape when cut in slices.

Golden Star: large, deeply winged fruit. Skin bright golden yellow, very waxy. Flesh juicy, crisp, mildly subacid to sweet in flavor, containing no fibers. High in carbohydrates and vitamins A and C. Tree bears well and regularly without cross pollination.

Hoku: selected by the University of Hawaii. Fruit 5–6 inches long. Bright yellow skin and flesh. Juicy, firm flesh with a sweet rich flavor, few seeds. Attractive star shape when cut in slices.

Kaiang: fruit 4–5 inches long. Bright yellow skin and flesh. Sweet, juicy, firm flesh with few seeds. Beautiful star shape when cut in slices.

Maha: Originated in Hawaii. Roundish fruit with light yellowish-white skin. Sweet, crunchy, white flesh with low acid content.

Sri Kembanqan (Kembangan): originated in Thailand. Elongated pointed fruit, 5–6 inches long. Bright yellow-orange skin and flesh. Juicy, firm flesh with few seeds. Flavor rich and sweet; excellent dessert quality.

Wheeler: medium to large, elongated fruit. Orange skin and flesh. Mildly sweet flavor. Tree a heavy bearer.

14.5 Climate

Carambola prefers a warm moist climate and can be grown on the hills up to 1,200 m. A well-distributed rainfall encourages normal growth and cropping. It can grow on any type of soil with good drainage, but deep rich soil supports better plant growth. Although it grows both in acid and alkaline soils, it prefers acidic soils. Carambola should be considered as tropical to sub-tropical because mature trees can tolerate temperatures as low as 27 °F for short periods of time with little damage. Like many other subtropicals, however, young plants are more susceptible to frost and can be killed at 32 °F. The carambola needs moisture for best performance and ideally rainfall should be fairly evenly distributed all year. Carambolas can be severely damaged by flooding or prevailing hot, dry winds. In Australia, it is claimed that fruit quality and flavor are best where annual rainfall is 70 in (180 cm) or somewhat more. The small trees make good container plants. Carambola is grown more as an ornamental than for its fruits.

14.6 Propagation

The carambola is widely grown from seed though viability lasts only a few days. Only plump, fully developed seed should be planted. In damp peat moss, they will germinate in one week in summer, requiring 14 to 18 days in winter. The seedlings are transplanted to containers of light sandy loam and held until time to set out. They are very tender and need good care. Seedlings are highly variable and hence much variability exists due to seed propagation. The seed derived tree takes 4–5 years for flowering.

Carambola can also be propagated vegetatively. Veneer grafting during the time of most active growth gives the best results. Healthy, year-old seedlings of 3/8–3/4 inch diameter are best for rootstocks. Graft-wood should be taken from mature twigs on which leaves are still present and, if possible, the buds are just beginning to grow. Cleft-grafting of green budwood is also successful. Top-working of older trees has been done by bark grafting. Air-layering has been practised and advocated though it is less successful than grafting. The roots develop slowly, and percentage of success often is low. Trees are small and rather weak when propagated by this method. Usually vegetative propagation is resorted to only for the propagation of the sweet-fruited varieties (Anon., 2001).

However, root formation is slow and later performance is not wholly satisfactory. Inarching is successful in India, shield-budding in the Philippines and the Forkert method in Java. Trees top-worked by bark-grafting is popular in Java. For mass production, side-veneer grafting of mature, purplish wood, onto carambola seedlings gives best results. The rootstocks should be at least one year old and 1–1.5 cm thick. Grafted trees will fruit in ten months from the time of planting out. Mature trees can be top-worked by bark-grafting.

Amin and Razzaque (1993) have successfully regenerated *Averrhoa carambola* plants *in vitro* from callus cultures of seedling explants. Kantharajah *et al*. (1992) have indicated roots as a source of explants for the successful micropropagation of carambola (*Averrhoa carambola* L.). Multiple shoots were induced in Woody Plants Medium or Murashige and Skoogs (MS) with 2 mg/l Benzyl adenine (BA) and 0.2 mg/l α-naphthalene acetic acid (NAA) with a multiplication rate of 2.1 shoots per month. Adventitious shoot formation from fully grown cotyledon prior to maturity was investigated. Explants produced callus and subsequently adventitious shoots on MS medium supplemented with 4.4–13.2 µM BA and 0.54–2.7 µM NAA (Khalekuzzaman *et al.*, 1995). These shoots were rooted in half strength MS medium with 2.46 µM Indole-3-butyric acid and established in soil. Plantlet regeneration from hypocotyl explants of *in vitro* grown seedlings have been reported by Islam *et al*. 1996).

14.7 Planting

Carambolas do best in a frost-free location and the tree needs full sun. It prefers warm humid areas, it can grow in most parts of the tropics and subtropics. Young trees need protection from cold wind. Generally they are tolerant of wind except for those that are hot and dry. A spacing of 6–9 m has been advocated depending on the soil, giving more space in fertile soils.

At the Research Center in Homestead, Florida trees 8–10 ft high respond well to

0.5 kg of N, P, K, Mg in the ratio of 6-6-6-3 given three to four times per year. If chlorosis occurs, it can be corrected by added iron, zinc and manganese. Some advisers recommend minor-element spraying four times during the year if the trees are on limestone soils. Moderate irrigation is highly desirable during dry seasons. Heavy rains during blooming season interfere with pollination and fruit production. Interplanting of different strains is usually necessary to provide cross-pollination and obtain the highest yields. Only light pruning is required.

14.8 Soils, water and nutrients

Carambola is not too particular as to soil, but does well on sand, heavy clay or limestone and will grow faster and bear more heavily in rich loam. It prefers a moderately acid soil (pH 5.5–6.5) and is sensitive to water logging. The plant often becomes chlorotic in alkaline soils. It needs good drainage and cannot stand flooding. Carambola need moisture for best performance. This means regular watering during the summer months and during dry spells.

In soils of low fertility young trees should receive light fertilizer applications every 60 to 90 days until well established. Thereafter, they should receive one or two applications a year in deep soils or three or more applications in shallow soils where nutrients are lost by leaching. Application at the rate of 0.5 kg per year for every inch of trunk diameter is suggested. Fertilizer mixtures containing 6–8% nitrogen, 2–4% available phosphoric acid, 6–8% potash and 3–4% magnesium are satisfactory. In the more fertile soils of California, this program can be reduced.

The tree is prone to chlorosis in deficient soils but responds to soil and foliar application of chelated iron and other micronutrients. Growth and physiological processes of carambola plants under soil flooding and root growth restriction (Ismail and Noor, 1996a; 1996b) and physiological changes as influenced by water availability (Ismail and Awang, 1992) have been studied.

14.9 Pests and diseases

The carambola is relatively pest free except for fruit flies. No diseases of sufficient importance are known. The fruit is damaged by fruit fly, fruit moths and fruit spotting bugs in those areas having these infestations. In fruit fly susceptible areas fruit can be bagged for protection. In Malaya, fruit flies (especially *Dacus dorsalis*) are so troublesome on carambolas that growers have to wrap the fruits on the tree with paper. Experimental trapping, with methyl eugenol as an attractant, has reduced fruit damage by 20%. In Florida, a small stinkbug causes superficial blemishes and a black beetle attacks overripe fruits. Reniform nematodes may cause tree decline (De *et al.,* 2000). Cold storage quarantine treatment for Hawaiian carambola fruit infested with Mediterranean fruit fly, melon fly, or oriental fruit fly (Armstrong *et al*.,1995) Caribbean fruit fly (Gould and Sharp 1990) (Diptera: Tephritidae) eggs and larvae have been suggested. Studies on gamma irradiation as a quarantine treatment for carambolas infested with Caribbean fruit flies have been made by Gould and Von (1991).

Ibrahim (1994) has studied the biology and natural control of the scale *Drepanococcus*

chiton (Green) (Homoptera: Coccidae), a minor pest of carambola in Malaysia. The larvae of *Diacrotuchia fascicola* and nymphs of *Schistocera gregaria* damage tender leaves of carambola (Anon., 1985). De *et al*. (2000) have reported the incidence of *Anastrepha obliqua* (Macquart) and *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) in star fruit in eight localities of the State of Sao Paulo, Brazil and an infestation rate of 31.7 puparia per star fruit.

Anthracnose caused by *Colletotrichum gloeosporioides* may be a problem in Florida, and leaf spot may arise from attack by *Phomopsis* sp., *Phyllosticta* sp. or *Cercospora averrhoae. Cercospora* leaf spot is reported also from Malaya, Ceylon, China and may occur in the Philippines as well. A substance resembling sooty mold makes many fruits unmarketable in summer. Black rot of fruit is caused by *Trichotheceum roseum.* Brown spot disease affecting the fruit is due to *Alternaria tenuri,* while *Cladosporium herbarum* causes black circular lesions. All the above diseases can be successfully controlled by fungicidal sprays (Anon., 1985). Fruit rotting caused by *Botrydeploidea theobromae* and *Phomepais* are serious problems during moist weather conditions.

Effects on fruit ripeness by infestation of carambolas by laboratory-reared Caribbean fruit flies (Howard and Kenney, 1987) have been studied and quarantine treatments like Methyl bromide fumigation (Hallman and King, 1231), hot water immersion Hallman and Sharp (1471) and vapor-heat treatment (Hallman, 1990) have been suggested for carambolas infested with Caribbean fruit fly. Hallman (1991) has further evaluated the quality of carambolas subjected to post-harvest hot water immersion and vapor heat treatments.

14.10 Harvesting and yield

Carambola trees flower several times a year, with a heavy crop during summer. Fruits change color slightly when they are ready for picking, but the best check for ripeness is to eat one and see how sweet the fruit is. Trees that receive adequate care and attention have yielded up to 45–135 kg of fruit.

In Malaya, they are produced all the year. In Florida, scattered fruits are found throughout the year but the main crop usually matures from late summer to early winter. Some trees have fruited heavily in November and December, and again in March and April. There may even be three crops. Weather conditions account for much of the seasonal variability. In India, carambolas are available in September and October and again in December and January.

The fruits naturally fall to the ground when fully ripe. Green or ripe fruits are easily damaged and must be handled with great care. Often the taste suffers if fruits are picked too green. Fruit are best when ripened on the tree, but will ripen if stored under refrigeration and will keep for 1–3 weeks if picked before fully ripe. Ripe carambolas are eaten out-of-hand, sliced and served in salads or used as a garnish. They are also cooked in puddings, tarts, stews and curries.

14.11 Keeping quality

For marketing and shipping they should be hand-picked while pale-green with just a touch of yellow. Fruit is very fragile and needs to be packed carefully. Carambolas

have been shipped successfully without refrigeration from Florida to northern cities in avocado lugs lined and topped with excelsior. The fruits are packed solidly, stem-end down, at a 45º angle, the flanges of one fruit fitting into the 'V' grooves of another (Campbell,1994).

In storage trials at Winter Haven, Florida, carambolas picked when showing the first signs of yellowing kept in good condition for four weeks at 50 ºF (10º C), three weeks at 60 ºF (15.56 ºC) and two weeks at 70 ºF (21.1 ºC). Waxing extends storage life and preserves the vitamin value. Campbell and Koch (1989) have studied the sugar/acid composition and development of sweet and tart carambola fruit. The post-harvest changes in sugars, acids, and color of carambola fruit at various temperatures *viz*., 5, 10, 15 °C have shown that fruits stored at 5 °C maintained better appearance, lost less weight, had fewer changes in soluble sugars or organic acid. Rewarming experiments proved an absence of any chilling injury (Campbell *et al*., 1989).

Volatile constituents of carambola were identified in ripe fruit extracts, the most abundant being methyl athranilate with grape-like flavor and the strong fruity aroma was considered to be due to major ester and ketones in extract (Wilson *et al*., 1985). Biochemical changes, chilling injury of carambola stored at various temperatures (Wan and Lam, 1984) have also been studied. The browning susceptibility and changes in composition during storage of carambola slices (Weller *et al*., 1997) were due to decrease in ascorbic acid content and increase in polyphenoloxidase activity. These changes were greater in slices than in whole fruit. Treating with 1–2.5% citric acid and 0.25% ascorbic acid prior to packing was effective in limiting browning. Ghazali and Leong (1987) have worked upon the polygalacturonase activity in starfruit and the changes in polygalacturonase activity and texture during its ripening (Ghazali and Peng,1993). Additional volatile constituents (Froehlich and Schreier, 1989) and non-odorous characteristics pertaining to fruit-piercing moth susceptibility (Fay and Halfpapp, 1993) have been reported.

14.12 Food uses

Carambolas can be sliced up into attractive star shapes, which can then be added as a garnish to fruit salad and fish. It is also a good fruit for juicing. One need not peel the fruit, but each rib should be trimmed and the darker green edge which is very bitter is removed. Ripe carambolas are eaten out-of-hand, sliced and served in salads, or used as a garnish on seafood. They are also cooked in puddings, tarts, stews and curries. In Malaya, they are often stewed with sugar and cloves, alone or combined with apples. The Chinese cook carambolas with fish. Thais boil the sliced green fruit with shrimp. Slightly under-ripe fruits are salted, pickled or made into jam or other preserves in India.

In mainland China and in Taiwan, carambolas are sliced lengthwise and canned in syrup for export. In Queensland, the sweeter type is cooked green as a vegetable. Cross-sections may be covered with honey, allowed to stand overnight, and then cooked briefly and, put into sterilized jars. Some cooks add raisins to give the product more character. A relish may be made of chopped unripe fruits combined with horseradish, celery, vinegar, seasonings and spices. Indian experimenters boiled horizontal slices with 3/4 of their weight in sugar until very thick, with a Brix of 68º. They found that the skin became very tough, the flavor was not distinctive, and the jam was rated as only fair. Sour fruits, pricked to permit absorption of sugar and

cooked in syrup, at first 33º Brix, later 72º, made an acceptable candied product though the skin was still tough. The ripe fruits are sometimes dried in Jamaica.

Carambola juice is served as a cooling beverage. In Hawaii, the juice of sour fruits is mixed with gelatin, sugar, lemon juice and boiling water to make sherbet. Filipinos often use the juice as a seasoning. The juice is bottled in India, either with added citric acid (1% by weight) and 0.05% potassium metabisulphite, or merely sterilizing the filled bottles for 1/2 hr in boiling water. To make jelly, it is necessary to use unripe 'sweet' types or ripe sour types and to add commercial pectin or some other fruit rich in pectin such as green papaya, together with lemon or lime juice. The flowers are acid and are added to salads in Java; also, they are made into preserves in India. The leaves have been eaten as a substitute for sorrel.

14.13 Food value

Carambola fruits are very sour due to the presence of a high oxalic acid content. Sweet varieties have a negligible oxalic acid content. The juice of some varieties has a pH of about 1.9–2.0 and about 15–16 mg of ascorbic acid per 100 gm of juice, hence it is a rich source of vitamin C. A wide variation in vitamin C is reported from various locations in India. Juice also contains iron and phosphorous. Herderich *et al*. (1992) had, for the first time, identified Carbon-13 norisoprenoid flavor precursors in starfruit. Several constituents are easily degraded upon heat treatment at natural pH conditions of the fruit pulp, thus rationalizing the formation of a number of C_{13} aroma compounds reported as starfruit volatiles. Glycosidically bound precursors of C_{13} odorants including a rare natural precursor of the potent aroma compound – damascenone has been identified and a pathway for its formation from non-allenic compounds has been proposed.

Ripening and storage studies were conducted at the Florida Citrus Experiment Station at Lake Alfred in 1966. They found significant differences in the acid make-up of mature green and mature yellow carambolas. Fresh mature green fruits of 'Golden Star' were found to have a total acid content of 12.51 mg/g consisting of 5 mg oxalic, 4.37 tartaric, 1.32 citric, 1.21 malic, 0.39 α-ketoglutaric, 0.22 succinic, and a trace of fumaric. Mature yellow fruits had a total acid content of 13 mg/g, made up of 9.58 mg oxalic, 0.91 tartaric, 2.20 α-ketoglutaric, 0.31 fumaric.

In 1975, 16 carambola selections and two named cultivars were assayed at the United States Citrus and Subtropical Products Laboratory, Winter Haven, Florida. The variety 'Dah Pon' was described as 'sweet, good and apple-like'. It also has a relatively high ascorbic acid content. Oxalic acid content of the 18 selections and cultivars ranged from 0.039 mg to 0.679 mg and four of the preferred carambolas were in the lower range. Puerto Rican technologists found the oxalic acid content of ripe carambolas to average 0.5 g per 100 ml of juice, the acid being mostly in the free state. Carambolas are suitable for individuals who may be adversely affected by small amounts of oxalic acid or oxalates (Table 14.1).

Other amino acids reported by the Florida Citrus Experiment Station at Lake Alfred and expressed in micromoles per g ($\mu m\ g^{-1}$) in mature green fruits (higher) and mature yellow fruits (lower), respectively, are shown in Table 14.2.

Analyses in India showed 10.40 mg ascorbic acid in the juice of a 'sweet' variety; 15.4 mg in juice of a sour variety. Ascorbic acid content of both waxed and unwaxed fruits stored at 50 ºF (10 ºC) has been reported as 20 mg/100 ml of juice. Waxed fruits

Table 14.1 Food value of edible portion of carambola fruits

Calories	35.7
Moisture	89.0–91.0 g
Protein	0.38 g
Fat	0.08 g
Carbohydrates	9.38 g
Fiber	0.80–0.90 g
Ash	0.26–0.40 g
Calcium	4.4–6.0 mg
Phosphorus	15.5–21.0 mg
Iron	0.32–1.65 mg
Carotene	0.003–0.552 mg
Thiamine	0.03–0.038 mg
Riboflavin	0.019–0.03 mg
Niacin	0.294–0.38 mg
Ascorbic Acid*	26.0–53.1 mg

* According to analyses made in Cuba and Honduras.

Table 14.2 Amino acid composition of mature fruits

Tryptophan*	3.0 mg
Methionine*	2 mg
Lysine#	26 mg
Asparagine	0.82–0.64 μm g^{-1}
Threonine	0.92–0.79 μm g^{-1}
Serine	3.88–2.00 μm g^{-1}
Glutamic acid	2.41–1.80 μm g^{-1}
Proline	0.23–0.09 μm g^{-1}
Glycine	0.20–0.10 μm g^{-1}
Alanine	5.40–1.26 μm g^{-1}
Valine	0.17–0.11 μm g^{-1}
Isoleucine	0.03–trace μm g^{-1}
Leucine	trace
Phenylalanine	trace
Gamma amino bytyric acid	0.77–0.55
Ornithine	0.11–0.13
Histidine	trace

*Amino Acids: (shown in Cuban analyses).

stored for 17 days at 60 ºF (15.56 ºC) had 11 mg/100 ml of juice. Unwaxed fruits had lost ascorbic acid.

The fruit extracts of the slow-ripening cv. B 10 carambola (*Averrhoa carambola* L.) contained a number of cell wall hydrolases (Chin *et al*., 1999). The predominant ones appeared to be β-(1,4)-glucanase (as carboxymethylcellulase), pectinesterase, β-galactosidase, and polygalacturonase (PG). Other significant hydrolases, the activity of which also increased with ripening were the glycosidases, α-arabinosidase, α-galactosidase, and α-mannosidase, and also the glycanases, β-(1,4)-galactanase and xylanase. Throughout ripening, as pectins and hemicelluloses were being differentially modified, the levels of buffered-phenol cell wall materials, total polyuronides as well as arabinose, galactose, xylose, and glucose decreased. At early ripening phase (days 0–12) there was no apparent pectin solubilization, and the loosely bound water- and chelator-soluble pectins were the first pectic polysaccharides to be affected. That of the former exhibited an upshift in their molecular size profiles.

At late ripening phase (days 12–24) when tissue firmness had declined substantially, dramatic changes involving pectins and hemicelluloses were evident. Pectins were solubilized, and this increased solubility was accompanied by depolymerization of all pectin classes and a decrease in the level of the Na_2CO_3-soluble polyuronides. Coincident with these marked modifications of the tightly bound, predominant polyuronide fractions and hemicellulose was the increase in activities of polygalacturonase and β-(1,4)-glucanase, suggesting that these enzymes may contribute to wall modifications late during ripening. Some of the other wall hydrolases, namely, α-arabinosidase, α-galactosidase and certain isoforms of β-galactosidase/galactanase, have been indicated to be relevant to the early ripening changes when pectin solubilization was limited (Chin *et al.*, 1999).

14.14 Medicinal uses

All parts of the Carambola tree are credited with medicinal properties (Anon. 1985). The root is administered as an antidote in snake poisoning. The crushed leaves or shoots are applied externally in chicken pox, ring worm, scabies and headache. They are reputed to be antiscourbutic. A decoction of leaves is used for aphtha and angina and to arrest vomiting. Flowers are said to possess wormicidal properties. The fruits are reputed to be laxative, antiscourbutic, febrifuge, antidysentric and antiphlogistic. The fruit juice is a good remedy for piles and is useful in relieving thirst and febrile excitement. The seeds are said to increase the flow of milk and in large doses act as an emmenagogue and cause abortion. They are generally administered as infusion, decoction or tincture. They have slight intoxicating and emetic properties. They are useful in treating asthma, colic and jaundice.

In India, the ripe fruit is administered to halt hemorrhages and to relieve bleeding hemorrhoids and the dried fruit or the juice may be taken to counteract fevers. A conserve of the fruit is said to allay biliousness and diarrhea and to relieve a 'hangover' from excessive indulgence in alcohol. A salve made of the fruit is employed to relieve eye afflictions. In Brazil, the carambola is recommended as a diuretic in kidney and bladder complaints, and is believed to have a beneficial effect in the treatment of eczema. In Chinese *Materia Medica* it is stated, 'Its action is to quench thirst, to increase the salivary secretion'.

14.15 Other uses

The acid types of carambola have been used to clean and polish metal, especially brass, as they dissolve tarnish and rust. The juice will also bleach rust stains from white cloth. Unripe fruits are used in place of a conventional mordant in dyeing. Carambola wood is white, becoming reddish with age; close-grained, medium-hard. It has been utilized for construction and furniture.

14.16 References

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15

Caraway

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15.1 Introduction

Caraway (*Carum carvi* L.) of the *Apiaceae* family, appears to have its origin in Asia Minor. The 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 a condiment for flavouring food preparations into Europe and the Middle East from ancient times. It is known to be cultivated in the Netherlands, Holland, Russia, Hungary, Poland, Denmark, Germany and Norway. The other producing countries are Romania, Bulgaria, Morocco, the USA, Syria, Turkey and India. The major commercial sources of caraway in the world are the Netherlands and Germany, where it is extensively cultivated.

There are about 25 species of *Carum* known to occur and only *Carum carvi* L. has an economic importance, being used and cultivated in several regions. It is commonly called caraway and is popular 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, *siah zeera* in India. All European countries have their own, however, to some extent similar names for this species and these names might be traced back to the Arabian '*karauya*' from the XII century (Rosengarten, 1969). It is also called *sushva*, *krishna jiraka* or black cumin in India. The caraway (*Carum carvi* L.) is usually confused with black caraway (*Carum bulbocastanum* Koch, *Bunicum persicum* Boiss) and Nigella (*Nigella sativa* L.) because of the common vernacular names, but they are botanically different from each other.

15.1.1 Classification

In a classification of plant organs used as spice, the caraway has been categorized as a seed spice because 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 the order *Apiales*, family *Apiaceae*, genus *Carum* and species *carvi*. The other

synonyms of *Carum carvi* L. mentioned in various literatures are *Carum decussatum* Gillib, *Carum aromaticum* Salisb. *Carum officinale* S.F. Gray, *Apium carvi* Crantz, *Seseli carvi* Lam, *Seseli carum* Scop. *Ligusticum carvi* Roth, *Sium carvi* Bernh, *Bunicum carvi Bieb, Foeniculum carvi* Limk, *Pimpinella carvi* Jessen and *Selinum carvi* E.H.L. As per the conventional classification of spices, out of five types, *viz*., hot spices, mild spices, aromatic spices, herbs and aromatic vegetables, caraway is classified as a mild spice and on the basis of plant organs used, it is known as seed because the dried fruits are mostly used as spices.

15.1.2 Description

The caraway plant is an erect, herbaceous, biennial herb with a thick tuberous rootstock. The plant height varies from 0.5 to 1.0 m. The stem is cylindrical, robust, divertically branched, aromatic, straight and leafy. The leaves are pinnately compound and ultimately segments of lower leaves are lanceolate. Flowers are minute, borne in terminally or axillary compound umbels producing clusters of white flowers. The flowers have bracts 1-3, small, linear or none; calyx teeth 5, small or none; petals 5, notched, often enlarged and erect. Carpels are rounded and narrowed upwards. Fruits are brown, cremocarp, 3–6 mm long, ovoid or oblong, glabrous and laterally flattened. Seeds are dorsally flattened smooth or slightly grooved on the inner surface. The fruit when ripe splits into narrow, elongated carpels 4 to 6.5 mm long, curved, pointed at the ends with five longitudinal ridges on the surface. The seeds have a warm, sweet, slightly sharp taste and flavour (Malhotra, 2004). The sematic chromosome numbers are 2n-20.

15.1.3 Production and international trade

Caraway is grown significantly on a large scale in the Netherlands, Germany, Poland, Ukraine, Hungary, and Romania. The Netherlands has an outstanding position in the world for caraway production. Some further countries Sweden, Norway, Spain and Austria, were mentioned as caraway producers, (Heeger, 1956), however, production seems not to be a determining factor today in the world market from these countries. From the last few decades, the production of caraway has shifted to new regions, such as Canada, USA, Finland, Syria and Morocco. The *Carum carvi* plant as natural flora is prevalent in North and Central Europe, England, East and Central France, South Spain, North Italy, Balkan Peninsula, Central Asia (Nemeth, 1998). It has spread as a result of human activity also in Holland, North Africa, North America and New Zealand.

The principal commercial source of caraway seed is the Netherlands. The seed is also cultivated in Bulgaria, Canada, Germany, Britain, India, Morocco, Newfoundland, Poland, Romania, Russia, Syria and the USA (Weiss, 2002). About 3500 metric tonnes of caraway seed and value added products are imported annually into the USA and about 80% of this tonnage arrives from the Netherlands, the remainder coming from Poland and Denmark. Switzerland and Austria get about 500 tonnes of caraway, 70% from the Netherlands and the remainder from Poland. The Netherlands, Poland and Germany are the major exporters in the world market and export caraway seed to the USA, Switzerland, Austria and Hungary. In India, caraway grows wild in the North Himalayan region and is cultivated as a winter crop in the plains and a summer crop in Kashmir, Kumaon, Garhwal and Chamba at attitudes of 2740 to 3660 m above mean sea level.

Average annual world production of caraway oil ranges from 30–40 t, with a total value of more than $1 million. Holland is one of the major producers and exporters of caraway essential oil. For many years Holland has been the world's principal supplier of caraway seed and oil, but now the Netherlands has attained supreme position in the global market. The international price of oil varies from 2000 Rupees (Rs) to Rs. 2500 per kg so it is a minor item in the export and import of oil in India. Approximately 200 kg of caraway oil, worth Rs. 0.01–0.02 million and caraway seed 200 tonnes, worth Rs.3.5 million are imported annually from India (Shiva *et al.,* 2002*)*. Around 30 t of essential oil of caraway is traded yearly in the world, the fifth largest amount amongst *Apiaceace* species. The world production of seeds may be assumed reach to around 15 thousand tonnes. Production, however, is rather variable and fluctuates from year to year both in quantities and in prices.

Production of caraway seed is significant in northern Europe, especially the Netherlands, and in Canada, the USA, Scandinavia, Russia and Germany. The tuberous roots of caraway are edible and somewhat popular especially among the inhabitants of higher hills in India and China, and further extending to the Caucasus, Persia, Tibet and Siberia. 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.

15.2 Cultivation

15.2.1 Climate

Caraway crop requires a dry temperate climate and thrives well in tilled soils, rich in humus at an elevation of 3000–4000 m. Caraway is basically a biennial but usually treated as an annual from crop production techniques. It grows as an annual at lower altitudes and as a biennial in higher altitudes up to 4000 m above sea level. It prefers a lot of sunshine and low temperatures (16/20 °C) for flowering and seed setting of biennial types (winter types), whereas annual types of caraway require more heat for seed production (Svab, 1992). High fruit yield of caraway requires plenty of sunshine especially in the first year of growth and also during the flowering stage. Low light levels will delay and decrease the fruit production (Bouwmeester *et al.*, 1995). The biennial types require a period of about eight weeks of temperature below 10 °C to induce flowering, whereas annual types attain flowering during long days (10 hours or more), the higher the temperature, the quicker flowering develops. 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 seven weeks was best for achieving 100% flowering in biennial type caraway plants in Hungary (Nemeth *et al.*, 1998). Commercial crops of caraway are usually located in moderate to high rainfall areas of the temperate region up to 1500 mm annually. Caraway can withstand frost after sowing in autumn. In general, light intensity is more important than day length and long periods of cloudy weather or shading from other crops at flowering substantially reduces seed yield (Putievsky, 1983). In warmer regions, caraway is grown at higher elevations i.e. near 3000 m in Kashmir, India.

15.2.2 Soil

Caraway grows in a variety of soils but yields best on deep and warm soils rich in

humus and nutrients. Commercial caraway crops are usually grown on free-draining clays or heavy loams soils provided moisture is adequate. The growers in northern Netherlands, the known high-productivity zone, harvest a high yield and quality of caraway under such soil conditions. Prolonged water-logged conditions may cause damage to the crop. Caraway grows well only on neutral or slightly alkaline soils, a soil reaction of pH 6.5–7.5 is preferred, and above or below this, yield is progressively reduced, although there may be no major difference in vegetative growth (Chotin and Szulgina, 1963). Below pH 6.0, caraway plants generally make poor growth and many die.

Liming can adjust soil acidity, but heavy application may induce manganese and born deficiencies thus acid or high alkaline soils should be avoided for the cultivation of caraway. Dry sandy and arid soils are not suitable for the cultivation of caraway. In the Netherlands, the highest yield of carvone (>70 kg/ha) was obtained on sandy loam, whereas on sandy soils the yield was about 40 kg/ha (Toxopeus and Lubberts, 1994). In India, the sandy loam and well drained soils are best for caraway cultivation and can be grown in fruit orchards in between the rows of the plants.

15.2.3 Sowing

Caraway is propagated through seeds and is usually sown at a row distance of 30–40 cm, during March–April in temperate areas and October–November in subtropical areas in India and the Mediterranean region. Biennial caraway can either be sown in late spring-early summer in areas with a relatively mild winter or in autumn where winters are more rigorous. In areas where there are very cold winters, caraway should be sown in late July to ensure vernalization occurs. In the Netherlands it is frequently sown in March–April, mostly the biennial type, but the annual cultivars should be sown as early as possible in spring when the ground has warmed after winter. A soil temperature between 10 and 15 °C gave the highest germination percentage in Israel and germination time was halved when seeds were leached with water and dried before sowing. Annual caraway can be sown under cover early in the year and grown mostly by direct seeding in the field. This produces an early herb crop or a high seed yield, but is profitable only near a high-value urban market or for domestic use.

About 6–8 kg good-quality seed is required for sowing in one hectare and significantly advanced and more uniform emergence can be obtained as a result of seed stratification at 0 °C for 20–25 days and also by warming up the seeds just before sowing (Chotin and Szulgina, 1963). A sufficient water supply for maintaining optimum soil moisture during germination is required for getting maximum germination level and uniform emergence of seedlings. In the moderate climate of Central Europe, two cultivation methods are used, mixed or pure crop. Mixed cultivation with a cover crop is usually preferred by the small farm holdings located under favourable soil and climatic conditions. The pure sowing can be delayed to the end of May and even to August (Ruminska,1981), whereas in mixed cultivation, possibly the earliest sowing (March, April) is just obligatory (Weglarz, 1998).

Seed sowing rate depends both on cultivation method and soil type, ranging from 6–8 kg to 8–10 kg per ha for mixed and pure crops, respectively. Sowing is performed in rows at a 35–50 cm spacing and sowing depth increase from 1.5 cm on heavy soils to 2–4 cm on relatively higher soils, exposed to fast drying of the top layer. The optimum stand density for caraway as worked out by Wander (1997) is 75–100 plants/m^2 for getting higher seed yield and quality. In Saskatoon, western Canada,

among the three cultivars tested, Karzo produced the highest seed yield (1648 kg/ha) and had the highest essential oil content (3.4%) and for best yields, Karzo required high sowing rates (about three times the current sowing rate used with other cultivars), a sowing depth of 2.5–4 cm, and sowing no later than 19 May, to ensure full seed maturity (Arganosa, *et al*., 1998). The different sowing dates (between 6 April and 21 June) and seed rates (5, 10 or 15 kg/ha) using *C. carvi* (cv. Sylvia) were standardized by Dragland and Aslaksen (1996) for its successful cultivation in various agro-climatic localities in Norway.

15.2.4 Fertilization

Nutrient intake by caraway plants is intensive and the pure crops require about 10–15 t/ha farmyard manure and the best fore crops for caraway are considered root and vegetable plants previously supplied with a full rate of farmyard manure (20–40 t/ha). Plants ploughed in for green manure could also be recommended. According to Schroder (1964), 85 kg N, 39 kg P_2O_5 and 94 kg K_2O per ha, yields 1.2 and 4.2 tonnes of fruits and straw, respectively. In Poland, caraway crop is usually provided with 60–80 kg N, 70–80 kg P_2O_5, 100–120 kg K_2O and 20–30 kg MgO, applied both in the first and second growing season (Ruminska, 1990). Full rates of P, K compounds together with half amount of N-fertilizers are applied prior to sowing in late autumn or early spring. The other half of nitrogen is provided after caraway emergence. However, the main sources of nutrients as mentioned above are mineral fertilizers, supplied in both years of cultivation in the biennial type.

Annual caraway responds very positively to N and P for increasing plant height, number of branches, seed weight and seed yield (Munshi *et al.*, 1990) and in Europe it was found that N is needed mainly during leaf development and K during flower stalk growth while the P and Ca uptake (as found in plant parts) was high during seed ripening (Lihan and Jezikova, 1991). The highest seed production has been obtained when a high level of N is applied before sowing or 50% before sowing and 50% of the total amount at mid-winter. In Israel, maximum seed yield has been obtained at 50 kg of N/1000 m^2 supplied as ammonium sulphate (Putievsky and Sanderovich, 1985). The highest seed yield and carvone yield were achieved with 30–60 kg N/ha under the Netherlands growing conditions (Wander, 1997).

15.2.5 Maintenance and care

The important agricultural practices for caraway production after sowing are loosening of soil, weed control, irrigation and plant protection. It is necessary to decrease the weed population to the minimum, not only to reduce competition with the crop, but also to maintain quality at harvest, because many weeds are *umbelliferous* and their seeds are difficult to separate from caraway fruits and ultimately reduces its value. Since the crop growth during the initial period of emergence is slow, crop should be kept free of weeds during the first two months by practising 2–3 weeding and hoeing for annual types (Malhotra, 2005). For biennial crops, one weeding and hoeing would be required in the first year when the crop has started sprouting and another during spring of the next year after over wintering in April. This practice helps in removal of weeds and loosening of soil for aeration.

In Russia, herbicides like prometryne and gasgard are used against dicot weeds. In pure crops of caraway, the use of linuron, prometryne and metobromuron have proved

effective for chemical weed control (Pruszynski, 1995). Weed control is a very important factor mainly during the early developing stages before the plants cover the field at early spring (March). The growing season of biennial caraway is much longer, therefore a wide range of weed control is needed for a longer time. The two most common herbicides used for weed control are afalon (linuron) and prometryne, mainly used after sowing before emergence (Putievsky, 1978), but can also be used before sowing (Pank *et al*., 1984).

It is important to maintain adequate soil moisture to get high seed yield. Depending upon the soil type and climate, the crop requires three to four irrigations. In biennial types, a first irrigation should be given when bolting starts and is followed by irrigation at flowering and seed formation, the most important stages for realizing a higher seed yield. In semi-arid regions where the annual caraway grows, two critical stages when irrigation is necessary are during the early period of growth from germination to establishment and seed formation. In Egypt, when rainfall is not sufficient, the farmers make use of the flooding system to irrigate the crop, while in Israel a sprinkler irrigation system is used for this purpose.

The caraway crop is affected by several diseases and insect pests but insects pose comparatively less of a problem than do the diseases. The aphid (*Hyadaphis coriander*i) is frequently recorded in caraway from the Middle East to India and is damaging in growing seasons. The most commonly recorded diseases are caused by *Fusarium* spp., *Verticillium* spp., *Sclerotinia* spp., especially *S. sclerotiorum*, which has a very wide host and geographical range and *Phomopsis* spp., especially *Phomopsis diachenii* and *Ramularia* spp. in Europe. A major disease of spring caraway in the Netherlands is the soil-borne *Sclerotinia* stem rot, which can be effectively controlled by following crop rotation. The *Anthracnose* due to *Mycocentrospora acerina* occurs widely in Europe. Suitable disease management as recommended for each disease and pests in various countries can be followed accordingly.

15.2.6 Harvesting and yield

The fruits of caraway, being highly susceptible to shattering, necessitate harvesting of crop at the appropriate time. In Europe, caraway is harvested in the period from late June to mid-July for biennial types. Depending on region and cultivar, biennial types are harvested from July to 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 crop is harvested in July after a crop duration of about 15 months. The crop is harvested when the oldest seeds start turning brown. Harvesting is done by sickle on small farms or by mowing machine, as is done on large farms in Holland. Caraway yield widely fluctuates from 1–3 t/ha for biennial types and 0.7–1 t/ha from annual type. In mixed cultivation with cover crops, the yields obtained may be 15–30% lower (Muller, 1990). In field tests carried out over several years in Vienna, Austria, by Bailer *et al*., (2001) on four annual and seven biennial caraway varieties, yielded 900 kg/ha in biennial caraway, and 1250 kg/ha in annual caraway. The yield of caraway fruits grown in experimental fields ranged from 984–2673 kg ha-1, depending on fertilizer content, cultivation area and cultivar under Lithuanian agro-climatic conditions (Venskutonis *et al*., 1999).

15.2.7 Post-harvest handling

The seed crop of caraway is collected after harvest and should be left in swaths or

sheaves for a period of 7–10 days before they are threshed. This short period from cutting to threshing is very essential, since then the fruits become finally formed and coloured. Warm weather favours this process, however, too intensive insolation is unwanted, when threshers are also used. However, transport of dry plants from the field usually increases yield losses (Hecht *et al*., 1992). In a study by Wander (1997) thresher drum speed had exhibited no adverse effect on seed or carvone yield while threshing caraway. After threshing and mechanical cleaning, the fruits should be re-dried down to 10–12% moisture content. Then for some time, the fruits should be kept loose in a thin layer, being frequently mixed, within a dry and aerated storeroom to finally establish their moisture content. Such prepared raw material is packed into sacks and if inadequately stored, can go musty and mouldy, thus becoming useless as raw material (Weglarz, 1998). Spices should be stored on a dry, cool and dark place in order to keep the aroma as long as possible. The shade-dried seed contains more oil content than the 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 the oil extraction unit for more recovery of essential oil content (Malhotra, 2006a,b).

15.2.8 Cultivars

There are annual and biennial forms of *Carum carvi,* existing with slight uncertain differences in morphological and anatomical characteristics between these two morphotypes of caraway (Hornok, 1986). Concerning essential oil content, there is a clear distinction between these two with about 3% for annual and 4% for biennial caraway (Bouwmeester *et al*., 1995)

Different cultivars have been recommended for cultivation in different provinces. The popular biennial type landraces and varieties of caraway are Noord-Hollandsche, Mansholts and Volhouden. In 1972, a non-shattering variety 'Bleija' was developed through Volhouden and Mansholts. Two spring type annual caraway varieties 'Karzo' and 'Springcar', were both registered in the years 1993 and 1995, respectively. In the Mediterranean region, varieties mostly originated from local wild populations and they are known as 'Balady' in Arabic. In order to get the highest seed, essential oil and carvone yields, the identified varieties/landraces popular in a province should be used for cultivation. There is one report of transgenic caraway from the Netherland (Krens, *et al*., 1997). A population of annual caraway was evaluated over nine years for quality parameters in comparison to biennial caraway in the Central German area. Annual caraway has the potential to reach yield and quality levels of biennial varieties. Plant height, 1000-seed weight, carvone content and taste were satisfactory, but earliness, homogeneity, yields, contents of essential oils and colour need improvement. Also, the causes of low seed germination (40%) have to be investigated (Pank and Quilitzsch, 1996).

Clear agro-botanical differences were observed between wild and cultivated populations. Cultivated populations were characterized by a longer growing period, differences in rosette growth habit, larger and heavier seeds, and a higher and more constant seed germination capacity. The essential oil content of all seeds was variable (2.3–7.6%); the average oil contents of wild and cultivated forms were 5.0 and 5.1%, respectively. The highest oil contents were found in a cultivated Swiss and a wild Finnish population (7.6 and 7.5%, respectively). The average oil content of wild Finnish populations was significantly higher (5.3%) than that of cultivated Finnish forms (4.8%). The main constituents of most of essential oil

samples were carvone (40–60%) and limonene (38–54%). High carvone contents were observed in a Norwegian and an Icelandic population. The carvone and limonene ratio of wild populations from northern parts of Finland was higher than that from southern parts.

Populations from higher elevations in the Alps also had high carvone:limonene ratios (Galambosi and Peura, 1996). The caraway selections A.Car-01-91 and A.Car-01-94 both annual types are being acclimatized to semi-arid conditions in India for high yield and essential oil content (Malhotra, 2005). The caraway, Bi-An, a new biennial cultivar which flowers in the first year of growth, was selected from commercial biennial varieties of caraway, and was grown in the field at Newe Ya'ar, Israel. The composition of the essential oil, hydro-distilled from fruits of the new cultivar, was analyzed. The main constituents of the essential oil were limonene (50.16%) and d-carvone (46.74%) (Putievsky *et al*., 1994).

15.2.9 Organic farming

In recent years, organic agriculture has gained importance and many farmers are showing interest in organic farming. The demand for organic caraway is steadily increasing, because many consumers have a preference for the organic product of this group of spices. The importance of organic farming can be inferred from the fact that some European countries are supporting organic agriculture by giving subsidies for conversion. Demand for organic spices varies considerably from country to country and the kind of spices in a particular country. The European countries, the USA, Canada and Japan are by far the largest markets and looking far organic spices from the high-productivity areas in the world. The newly emerging markets for organic spices are Australia, New Zealand and some other European countries.

No reliable published data is available for caraway organic seed production and export but as a whole it is not more than 20 tonnes as assessed from important buyers. The major organic caraway-producing countries are the Netherlands, Germany and Norway. Keeping in mind the growing demand for organic spices in the global market, it is necessary to give a boost to the organic farming of spices by tackling a few issues related to the costly and cumbersome land certification system and availability of the technical knowhow especially on production, processing, storage and market information. The future demand for organic spices appears to be bright. The general and specific guidelines for organic production of seed spices including caraway have been detailed by Malhotra and Vashishtha, (2004).

15.3 Chemical structure

Chemical composition varies with variety region, stage of harvest and method of distillate. 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. Caraway grown in the northern latitudes yields higher quantities of volatile oil than that cultivated in the warmer climates. The essential oil content varies with stage of harvest, variety of caraway and geographical region and has been reported by various workers ranging from 0.99% to 8.1% (El Wakeil *et al.,* 1986; Atal and Sood, 1967). The chief constituents of essential oil of caraway range from 47–81.17%, carvone and 9.4–48.7%, limonene to which chiefly

the odour and flavour are attributed. The essential 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 chemical constituents of caraway can be classified as primary and secondary metabolites. The first group comprises substances playing a vital role and necessary in normal cell life processes, the second is usually of broader interest due to the presence of bioactive substances contributing to flavour, fragrance and medicinal value. Our major concern is with secondary metabolites as they yield bioactive substances specific to a crop species. Thus, the growing interest nowadays is for secondary metabolites, viz., terpenes, flavonoids, coumarins and phenolic constituents of *Carum carvi* due to their antioxidative properties. The main primary metabolites identified and characterized for caraway samples by various workers are saccharides (monosaccharides – glucose, fructose, disaccharides-sucrose; trisaccharides – umbelliferore (Hopf and Kandler 1976), lipids (triglycerides, 66%; free fatty acids, 5.1%, steroids, 0.4%, hydrocarbons, 0.2%; chlorophyll, 0.2%; waxes,0.1%; free alcohol 0.1% (Stepanenko, *et al*.,1980), amino acids such as alanine, phexylalanine, methionine, glutamic acid, serine and valine (Perseca *et al*., 1981), endogenous abscisic acid (ABA) 120 µg/kg of d.wt (Mendez, 1978) and other minor miscellaneous constituents, caraway choline, 0.03–0.15% (Matsuzawa and Kawa, 1996). A linear relationship between ABA content and dormancy degree in caraway seed has been noticed (Hradlik and Fiserova, 1980). The constituents carvacrol, cumin alcohol and cumin aldehyde found in volatile parts of caraway essential oil are phenolic substances.

Research on the constituents responsible for the antioxidant properties of *Carum* has led, among others, to carvacrol (Lagouri and Boskau, 1995) and dihydro-derivatives of main terpenes-dihydrocarbon and dihydrocarvecol are the important mixtures of stereoisomer. The contents of other minor and trace substances in the oil may vary within broad limits as shown in Table 15.1., which presents an analysis of seed samples from Egyptian origin and mid-European countries. Upon hydro-distillation, 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%) Chowdhury (2002). The other constituents identified were citronellyl acetate, dihydrocarvone, eugenol, isolimonene and limonene oxide, δ 3-carene, camphene, caryophyllene, carveol, ρ-cymene, dihydrocarveol, linalool, ρ-mentha-2,8-dien-1-ol, myrecene, α-pinene, β-pinene, phellandrene, sabinene, α-terpinene and terpinelene and were isolated in trace amounts.

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. Solvent extraction of the crushed seeds with hexane, a method using triple extraction and ultrasonic treatment, led to nearly identical results as hydro-distillation with dill, but to carvone values 16% lower with caraway (Bailer *et al.,* 2001). The four varieties (Gintaras, Rekord, Chmelnickij and Prochana) were studied by Venskutonis *et al.,* (1999) under different nitrogen fertilizer regimes (0–120 kg ha^{-1}) and found that total content of essential oils in fruits 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. These compounds accounted for more than 96% of the total essential oil of all analyzed samples.

Table 15.1 The constituents of caraway essential oil

Constituents	Contents (% of dry weight)	
	El Wakeil *et al.,* 1986)	Pushman *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

Flavonoids (flavonoid glycosides) are the other important secondary metabolites of *Carum* and seed flavonoids occur in the form of 3-0-glycosides in *Carum carvi*. Few crystalline compounds were obtained from caraway seed methanolic extract and are terpenoid constituents, predominating the oil constituent is S(+) carvone (formerly carvol and δ-carvone), results mostly from allylic oxidation of R(+) limonene with carveol. The monoterpene R(+) limonene ratios vary from 3:2 up to 3:1, depending on variety of plant and storage conditions. The following compounds were obtained crystalline from caraway seed methanolic extract (Glidewell, 1991; Kunzemann and Herrmann, 1977; Ruszkowska, 1998). From the water-soluble portion of the methanolic extract of caraway fruits, an aromatic compound glucoside and a glucide were isolated together with 16 known compounds. Their structures were clarified 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 listed below.

quercetin 3-glucuronide
isoquercitrine = quercetin-3-O-B-glucopyranoside
quercetin-3-0-Caffeylglucoside
kalmpferol-3-glucoside

Isoquercitrine is the predominating constituent (80 mg/kg dry weight) present in caraway seed while others are found between two and ten times lower than this quantity. The above-mentioned flavonoids are known to possess antioxidative properties and activate enzymes detoxifying carcinogenic substances and metabolites in the cells.

The enantiomeric composition of limonene and carvone caraway seed oils was determined by chiral gas chromatography. Two different gas chromatography chiral

columns were used to obtain enantiomeric separation of both aroma compounds and two varieties of caraway were used for investigation. In Plewicki, the concentrations of limonene and carvone were 31.41 and 36.24 mg/g, respectively, and in Konczewicki they were 17.60 and 22.46 mg/g, respectively. The enantiomeric ratio was stable for both compounds in the analyzed samples. The purity, expressed as a percentage of + optical form to total, was high for R(+)-limonene (99.1–99.5%) and S(+)-carvone (99.4–99.8%) in caraway seed oils (Zawirska, 2000). Microsomal preparations from fruits of annual (cv. Karzo) and biennial (cv. Bleija) forms of *C. carvi* catalyse the C-6 hydroxylation of (+)-limonene to (+)-trans-carveol, the key intermediate in the biosynthesis of carvone ((+)-limonene-6-hydroxylase activity) as reported by Bouwmeester *et al*., (1998, 1999).

The biosynthesis of the monoterpenes limonene and carvone in the fruits of caraway (*Carum carvi*) proceeds from geranyl diphosphate via a three-step pathway. First, geranyl diphosphate is cyclized to (+)-limonene by a monoterpene synthase. Second, this intermediate is stored in the essential oil ducts without further metabolism or is converted by limonene-6-hydroxylase to (+)-*trans*-carveol. Third, (+)-*trans*-carveol is oxidized by a dehydrogenase to (+)-carvone. The presence of antiproliferative polyacetylenes was suggested in *Carum carvi* (fruit and root) and were successfully isolated by Nakano *et al*., (1998).

The coumarins in caraway seed were identified as umfelliferone, coumarin and scopoletin, (Nielsen,1970) whereas the furocoumarins reported are 8-methoxypsoralen (8-MOP). Five methoxypsoralen (5-MOP) were detected of bioassay of caraway seeds of low quality of 0.005 μg/g of dry weight (Ceska *et al.,*1987). The coumarins and furocoumarins are known to have antibacterial, potent photosensitizers when activated by near UV light and thus they are phototoxic, mutagenic and photo-carcinogenic and 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 preparation.

The presence of a high content of phenolic substances is attributed significantly as a stabilizing effect of some spices on food especially on meat products. The phenolic functional group is known to have antimicrobial or antioxidant properties of active substances. The phenolic compounds identified in *Carum* seed are flavonoids, glycosides, derivatives of quinic acid, proteids and tannins. The isolation of a flavone from the methanolic extract of the seeds of *Carum carvi* was characterized by Rahman and Hossain (2003) as 4′,5,7-trihdyroxy-2′-methoxyflavone.

The major constituents of essential oil of caraway are carvone and limonene, which are known to possess insecticidal or insect-repellent effects (Zuelsdorff and Burkholder, 1978, Su, 1987), antibacterial and antifungal effects (Janssen, *et al*., 1988), inhibition of seed germination (Asplund, 1968) and sprouting in potatoes (Beveridge, *et al*., 1981). The chemical structures of carvone and limonene, the major compounds of caraway essential oil, are illustrated, in Fig. 15.1.

15.4 Main uses in food processing

Caraway is widely used as a spice for culinary purpose and for flavouring various food products. The main caraway products are fruit (generally known as seed), herb and seed oils. It was popular from ancient times for its use in folk medicines and the entire plant of caraway has its herbal value but commercially it is valued for fruit.

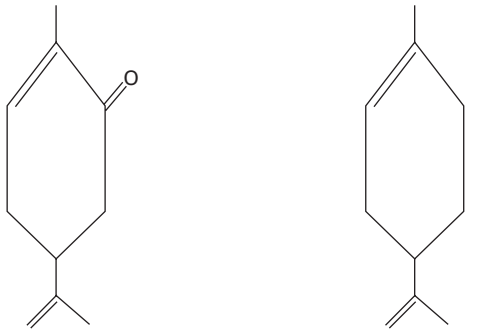

Fig. 15.1 The major compounds of caraway essential oil.

Caraway is grown widely in the Netherlands, Germany, Poland, Ukraine, Hungary and Romania for seed purposes and is reported to have been used as a condiment for flavouring and food preparation in Europe and Middle East in ancient times. The main processed products of caraway are whole seed, essential oil, oleoresin, powder, and a few others. They are used in the food industry, cosmetics, beverage and pharmaceutical industries primarily for flavouring and medicinal purposes (Malhotra, 2006a). The main processed products from caraway seeds and their uses in the food processing industry are described below.

15.4.1 Whole seed

The caraway seed has a characteristic distinct warm, slightly sweet, very sharp somewhat acrid but pleasant aroma. Caraway seed is processed for drying, cleaning, grading and is mostly traded in this form in the international market. Due to its inherent preserving qualities it is known to possess good storage life similar to pepper. Caraway seed is widely used as a spice for seasoning, at both the household and commercial levels.

Use at household level

In middle Europe caraway is used as a common spice, the Germans use caraway seed in many of their baked breads, piecrusts, sauces and their famous sauerbraten, whereas the Austrians like it in stews. Italians boil hot chestnuts with caraway seed before roasting them. Caraway masks the smell of heavy foods like spare ribs, roast goose, pork, mutton, oxtail stew or other hearty meat dishes, and adds an interesting sweetness to apples, pound cake and cheeses. Caraway seed is used in canapés, onion bread, cheese spreads, omelettes, 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).

Use at commercial level

Caraway seed is mostly used in bakery products and alcoholic beverages for adding taste and aroma. In the bakery industry, caraway seed is not only mixed into white and rye bread but is also sprinkled on the dough before baking for better dispersed aroma and for enhancing the taste impression (Daffershofer, 1980).

The flavouring of different kinds of alcoholic beverages has a long tradition particularly from Denmark and other Scandinavian countries. The popular products described as *akvavit* or *aquavit* are flavoured using neutral alcohol distillates of caraway. Caraway is added only before distillation (Ney, 1987) and in this way the flavour of the drink is attributable to the distillates of caraway. Some well-known alcoholic beverages world wide are listed in Table 15.2. In American Gin the flavour additives mostly used include juniper berries and cardamom as well as caraway seeds (Cole and Nobel, 1995).

15.4.2 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 oil, oleoresin and other extractives. The pre-chilling and reduced grinding can be used to overcome the loss of volatiles. Cryo-grinding, can better help in reducing the oil loss during the process of grinding and maintaining the particle size to optimum so as to ensure the free flow for the duration of its shelf-life (Russo, 1976). Moreover, cryoground caraway dispenses more uniformly in spice formulations and is therefore used as a spice for seasoning, at both the household and commercial levels. Ground caraway is mostly used for adding taste and aroma to various food preparations at home level, in bakery products and alcoholic beverages.

15.4.3 Essential oil

Caraway essential oil is obtained by steam distillation or hydro-distillation of fruits or chaff or herb and root according to the market requirement and particular use. But the essential oil extracted from seed is superior in quality and commercially valued more. In general, the essential oil content in caraway seed ranges from 2.9 to 5.1% with major components of d-carvone up to 65% and d-limonene up to 40% but these proportions are variable. The high-quality seed may contain up to 7% volatile oil and up to 15% fixed oil. Sedlakova *et al*., 2003b reported that seed samples collected before maturation had lower essential oil content than samples harvested in full ripeness. Samples collected before harvest had elongated, narrow seeds, while those gathered after ripeness had rounder seeds. The recovery of essential oil content was also more from supercritical fluid extraction than steam distillation on caraway and the essential oil content extracted was comparatively more from ground caraway rather than whole seeds in both of the methods of essential oil extraction.

In whole caraway extracts, the carvone content was 81.53%; in ground caraway

Table 15.2 Popular alcoholic beverages using caraway (Clutton, 1995)

Beverage name	Origin	Remarks
Akvavit or aquavit	Scandinavia	Caraway with aniseed and fennel 40% alcohol
Allash	Russia	Sweet kummel with bitter almonds and aniseed
Cloc	Denmark	Kummel 31% alcohol, colorless
Kummel	Netherlands	Caraway with some anise and cumin minimum 5% alcohol, one of the oldest liqueurs with digestive properties.

extracts, the carvone content declined to 66.37%. Among the three types of seed mills evaluated by Sedlakova *et al*., (2003a) (ETA 0067 with millstones, splintery VIPO mill and cryogenic mill Vibrom), the highest amount of extracted essential oil was obtained with the splintery mill VIPO (2.55%). The essential oil is a mobile liquid, almost colourless to pale yellow with a warm, spicy hot taste. The oil has virtually replaced the seed in processed foods, and is extensively used as a flavour component in processed meats, pickles, sauces, seasonings and similar preparations in alcoholic and non-alcoholic drinks.

The fresh seed can be crushed and immediately processed for distillation to avoid evaporation losses and recovery of more essential oil content. The average essential oil yield, as assessed on laboratory scale, was around 70 kg/ha with a top yield of 160 kg/ha (Dachler *et al*., 1995). The oil has a strong characteristic odour due to the carvone content and the rectified oil received from the process of double distillation is colourless to pale yellow and has a strong odour and more biting taste. Caraway essential oil has a ready market in the food, cosmetic and pharmaceutical industry. It is used in all major categories of foods including alcoholic and non-alcoholic beverages, frozen dairy desserts, candy, baked goods, gelatins and puddings, meat and meat products, condiment and relishes and others. 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 toothpastes, mouthwashes, soaps, creams, lotions and perfumes, with a maximum use level of 0.4% reported in perfumes (Leung and Foster, 1996).

Caraway essential oil may be used as animal food (pasture) for milking cows and sheep according to old publications by Heeger, (1956) but nowadays it is uncommon. Dried seeds after crushing are processed for distillation in order to obtain a better yield and higher quality of oil; crushed seeds are spread evenly on perforated grids provided in the still so that complete penetration of the steam is allowed. It takes about 6–8 hours for optimum distillation of one batch. According to Bentley and Trimen (1999) the caraway derived from a northern or elevated locality, yield the most oil. Moreover, the oil distilled from grown caraway is preferred, and is alone recognized in the British pharmacopoeia. The Dutch oil is also regarded as better than that distilled in the southern parts of Germany.

15.4.4 Fatty oil

The fatty oils produced from the distillation process of caraway seed has been reported to 15% and particularly rich in petroselinic acid. The fatty acid profiles of the oils were analysed by automated GC and petroselinic and *cis*-vaccenic acids were obtained as the major components (Reiter *et al*., 1998). The petroselinic acid is an important raw material for oleochemical processes and can be easily cracked into lauric and adipinic-acid (Lechner, 1997) in the related industry. The fatty oils produced from caraway seed have their use in various pharmaceutical and cosmetic products. Caraway fatty oils are primarily used in the soap industry, for flavouring and as a deodorant in the manufacture of perfumed disinfectant soaps.

15.4.5 Caraway chaff oil

Caraway chaff oil is distilled from the husks and stalks that remain after threshing and is considered inferior in quality compared with oil extracted from seeds. The

dried exhausted and pulverized caraway chaff contains 20–23.5% crude protein of which 75–85% is digestible and 14–16% is fat and can be used as an ideal cattle feed. The chaff oil is obtained by steam distillation of material left after threshing of fruits and contains less carvone and more terpenes. It has less of the characteristic odour of the seed oil and is harsher with a somewhat bitter taste. This is produced on a very small scale and is also used as an adulterant of the fruit oil.

15.4.6 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 the distillation process and stems are removed. Some growers feel that it should be harvested before flowering and others say it is better afterwards. Caraway herb oil has similarity in flavour with oil extracted from seed and 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 it oxygenated compounds with aldehydes up to 70% including octanal, nonanal, *cis*-dec-4-enal and *trans*-dec-2-enal. The complete analysis of herb oil and root oil in comparison to essential oil extracted from seed is not available but it is considered that it is inferior in quality to seed oil and is also used as an adulterant of fruit oil.

15.4.7 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 and slightly floral. The taste is sweet, spicy and bread-like. The carvone reportedly has certain cancer-preventive properties and anthelmintic properties. Pure carvone is prepared by decomposing crystalline compounds of carvone with hydrogen-sulphide. Carvone also has uses in the soap industry for addition of natural aroma. Demand for carvone fluctuates and is confined to a particular segment of the market but regular extraction of carvone can become an alternative to caraway seed in the food-processing and pharmaceutical industries.

15.4.8 Decarvonized oil

Decarvonized oil consists of limonene with traces of carvone and is sold on the market as light oil of caraway. It finds use in scenting soaps. At the beginning of distillation the essential oil has higher carvone, whereas at the end limonene predominates. The reason is that carvone is an oxygen-containing compound and is several times more soluble in water than limonene.

15.4.9 Caraway oleoresin

The oleoresin of caraway fruit 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 oleoresin as per the ISO, as well as the fixed maximum permissible limits for the approved solvents of the importing countries. Caraway oleoresin is one of the most valuable flavouring agents as it imparts warm, aromatic

and pleasing flavours to food products. It contains essential oil, organically soluble resins and other related materials present in the original spices, is usually a greenish shade of yellow, and normally contains 20–25% volatile and 60–75% fixed oil as reported by Weiss (2002). The effects of polar solvent or modifier (methanol, ethanol, acetone, acetonitrile, hexane, dichloromethane (methylene chloride), chloroform or toluene)) application during extraction on essential oil yield were studied in cv. Kepron and all modifiers significantly increased the essential oil yield. The use of chloroform was most effective, increasing the amount of extracted essential oil by approximately 91% compared to steam distillation (Sedlakova *et al*., 2003b). 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.

15.5 Functional properties

Caraway fruits are aromatic and are quite spicy in taste. An analysis of caraway seed samples shows it contains small amounts of protein, fat, carbohydrates, minerals and vitamins. The nutritive constituents present in caraway seed are given in Table 15.3. The nutritive value of caraway seed if consumed as such is small but it is valued more for the peculiar flavour and specific medicinal properties.

Caraway seeds and extractives are known to possess a number of functional properties and are therefore valued as folk medicines for curing various ailments and as contemporary medicine in the cosmetic industry. They are

- carminative, stimulant and expectorant
- antiflatulent and antispasmodic

Table 15.3 Proximate composition of caraway seed (100 g edible portion)

Composition	Content	
	Farrell, 1999	Bakhru, 2001
Water (g)	9.9	4.5
Food Energy (KCal)	333	465
Protein (g)	19.8	7.6
Fat (g)	14.6	8.8
Total carbohydrate (g)	49.9	60.2
Fibre (g)	12.7	–
Ash (g)	5.9	3.7
Calcium (mg)	689	1000
Iron (mg)	16	90
Magnesium (mg)	258	–
Phosphorus (mg)	568	110
Potassium (mg)	1351	1900
Sodium (mg)	17	20
Zinc (mg)	6	–
Niacin (mg)	4	801
Vitamin A (IU)	363	580
Thiamine (mg)	–	3.38
Riboflavin (mg)	–	0.38
Vitamin C (mg)	–	12

- mildly antibacterial and antifungal
- antidyspepsic
- emmenogogues and lactogogues

The seeds of *Carum carvi* are like those of many other umbelliferous plants, aromatic and stimulant and are perhaps the most commonly used of any and are excellent carminatives and stomachics. Caraway fruit is mentioned by pharmacopoeias of numerous European countries, the USA and others, and most of all is used as a component of herbal mixtures recommended as digestives, carminatives and galactagogues. 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 preparations solely (Lutomski and Alkiewicz, 1993; Ozarowski and Jaroniewski, 1987) and or in composition with other herbs and spices (Sadowska and Obidoska, 1998) are given in Table 15.4.

The other important functional properties reported for the caraway seed and essential oil are as antiflatulents and antispasmodics. In colic and gastrodynia, a few drops of this oil or half a teaspoonful of the seeds are sovereign remedies. A liniment formed by adding a few drops of this oil to a small quantity of olive oil is rubbed over the pit of stomach or the abdomen in cases of colic (George, 1996). Being antispasmodic, the seeds soothe the digestive tract, acting directly on the intestinal muscles to relieve colic and gripping as well as bloating and flatulence. The presence of d-limonene and d-carvone probably contribute towards caraway's antispasmodic action. Duke *et al*., (2002) have mentioned ED 5O caraway oil as a confirmed antispasmodic when used at a dose of 20 mg/l. In a study tablets containing a combination of 100 mg of each of peppermint leaves, caraway and fennel fruits, and 30 mg gentian root were administered to patients with idiopathic dyspepsia. In the first study, administration of three, six or nine tablets (or a placebo) to patients with acute symptoms immediately after a meal showed that three tablets were sufficient to reduce these after an hour. In the second, patients with chronic symptoms were each given two tablets three times a day for 14 days, or a placebo. Relief was obtained in the experimental patients after a week, with a further improvement in the second week (Uehleke *et al.*, 2002). The enteric-coated combination preparation consisting of (2x1 capsules containing 90 mg peppermint oil + 50 mg caraway oil) per day as compared with cisapride, provide an effective means for treatment of functional dyspepsia (Madisch *et al.,* 1999; Freise and Kohler, 1999).

Caraway is recommended as a remedy for digestive tract disorders like flatulence, eructation, stomach aches, constipation, lack of appetite and nausea. In small children caraway is used to treat flatulence and stomach aches, in the elderly for bile flow disorders, intestinal atony and vegetative neurosis (Ozarowski and Jaroniewski, 1987). Fruits of caraway ingested orally produce an effect on the digestive tract, bile ducts, liver and kidneys. They have spasmolytic properties, bile ducts and the sphincter regulating the flow of bile and pancreatic juices to the duodenum. They act as a cholagogue and increase the secretion of gastric juices, which results in appetite and digestion stimulation.

The 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 the antigripping quality present in it. The component acting as a galactagogue in caraway seed has not been identified but limonene and carvone, the main components of caraway seed having antigripping qualities, were found in the essential oils of goat milk when goats had consumed 3.5 g caraway seeds

Table 15.4 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 TSF honey	Carminative	2–4 times a day
b. Caraway tea	Pour 1.5 glass (capacity 0.35 l) of boiling water over 1 TSF of pulverized fruits.	Carminative	Drink 0.5 glass 2–3 times a day after meals
c. Caraway syrup	Pour 1 glass (capacity 0.25 1) of boiling water over 1 TSF of pulverized fruits keep covered for 30 min, strain and add honey.	Carminative for children	Serve 1 TSF after each meal
2. Caraway Herbal composition (Lutomski and Alkiewicz, 1993)			
a. Mixture of fruits of caraway, anise, peppermint chamomile and thyme in equal proportion	Pour a glass (capacity 250 ml) of boiling water over 1 TSF of herbs keep covered for 30 min.	Carminative	Drink 0.5 glass 2 times a days after meals'
b. Mixture of fruits of caraway anise fennel in equal proportion	Pour a glass (250 ml) of boiling water over 1 TSF 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 (250 ml) of boiling water over 1 TSF 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 glasse (500 ml) of boiling water over 1.5 TSF of herbs in thermos keep covered for 1 hr.	Digestive (improves appetite)	Drink 0.5 l glass 30 min before meals
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 white, dry wine over 3 TSF of herbs leaves for 2 weeks (Shaking from time to time)	Digestive (improves appetite)	Drink about 50 ml two times a day after meals

Table 15.4 Continued

Preparation	Dose formulation	Properties as medicine	Dose
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 TSF of herbs in a thermos and keep closed for 30 min.	Digestive (improves appetite)	Drink about a 0.5 l glass 3 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
7. Caraway formulations: (Duke *et al.,* 2002)			
a. Caraway seed	1.5–6 g fruit	Antiseptic	2–4 times a day between meals
b. Caraway seed powder	1–2 TSF crushed seed/cup water or Chew 1 tsp seed	Antianemic Antibacterial Anticancer Antihistamine Antispasmodic Carminative Digestive	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 3–4 ml liquid extract	Stimulant	3–4 times a day –
e. Caraway essential oil	3–6 drops oil or 0.05–0.2 ml	Stimulant	

daily supplemented with the diet (Molnar *et al*., 1997). The addition of 50 g caraway seeds to the basic diet daily to lactating buffalo continued for 12 weeks of lactation, increased the milk yield, daily fat, SNF, lactose and protein yield significantly (El-Alamy *et al*., 2001). Caraway 50–100 g diet supplemented daily with ground caraway seeds to Black Pied cows had a favourable effect on the milk yield and milk quality (Portnoi, 1996). Caraway possesses antioxidant properties and in a report by Farag

and El-Khawas, (1998) the essential oils extracted from the gamma-irradiated (10 KGy) caraway fruits were more effective as antioxidants than those produced from microwaved fruits (low oven power setting for one minute).

Caraway essential oil or carvone, owing to antifungal and antibacterial properties, is recommended for external use for the control of dermal mycosis and scabies. The inhibitory properties of caraway extractives have been reported against *Staphylococcus aureus*, *Esherichia coli*, *Salmonella typhi* and *Vibrio cholerae* (Syed *et al*., 1987) and *Mycobacterium tuberculosis* (Mishenkova *et al*., 1985). These properties give caraway industrial importance in scenting soaps to be used as deodorants. 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 for taking orally (Pruthi, 2001, Bakhru, 2001), who further reported caraway seed, seed oil and carvone to possess anthelmintic properties, especially in removing hookworms from the intestines. In Indonesia the leaves mixed with garlic and spat on the skin are recommended to treat inflamed eczema (Perry, 1980).

The taste of caraway being warm, pungent and aromatic makes it suitable for overcoming bad breath or insipid taste and thus is used in oral preparations for control of unpleasant odour or taste. Caraway has been proved as an adjuvant or corrective for medicines and is recommended as a remedy curing digestive tract disorders such as relieving gas from the stomach. It is also known to counter any possible adverse effects of medicines and masks the foul smell of foods. Caraway has also been reported to play a therapeutic role by showing advantageous effects on intestinal iron absorption (El Shobaki *et al*., 1990). The essential oil from caraway has been reported to be potentially anti-carcinogenic (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). They further reported that carvone and limonene are the compounds responsible for the above mentioned property while carvone exhibited even higher activity as a GST inducer. Higashimoto *et al*., (1993) also reported potent 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 diet may even inhibit the early stages of carcinogenesis. Caraway has been reported to be used in the form of poultices for the control of swellings in the breast and the testicles.

15.5.1 Use as veterinary medicine

Due to the presence of several functional properties in caraway such as being carminative, antiflatulent, antispasmodic, antibacterial, antifungal and galactagogue, the use of *Carum carvi* seed and extractives is very popular in the treatment of animals for various ailments. As a veterinary medicine for animals, the caraway herb is more a popular remedy than the fruit. The use of caraway, as decoction of fruit and herbs for animals, improves digestion by promoting gastric secretion and stimulates appetite. It is also used to cure gastrointestinal disorders like flatulence, stomach aches and gripes. Caraway fruit coarse powder or dry herb mixed together, when fed to cows, mares and other animals, enhanced lactation (Voloshchuk, *et al*., 1985, Sadowska and Obidoska, 1998). The decoction of fruits is a good remedy for rabbits, piglets and other animals against verminous disease. The effectiveness of caraway extract has been reported by Gadzhiev and Eminov (1986) against trichostrongyle larvae in rams. An ointment made from powdered fruits mixed with vaseline is

recommended against scabs, manges, mycosis and other dermal diseases. Due to its antibacterial and antifungal functional properties, caraway is also used to heal infected injuries and burns. Caraway diet supplementation 12 g/kg diet in New Zealand white rabbits, improved reproductive efficiency, doe milk yield and pup pre-weaning mortality (Rashwan, 1998). Lipid oxidation was effectively inhibited in chicken meat treated with marjoram (*Origanum*). Wild marjoram and caraway (*Carum carvi*) were the most effective dry spices (El-Alim *et al*., 1999).

15.5.2 Natural potato sprout inhibitor

Besides the use of caraway seeds, caraway seed powder and essential oils in the food and pharmaceutical industries, it has proved to be an important natural sprout inhibitor in potato by extending the dormancy period and quality after storage. Caraway as a natural sprout inhibitor had a positive effect on the reduction in respiration intensity dry matter, reducing sugars and starch contents after seven months during storage (Zabaliuniene, *et al*., 2003). A few monoterpenes from caraway, including S-carvone (the safe food ingredient), were found to suppress sprout growth under warehouse conditions 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.

15.6 Toxicity

Caraway seed and essential oil do not appear to have any significant toxicity to human beings. Most authors agree that caraway shows no toxic affect towards people and is well tolerated in medicinal doses and as a spice. However Lewis (1977), while discussing the problem of allergy, mentioned carvone as a 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) and thus are not harmful. The residues of nitrate, nitrite and pesticides in herbs can be transformed by bacteria to toxic nitrites which can cause blood circulation disorders and methemoglobinemia, but analysis of caraway samples has exhibited no contents of nitrites but nitrates content was noticed in relatively small quantities (Gajewska *et al*., 1995). Similarly, analysis of pesticide residues through gas liquid and thin layer chromatography tests showed that HCH was the main compound found and residue did not exceed the maximum limit of 0.2 mg/kg.

Duke *et al*., (2002) have mentioned that caraway hazards and/or side effects are not known for proper therapeutic dosages. The drug 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. Caraway essential oil has proved toxic to mites and insects. It has been reported to inhibit allergy-causing mites *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 has shown acaricidal properties for inhibiting *Tyrogphagus putrescentiae* mite (Afifi and Hafez,1988) and toxicity to some insects causing larval inhibition in *Musca domestica, Culex pipiens,* fatigans and mosquito (Deshmukh and

Renapurkar,1987) and fifth instar larvae of *Spodoptera littoralis* (Antonious and Hegazy, 1987).

Volatile toxicity of caraway was recorded by setting up a bioassay, with experimental units of 0.5 l, which took into account the storage pests, the mode of oil application (vapours only, avoiding direct contact) and the stored product, causing 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 *et al.,* 2002). The vapours of the essential oils (80–160 ppm) of caraway (*Carum carvi*), exhibited antifungal properties against *Mycocentrospora acerina, Fibularhizoctonia carotae* (*Rhizoctonia carotae*) and *Sclerotinia sclerotiorum*, three important post-harvest pathogens of carrots. Horberg (1998) also reported that high dosage levels were more important than exposure time for the fungicidal activity of the plant extracts.

Numerous species of fungi are known to produce toxic and carcinogenic mycotoxins during storage, they can be consumed with contaminated fruits, cumulated in liver and can result to cancer. The fungi like *Aspergillus flavus, Aspergillus niger* and *Fusarium moniliforma* can cause biochemical changes in caraway fruits and can lead to reduction in protein, carbohydrates, and total oil and increase in fatty acids (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 *A. flavus* and *A. parasiticus,* and at 3000 ppm against *A. ochraceus* and *F. moniliforma*, the mycotoxigenic fungi (Soliman and Badeaa, 2002) and use of caraway oil (4%) also showed high antimicrobial activity against *A. tumefaciens*, *R. solanacearum* and *Erwinia carotovora* (Hassanein and Eldoksch, 1997).

The application of caraway essential oil has shown inhibitory effect on three strains of Gram-negative and four Gram-positive bacteria. Thus according to Farag *et al*., (1989b), the use of natural essential oils can be of great importance practically as anti-microbial agents to prevent deterioration of stored foods by bacteria and will not cause health problems to the consumer and handler. Likewise, *Carum carvi* essential oil causes inhibition of mycelial growth and aflatoxin production of *Aspergillus parasticus* and can prove to be an alternative to chemical preservatives such as potassium fluoride, acetic acid and potassium sulphite addition in foods (Farag *et al.,* 1989a). Such toxic properties of caraway to bacteria, fungi and insects and non-toxic behaviour to human beings offers great scope as a botanical inhibitor for crop raising and safe storage under the organic production system.

15.7 Quality specifications

15.7.1 Specification for whole seeds

The physical description of the quality of caraway seeds depends mainly on

- Quantity of mature, undamaged seeds with external appearance that provides visual perception of quality such as 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 scent from seeds is very aromatic, sweet, spicy, fresh, characteristic, agreeable, slightly minty, with a penetrating medicinal effect resembling anise.

- The 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 below (Pruthi, 2001).

Whole seed
Caraway whole seed means the dried seed of the plant (*Carum carvi* Linn). Extraneous matter including foreign edible seeds, chaff, stem straw, dust, dirt, stones and lumps of earth shall not exceed 5% by weight. The amount of insect damaged matter shall not exceed 5% by weight. It shall be free from added colouring matter.

15.7.2 Caraway powder

Caraway powder means the powder obtained from the dried seed of *Carum carvi* (L). It may be in the form of small pieces of the seeds or in finely ground form. It shall 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%

15.7.3 Essential oil and fixed oil

The essential oil content of caraway seed generally ranges between 2–5% and it primarily contains carvone (47–81%), limonene (9–48%) and fixed oil (15%). Caraway oil is a mobile liquid, almost colourless to pale yellow, although it may become brownish to dark brown depending upon time. The physico-chemical properties of caraway seed oil are as follows (Singhal *et al.,*1997)

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 volumes of 80% alcohol, clearly soluble in equal volumes of 90% alcohol.

Table 15.5 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 15.6 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).

The quality standards as prescribed by the American Spices Trade Association (ASTA) and ISO are given in Table 15.5 and 15.6.

15.7.4 Adulteration

Caraway seed is available both whole or in ground form and is subjected to adulteration by the addition of exhausted or spent seed (from which oil or oleoresins have been extracted), excess stems, chaff and earth or dust. Caraway essential oil is also adulterated with 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 an enantiomeric ratio ranging between 0.75–1.00. If chaff oil is added than the enantiomeric ratio will be 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 and requires further study to standardize such quality parameters for judging 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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16

Cayenne/American pepper

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16.1 Introduction

American pepper (synonyms: chilli, chile, azi, cayenne, hot pepper, sweet pepper) is a popular commercial crop valued for its fruit colour, flavour, spice, vegetable and nutrition it provides to several food items. Plants are a dicotyledonous and short-lived perennial herb of the Solanaceae (nightshade) family and are commercially cultivated as an annual and perennial in kitchen gardens. Among the five cultivated species of *Capsicum*, *C. annuum* is the most commonly cultivated for pungent (hot pepper) and non-pungent (sweet pepper) fruits and has worldwide commercial distribution. The sweet pepper is often called bell pepper because the majority of sweet pepper cultivars grown worldwide have bell-shaped fruits. India, China, Korea, Hungary, Spain, Nigeria, Thailand, Turkey, Kenya, Sudan, Uganda, Japan, Ethiopia, Indonesia, Pakistan, Mexico are the major pepper-growing countries.

The tap root consists of a main root with lateral roots with uniform distribution on the main axis and the occurrence of adventitious roots is very rare in pepper. Stems are branched, erect or semi-prostrate, fleshy often woody at the base, round or slightly angular growth normally indeterminate. Flowers are small, terminal but due to form of branching, appear to be axillary, small calyx, rotate companulate corolla and 5–6 stamens, which are inserted near the base of corolla. Unlike other members of the nightshade family, *viz.*, tomato, eggplant and potato, pepper leaves uniquely lack phenols. Hence it has been postulated that nature has provided a pepper capsaicinoids (pungency) pathway to protect plants from enemies. This could be viewed as an analogue of the phenol pathway present in other members of the nightshade family.

Nutritional compositions of pepper fruits depend on the genotype and fruit maturity stage. In general, 100 g of green fruits contain 85.7 g moisture, 2.9 g protein, 0.6 g fat, 1.0 g minerals, 6.8 g fibres, 3.0 g carbohydrates, 30 mg calcium, 24 mg magnesium, 0.39 mg riboflavin, 67 mg oxalic acid, 0.9 mg nicotinic acid, 80 mg phosphorus, 1.2 mg iron, 6.5 mg sodium, 217 mg potassium, 1.55 copper mg, 34 mg sulphur, 15 mg chlorine, 0.19 mg thiamine, 292 IU vitamin A and 111 mg vitamin C. Green fruits of hot and sweet peppers are one of the richest sources of antioxidative vitamins such

as Vitamin A, C and E. In fact, vitamin C was first purified from *Capsicum* fruits in 1928 by Hungarian biochemist Albert Szent Gyorgyi, which helped him to receive the Nobel Prize in physiology and medicine during 1937.

In this chapter, attempts have been made to describe in brief the taxonomic status of pepper and elaborate innovative uses of carotenoids and capsaicinoids present in the pepper fruits and their biosynthetic pathways. General cultural practices of growing pepper under open field conditions have also been briefly described.

16.2 The genus *Capsicum*

The genus *Capsicum* perhaps comes from the Latin word 'capsa', meaning chest or box because of the shape of fruits, which enclose seeds very neatly, as in a box (Berke and Shieh, 2000). The *Capsicum* ($2n = 24$) encompasses a diverse group of plants producing pungent or non-pungent fruits. At present, it is widely accepted that the genus consists of approximately 25 wild and five cultivated species. Based on the gene flow through natural and conventional hybridization, the *Capsicum* species are grouped in three species complexes (Table 16.1). Among the cultivated species, *viz.*, *C. annuum, C. frutescens, C. chinense, C. baccatum* (var. *baccatum*), *C. pubescens,* cultivation of *C. annuum* is the most widely spread all over the world. *C. annuum* was domesticated in the highlands of Mexico and includes most of the Mexican chile (syn. chilli), most of the chilli of Asia and Africa and sweet peppers of temperate countries. However, due to the non-adaptability of *C. annuum* in lowland tropics of Latin America, its cultivation was replaced by *C. frutescens* and *C. chinense* (Pickersgill, 1997). The cultivation of *C. baccatum* and *C. pubescens* is mostly restricted to Latin American countries like Peru, Bolivia, Columbia and Brazil. In India also, although *C. annuum* is most widely cultivated, *C. frutescens*, *C. chinense* and *C. baccatum* are also grown in specific regions. Except for *C. pubescens*, wild forms of the remaining four cultivated species are known.

All the five cultivated species of *Capsicum* are represented by genotypes with pungent (hot pepper) or non-pungent (sweet pepper) fruits. Furthermore, these species have huge variability for fruit size/shape and pungency and often genotypes with similar fruit morphology exist across the species. Hence assigning a given genotype to a specific cultivated species based on fruit size, shape and pungency is difficult. Nonetheless, certain flower and fruit descriptors may be used to assign a genotype to a cultivated species without much doubt (Table 16.2). Recently, molecular markers associated with specific species within *C. annuum* the complex have been developed.

Table 16.1 Three recognized species complexes of the genus *Capsicum*

Complex	Species
C. annuum complex	*C. annuum** L., *C. frutescens** L., *C. chinense** Jacq., *C. chacoense* Hunz., *C. galapagoense* Hunz.
C. baccatum complex	*C. baccatum** L., *C. praetermissum* Heiser & Smith, *C. tovarii*
C. pubescens complex	*C. pubescens** Ruiz & Pav., *C. cardenasii* Heiser & Smith, *C. eximium* Hunz.

* Cultivated species.

Table 16.2 Distinguishable morphology of five cultivated species of *Capsicum*

Cultivated species	Distinguishable morphology
C. annuum	White corolla and white filaments
C. frutescens	Yellow/greenish corolla and purple filaments
C. chinense	Annular constriction on pedicel attachment and yellow/greenish corolla
C. baccatum	Yellow or greenish yellow spots on corolla
C. pubescens	Hairy stems/leaves and black/brown seeds

16.3 Pod types and quality breeding goals

Tremendous morphological variability exists for flower morphology, especially corolla and anther colour and fruit colour, size, shape and pungency. Based on fruit size, shape and degree of pungency, a large number of horticultural types are recognized worldwide and at least 20 types are predominantly cultivated on a large scale in other parts of the world. Some of these fruit types such as ancho, bell, jalapeño, pasilla, New Mexican and yellow wax have a specific trait for processing, fresh use, flavour and pungency (Bosland and Votava, 2000). The breeding objectives for quality traits of hot pepper and sweet pepper could be described on the basis of five market types, *viz*., (i) fresh market (green, red, multi colour whole fruits), (ii) fresh processing (sauce, paste, canning, pickling), (iii) dried spice (whole fruits and powder), (iv) oleoresin extraction and (v) ornamental (plants and/or fruits) (Poulos, 1994). The current pepper breeding programmes have relied on a relatively narrow genetic base within cultivars of various market types, although huge morphological diversity exists within (intraspecific) and between (interspecific) species. This is because of (i) traditional market demand for specific fruits size and shape and (ii) the use of pure line or back cross breeding within open pollinated commercial varieties and development of inbreds from the commercial hybrid and their utilization as recycled parental lines (Poulos, 1994).

16.4 Uses in food processing

Pepper is a most popular and widely used condiment all over the world. Fruits are consumed in fresh, dried or processed forms, as table vegetable or spice. Fruits are extensively pickled in salt and vinegar. Fruit carotenoids (colour), capsaicinoids and flavour extracts are used in food, feed, medicine and the cosmetic industries. Sweet peppers are widely used at green-immature or mature stage as a vegetable. The fruits of the genus *Capsicum* have many versatile and innovative uses and diversity (Bosland, 1996; Dewitt, 1998; Bosland, 1999; Table 16.3).

16.4.1 Pungency (capsaicinoids)

The pungent-oily substances from the fruits of hot pepper were first discovered and isolated by Bucholz in 1816 and the most active ingredient (named capsaicin) was isolated by Thresh in 1846 (Govindarajan, 1986). The burning sensation (pungency) one gets from eating pepper fruits is caused by alkoloids called capsaicinoids, which are uniquely produced in *Capsicum*. Capsaicinoids are acid amides of C_9-C_{11} branched chain fatty acids and vanillylamine. The pungency is expressed in Scoville Heat

Table 16.3 Versatile and innovative uses of pepper

I. Fresh uses: immature green fruits, mature red fruits and leaves
- Green or red ripe fruits with variable degrees of pungency are invariably added in most South Asian curries.
- Immature or mature non-pungent fruits are exclusively prepared as vegetables.
- Immature non-pungent fruits are added in many Chinese cuisines.
- Immature mild pungent fruits are deep fried with gram flour and consumed in India.
- Fresh green non-pungent or mildly pungent fruits are consumed as salads.
- In the Philippines, leaves are added to soup and stew and consumed. The upper shoots of the plants are sold in bunches, just like other leafy vegetables (Bosland, 1999).

II. Fresh processing: sauces, pastes, pickles, beer
- Green or red ripe fruits with variable degrees of pungency are used to prepare sauce.
- Red ripe and mildly pungent fruits are stuffed with certain spices in North Indian states and prepared as pickles. Similarly, green fruits are pickled in edible oil and red ripe fruits are preserved in vinegar/citric acid for several years.
- In the US, mildly pungent fruits are prepared as salsa and consumed with snacks.
- Red ripe fruits are used in the preparation of tomato ketchup to improve its colour.
- The Black Mountain Brewing Co. in Arizona developed a pepper beer with the idea of producing a spicy beer for a local Mexican restaurant. The idea worked (Bosland, 1992).

III. Dried spice: mature whole fruits and powder
- Dry intact fruits or ground powder are invariably added in almost all South Asian chicken, egg and vegetable curies.

IV. Colouring and flavouring agents: oleoresins (carotenoids) extracts or powder
- Paprika oleoresin (colour extract from non-pungent fruits), a natural colouring agent, is considered to be the best substitute for synthetic colours used in the food and cosmetic industries.
- Cosmetic industry uses non-pungent oleoresin to prepare its products.
- In food-processing industries, especially in the meat industry, oleoresins are added in processed meat to impart attractive colour.
- In the beverage industries, oleoresins are used to improve colour and flavour of products.
- In countries like Japan, South Korea, etc., oleoresins are mixed with chicken feed to impart an attractive red colour to the skin and egg yolk.
- Oleoresins are mixed with the feed of flamingoes in zoos for improving feather colour and koi in aquariums (Bosland, 1996).

V. Ethno-botanical/traditional medicine: fruit extracts and powder (pungent fruits)
- Fruits are consumed to stimulate digestion (flow of saliva and gastric juice), raise body temperature and used for the treatment of the common cold.
- Mayas mix fruits with corn flour to produce 'chillatolli', a treatment for the common cold. Mayas also use them to treat asthma, coughs, and sore throats. The Aztecs used fruit pungency to relieve toothache (Bosland, 1999). In many African countries, fruits are consumed in the belief that it improves the complexion and increases passion (Bosland and Votava, 2000).
- Fruits are added to rose-gargles to cure pharyengitis. Fruits are also consumed for their carminative effects. West Indians soak fruits in water, add sugar and sour orange juice and drink it during fever (http.//www.dominion.com).
- The Tukano natives of Columbia, pour a mixture of crushed fruits and water into their noses to relieve a hangover and the effects of a night of dancing and drinking alcoholic beverages (Bosland, 1999).
- In Columbia and India, victims of snake bite are given pungent fruits to taste in order to sense the functioning of the nervous system affeted by snake venom. In similar fashion, freshly crushed fruits or powder are used to reduce swelling and draw out the poison of bee strings, spider bites and scorpion stings (Dewitt *et al*., 1998).

VI. Modern medicine/pharmaceuticals: capsaicinoids and carotenoids extracts
- The pharmaceutical industry uses capsaicin as a counter-irritant balm for external applications (Carmichael, 1991).

Table 16.3 Continued

- Capsaicinoids (mainly capsaicin) are an active ingredient in 'Heet' and 'Sloan's Liniment', massage liniments used for sore muscles. Capsaicinoids are used in the preparation of powder, tinctures, plaster ointments and medicated wools (Bosland, 1996).
- The pharmaceutical industry uses capsaicinoid extracts to prepare certain drugs (sprays), which are applied externally to stop the pain of arthritis (rheumatoid arthritis, osteoarthritis), artery diseases (peripheral neuropathies) and to relieve cramps (Cordell and Araujo, 1993; Bosland, 1996).
- Application of creams containing capsaicin reduces post-operative pain of mastectomy patients and its prolonged use helps in reducing the itching of dialysis patients, pains from shingles (Herpes zoster) and cluster headaches (Bosland, 1996).
- Pepper fruit carotenoids, *viz.*, β-carotene, acyl derivatives of capsanthin, acyl derivatives of capsorubin) have been shown to inhibit LDL oxidation *in vitro* with probable lowering of the 'atherogenic' LDL subfraction production (Medvedeva *et al*., 2003).
- Capsanthin and capsorubin can improve the cytotoxic action of anticancer chemotherapy and is considered to have the potential of carotenoids as possible resistance modifiers in cancer chemotherapy (Maoka *et al.*, 2001; Molnar *et al*. 2004).
- Lutein, zeaxanthin, capsanthin, crocetin and phytoene have shown more potent anticarcinogenic activity than β-carotene and is useful for cancer prevention and may be applicable as the concept of 'bio-chemoprevention', which involves transformation-assisted methods for cancer chemoprevention (Nishino *et al*., 2002).
- The water extract of 'paradicsompaprika' (mainly containing capsanthin) has been considered as a new anticancer agent and a fat-soluble component of this drug has been regarded as an anti-promoter of cancer (Mori *et al*., 2002).
- Capsaicin has recently been tried as an intravesical drug for overactive bladder (bladder cancer) and it has also been shown to induce apoptotic cell death in many cancerous cells (Lee *et al*., 2004).

VII. Insecticide/repellent: capsaicinoids
- Capsaicin extracts are used as an effective repellent against mice damaging underground cables and protecting germinating seeds from squirrels (Bosland, 1996).

VIII. Spiritual: whole fruits
- In India, fruits are stringed on a thread along with a lime fruit and hung at the entrance of houses/shops with the belief that it will keep evil away (Kumar and Rai, 2005).
- Red dry fruits are used to remove the bad consequences of evil eyes on younger babies in North Indian states.
- Traditionally, in New Mexico, mature fruits are stringed (called 'ristras') and hung at the entrance of houses as a symbol of hospitality (Bosland, 1992).

IX. Ornamental: whole plants or fruits
- Certain genotypes of pepper are grown for their attractive plant shape, dense and colourful foliage and fruits. Several colours of fruit (at various maturing stages) can be found on a single plant making it an attractive ornament (Bosland and Votava, 2000).

X. Defence/punishment: capsaicin extracts/or fruit powder
- In India, traditionally villagers keep powder in the house as a defence weapon against dacoits.
- Capsaicin sprays are being used by people, especially women to protect themselves from several types of criminal offence.
- Capsaicin spray has replaced mace and tear gas in the police departments of many countries to control unruly mobs and criminals.
- In Mexico, India and several Latin American countries, pepper powder is rubbed on children's thumbs to prevent sucking (Dewitt *et al.*, 1998). Similary in India, fruit paste is applied on the mother's nipple to discourage prolonged breast feeding.
- Maya threw powder into the eyes of young girls who stared at boy or men and squirt fruit juice on the private parts of unchaste women (Dewitt *et al*., 1998).

Units (SHU) and the organoleptic test was the first method to measure it. But nowadays the most common and reliable method to estimate pungency (capsaicin) is by high-performance liquid chromatography (HPLC). HPLC analysis has become the standard method for routine analysis of samples because it is rapid and a large number of samples can be handled. The capsaicinoid contents (ppm) are multiplied by 15 to convert it to SHU.

Biosynthetic pathways

More than 15 different capsaicinoids are known to be found in pepper fruits, which are synthesized and accumulated in the epidermal cells of placenta of the fruits. Among these, capsaicin and dihydrocapsaicin accounts for more than 80% of the capsiacinoids that determine pungency (Bosland and Votava, 2000). These two most common capsaicinoids differ in the degree of unsaturation of a 9-carbon fatty acid chain and other naturally occurring capsaicinoids differ in chain length as well as degree of unsaturation (Curry *et al.*, 1999).

Two pathways are involved in the biosynthesis of capsaicinoids (i) fatty acid metabolism and (ii) phenylpropanoid pathway (Ochoa-Alejo and Gomez-Peralta, 1993). The phenolic structure comes from the phenylpropanoid pathway, in which phenylalanine is the precursor. The formation of ferulic acid from phenylalanine is well understood in other higher plants. Four enzymes, phenylalanine ammonia-lyase (PAL), cinnamic acid-4-hydroxylase (C4H), r-coumaric acid-3-hydroxylase (C3H), and caffeic acid-*o*-methytranferase (CAOMT) are involved in the process. Capsaicinoids are formed from vanillylamine and isocapryl-CoA via capsaicinoid synthetases (CS) (Fujiwake *et al*., 1982; Sukrasno and Yewman, 1993; Curry *et al*., 1999).

During fruit ripening, capsaicin concentration reaches a maximum and later degrades to other secondary products (Bernal and Barceló, 1996). Most peroxidase activity occurs in the placenta and the outer layer of pericarp epidermal cells. As determined by gel permeation chromatography, the major oxidative products were 5, 5′-dicapsaicin and 4′-*O*-5-dicapsaicinether (Bernal *et al*., 1995). Peroxidase activity increased at the time when the concentration of capsaicinoids started to decrease (Contreras-Padilla and Yahia, 1998). It is assumed that peroxidases catalyze capsaicinoid oxidation and play a central role in their metabolism. Water deficit affects phenylpropanoid metabolism and the pungency of fruits (Quagliotti, 1971; Estrada *et al*., 1999). PAL, C4H, and CS are involved in capsaicinoid biosynthesis and peroxidase isoenzyme B6 directly affects capsaicin degradation. Higher concentrations of PAL are followed by an increase in the pungency of fruits about ten days later.

At the arrest of fruit growth, increased PAL activity in the fruit accelerates the degradation of phenylalanine and the concentration of cinnamic acid and capsaicinoids increases. Large amounts of cinnamic acid are synthesized seven days after flowering in the presence of PAL, demonstrating that PAL is a key enzyme in the phenylpropanoid pathway (Ochoa-Alejo and Gómez-Peralta, 1993). Cinnamic acid-4-hydroxylase (C4H) hydroxylates cinnamic acid to r-coumaric acid. Capsaicinoid synthetase (CS), the last enzyme involved in the biosynthesis of capsaicin, combines vanillylamine and isocapryl-CoA to make capsaicin (Fujiwake *et al*., 1982). Capsaicin concentration begins to decline 50 days after flowering. Cumulative evidence supports that capsaicinoids are oxidized in the fruits by peroxidases. Peroxidases are efficient in catalyzing *in vitro* oxidation of capsaicin and dihydrocapsaicin. These enzymes are mainly located in placental and the outermost epidermal cell layers of the fruits, i.e., at the site of capsaicinoids. The products of capsaicin oxidation by peroxidases have

been characterized *in vitro* and some of them have been found to appear *in vivo* in the fruits (Di *et al*., 2000).

Genetics and markers

It has long been known that a single dominant gene, *C*, controls the presence or absence of pungency in the fruits (Blum *et al*., 2002). However, in the pungent types, the degree of pungency is quantitatively inherited and highly affected by the environments (Zewdie and Bosland, 2000). The molecular linkage maps of *C* locus have been prepared and a pungency-related gene has been found to be located on chromosome 2 (Lee *et al*., 2005). The genes of capsacinoids biosynthetic pathway have been isolated and characterized. Curry *et al*. (1999) isolated genes encoding a putative aminotransferase (*pAmt*) and a 3-keto-acyl-ACP synthase (*Kas*). Kim *et al*. (2001) identified three genes coding for enzymes, *viz.*, SB2-66, a putative capsaicinoid synthase (*CS*), SB2-149, an aminotransferase and SB2-58, a keto-acyl-ACP synthase. SB-2-66 (CS) has been found to be linked with pungent (*C*) locus and the non-pungent locus has a deletion. Based on sequence of CS, sequence characterized amplified region (SCAR) markers have been developed and their usefulness in early detection of pungent or non-pungent genotypes has been demonstrated (Lee *at al*., 2005).

16.4.2 Colour (carotenoids)

The green, orange and red fruit colour originates from the carotenoid pigments. More than 30 different pigments have been identified in the fruits (Bosland and Votava, 2000). These pigments include the green chlorophyll (a, b), the yellow orange lutine, xeaxanthin, violaaxanthin, anthrakanthin, β-ryptoxanthin and β-carotene and the red pigments capsanthin, capsorubin and cryptocapsin, which are exclusively produced in pepper fruits. In general, the capsanthin and capsorubin constitute more than 60% of the total carotenoids present in the fruits. The contents of capsanthin and capsorubin increase proportionally with advanced stages of ripening with capsanthin being the more stable (Bosland, 1996). The most highly valued characteristic of pepper genotype for oleoresin (colour) extraction is the very high carotenoids content. This is because, ultimately the commercial value of paprika (non-pungent oleoresin) depends on its colouring capacity, which depends directly on relative pigment richness. Other characteristics of interest are very low content of capsaicinoids, low moisture content and a relatively thin pericarp of the fruits. A thin pericarp shortens the drying time of the fruits before processing, thereby reducing the cost.

Chemistry

The basic carotene structure can undergo several structural modifications, namely, cyclization, hydroxylation and epoxidation, yielding the great variety of carotenoids (more than 600) in nature. During ripening of the fruits, there is a spectacular synthesis of carotenoids. All the carotenoids present in the fruits are C40 isoprenoids containing nine conjugated double bonds in the central polyenic chain, although with different end groups (3-hydroxy-5, 6-epoxide). This changes the chromophore properties of each pigment and allows them to be classified in two isochromic families: red (R) and yellow (Y). The red fraction contains the pigments exclusive to the *Capsicum* genus (capsanthin, capsanthin-5, 6-epoxide, and capsorubin), and the yellow fraction comprises the remaining pigments, *viz.*, zeaxanthin, violaxanthin, antheraxanthin, β-cryptoxanthin, β-carotene, and cucurbitaxanthin.

Genetics and markers
Early studies demonstrated that mature red fruit colour is dominant over yellow and is controlled by a single gene (*Y*) and later it was found that mature fruit colour is under the control of three independent pairs of genes, viz. *cl, c2* and *y*. The presence of dominant alleles at these three loci results in red mature fruits, while the presence of recessive alleles at three loci results in white mature fruits (Popovsky and Paran, 2000). The predominant pigments of the fruits, i.e., capsanthin and capsorubin, are synthesized by the enzyme capsanthin-capsorubin synthase (CCS).

The intronless cDNA clone of CCS enzyme has been isolated and studies indicate that the expression of CCS is induced during chloroplast differentiation at the time of fruit ripening and is not expressed in the leaves or green immature fruits (Bouvier *et al*., 1994; Houlne *et al*., 1994; Hugueney *et al*., 1996). The absence of capsanthin and capsorubin in yellow fruits correlates with the lack of expression of CCS enzyme in yellow fruits (Bouvier *et al*., 1994; Houlne *et al*., 1994). Co-dominant DNA markers for the identification of red and yellow-fruited genotypes at seedling stage have been developed (Popovsky and Paran, 2000).

16.4.3 Flavours

Although pepper fruits are commonly known for pungency, they are often used in meals for their flavour. The pyrazine 2-methoxy 3-isobutyl-pyrazine, the green bell pepper smell, is one of the most potent volatiles known so far. The human can detect this smell at two parts per trillion (Bosland and Votava, 2000). In *C. annuum* and *C. frutescens*, 102 volatiles have been found (Keller *et al*., 1981). The aroma compounds vary greatly between the cultivated species and also between genotypes within the same species. For example, tabasco (*C. frutescens*) contains no pyrazine compounds, while its presence is the characteristic feature of sweet pepper (*C. annuum*). The delicate flavours of the fruits can be differentiated after a few years of experience. For example, ancho is sweetish, mulatto is chocolaty, mirasol is fruity and chilpotle is smokey. Grinding the fruits produces one flavour, roasting produces another and soaking the fruits in water produces yet another flavour (Bosland, 1996).

16.4.4 Spice production and quality

Pepper spices are the powders that are derived from the pungent, mild pungent or non-pungent fruits. Therefore, the main fruit quality parameters are colour and pungency. Apart from these, colour retention during storage, fruit wall thickness, fruit size, shape and weight are also important quality parameters. Yet another important quality concern is the development of aflatoxin in both raw and processed pepper spice. The aflatoxin level should be checked at less than 5 μg/kg. Fruit peduncle should be removed to get a good powder quality. Colour contents and quality are influenced by stage of fruit ripeness at harvest, processing and storage of the powder. Similarly, besides being genotype dependent (Table 16.4), pungency is highly influenced by the environment. For spice purpose, fruits need to be maintained on the plant until they become dark red and slightly shrivelled to obtain the maximum possible colour for the spice product. But it is not possible to leave a crop in the field until all fruits become shrivelled. Therefore, a more realistic aim is to harvest fruits when 80% or more fruits reach a dark red and slightly shrivelled stage. In order to achieve best overall colour, only those fruits should be processed into spice powder that are

Table 16.4 The names of some popular genotypes with their pungency levels

Name	Pod type	Species	Scoville units
Naga Jolokia	–	*C. chinense*	455,000
Orange Habanero	Habanero	*C. chinense*	210,000
Red Habanero	Habanero	*C. chinense*	150,000
Tabasco	Tabasco	*C. frutescens*	120,000
Tepin	Tepin	*C. annuum*	75,000
Chiltepin	Tepin	*C. annuum*	70,000
Thai Hot	Asain	*C. annuum*	60,000
Jalapeno M	Jalapeno	*C. annuum*	25,000
Long-Slim Cayenne	Cayenne	*C. annuum*	23,000
Mitla	Jalapeno	*C. annuum*	22,000
Santa Fe Grande	Hungarian	*C. annuum*	21,000
Aji Escabeche	Aji	*C. baccatum*	17,000
Long-Thick Cayenne	Cayenne	*C. annuum*	8,500
Cayenne	Cayenne	*C. annuum*	8,000
Pasilla	Pasilla	*C. annuum*	5,500
NuMex Primavera	Jalapeno	*C. annuum*	5,000
Sandia	New Mexican	*C. annuum*	5,000
NuMex Joe E. Parker	New Mexican	*C. annuum*	4,500
Serrano	Serrano	*C. annuum*	4,000
Mulato	Ancho	*C. annuum*	1,000
Bell	Bell	*C. annuum*	0

physiologically matured and properly dried. The energy efficient heat pump dryers are well suited for drying of fruits because they operate at low temperatures.

Fruit drying at low temperature should be preferred because at higher temperatures spice powder will become brown instead of bright red. The drying temperature should be below 60 °C (optimum drying regimes should be 40 °C at 20% relative humidity) for heat pump dryers. To accelerate drying, fruit should be cut into small and regular pieces. A final moisture content of about 8% is considered to be ideal, as moisture content above 11% allows mould growth and below 4% causes excessive colour loss. Seeds of different cultivars have varying effects on the rate of colour loss, which is most likely due to the presence of varying antioxidant contents in the seeds. For instance, vitamin E, a fat-soluble antioxidant, has an effect on reducing colour loss. Selecting cultivars with seeds having high antioxidant contents, therefore, is necessary to produce a colour-stable spice powder. During storage, carotenoid pigments (red colour) are readily oxidized and the spice powder becomes less intensely coloured. The selection of appropriate cultivars, standardization and adoption of drying and storage methods are the management strategies to reduce the instability of the spice powder colour.

16.5 Cultivation

Pepper cultivars display a wide range of plant and fruit traits and production practices vary greatly from region to region. Therefore, cultural practices for growing pepper standardized for one region may need modifications considering the type of soil and its fertility status, weather conditions and prevalence of pests and diseases (Berke *et al*., 2005).

16.5.1 Climate and soil requirements

Hot peppers are better adapted to the warm weather than the sweet pepper, but normal fruit setting is hampered when night temperatures are more than 24 °C and lower than 16 °C. The optimum day temperatures for hot pepper growth range from 20 to 30 °C. When the temperature falls below 15 °C or exceeds 32 °C for extended periods, growth and yield are reduced. Peppers are photoperiod-insensitive and grow best in loam or silt loam soil with good water-holding capacity, but can grow on many well-drained soil types. Soil pH should be between 5.5 and 6.8 (Berke *et al*., 2005).

16.5.2 Selection of cultivar

As described previously, a number of market types are grown worldwide, therefore, selection of the cultivars should be based on the regional preferences, especially with respect to trader and consumer preferences for the shape, colour and degree of pungency of the fruits. The occurrence of local pests and diseases should also be taken into account during the cultivar selection (Berke *et al*., 2005).

16.5.3 Seed bed preparation and sowing

Approximately 150–200 g seeds are required for raising seedlings to transplant 1 ha at the density of 30,000 plants/ha. For seedling raising, about ten seed beds of 7×1 m^2 size would be sufficient. Seed beds should be prepared on well drained and raised (20–25 cm) lands. One kg compost or farm yard manure (FYM) and 1 g Furadan (carbofuran) per m^2 should be applied. Seeds should be treated with carbendazim 50% (Bavistin) (@2 g/kg seeds) before sowing. Beds should be drenched from Captan 50% WP (@ 2 g/lit.) and sowing should be done after one or two days in the available moisture created by drenching. For raising healthy seedlings, dense sowing should be avoided. The compost or FYM are used to cover the seed followed by mulching of beds by grasses or stalks. Irrigation is applied by watering can. Need-based pesticides and fungicides should be applied and regular irrigation and weed control practised.

16.5.4 Fertilizer application

Peppers are fertilizer responsive and for an average fertile soil, well rotten 20 t/ha FYM should be added preferably about 2–3 weeks before field preparation. NPK dose should be determined based on soil test. A full dose of phosphorus, potash and 1/3 dose of nitrogen are applied as basal. The remaining dose of nitrogen should be applied as top dressing at 30 and 50 days after transplanting.

16.5.5 Transplanting and mulching

Plant spacing varies depending on the cropping system, soil type and cultivar. Thirty-days-old seedlings (4–5 true leaves stage) should be transplanted on raised beds. The bed should be 30 cm high with width of 1–1.5 m (furrow to furrow) and two rows per bed transplanted usually at a distance of 55×45 cm. Just prior to transplanting, three to four granules of carbofuran (Furadan 5G) should be applied in each hole. Mulching is recommended to reduce weed competition, soil compaction, soil erosion and conserve

soil moisture. Rice straw or other organic material, polyethylene plastic or their combinations are used for mulching (Berke *et al*., 2005).

16.5.6 Irrigation and weed control

Pepper plants are fairly shallow-rooted and have low tolerance to drought or flooding. The first light irrigation is given soon after transplanting followed by a second irrigation after 3–5 days. Subsequent irrigations are given at weekly or fortnightly intervals, depending upon the soil type and weather. Plants generally wilt and die if water stagnates in the field for more than 48 hours. Phytophtora blight and bacterial wilt may cause total crop loss following prolonged flooding (Berke *et al*., 2005). If weed control is not adequate through mulch or mulch is not available, any one of the following herbicides can be sprayed: Lasso (alachlor 43EC), Amex (butralin 47EC), Devrinol (napropamide 2E or 10G) or Dual (metolachlor 8E or 25G). Usually herbicide is applied 2–3 days after transplanting (Berke *et al*., 2005).

16.5.7 Crop protection measures

A number of diseases and insects attack pepper crops and many of them are common throughout the world, while a few are specific to certain regions. A brief description on the symptoms and control measures of economically important diseases and insect pests have been summarized (Table 16.5). More detailed information may be obtained from other sources (Bosland and Votava, 2000; Berke *et al*., 2005).

16.5.8 Harvesting and storage

Pepper fruits can be harvested either at the green immature or red mature stage. Under favourable growing conditions, fruit production can continue for several months. Fruits are stored in a cool, shaded, dry place until they are sold. At typical tropical ambient temperature and humidity (28 °C and 60% RH), fruits may last unspoiled for 1–2 weeks. Anthracnose is the major cause of spoilage of dry fruits. Drying of fruits in the sun is a common practice, but this tends to bleach the fruits, and rainfall and dew promote fruit rot (Berke *et al*., 2005).

16.6 Conclusions

The impact of the discovery by Columbus of a pungent spice was beyond imagination as it was confused with black pepper of the East Indies. Nevertheless, today hot peppers dominate the world spice trade and are commercially grown everywhere in the tropical and subtropical regions (Eshbaugh, 1993). Similarly, sweet peppers have become indispensable vegetables in the temperate regions and are gaining vast popularity in the tropical regions as well. Furthermore, pepper is also emerging as an industrial crop as fruits are used as raw materials in the food, feed, cosmetic and medicine industries. The recent discovery of new medicinal properties of carotenoids and capsaicinoids present in pepper fruits could be seen in the light of the huge potential of this crop of New World origin to become an even more versatile crop in world agriculture.

Table 16.5 Symptoms and control measures of major diseases and pests of pepper (partially adapted from Berke *et al*., 2005)

Stress/symptom	Control measures
Damping off (*Rhizoctonia solani, Pythium* spp*., Fusarium spp*.)	
Occurs mostly in the nursery. Seedlings become infected near the soil surface after emergence. The tissues become soft, water-soaked and weak causing the seedlings to fall down and die later.	Soil treatment with Captan @ 0.3%. Seed treatment before sowing and foliar spray with Bavistin @ 0.25%.
Die-back or Anthracnose (*Colletotrichum gloeosporioides*, *C. capsici, C. acutatum*, *C. coccodes*)	
Necrosis and withering of twigs from apical top to the bottom of the plant. The twigs are water soaked, brown and die back. Small, irregular, sunken, dark yellow to light brown lesions on the mature fruit.	Seed borne. Seed treatment with Bavistin @ 0.25 %. Seedlings spray with Captan @ 0.2% or Bavistin 0.3%. Use of pathogen-free seed, crop rotation and frequent picking of mature fruits are management practices.
Powdery mildew (*Leveillula tauricai*)	
White powdery growth on lower surface of leaves resulting in wilting of plants.	Spray of Karathane 1.0 to 1.5 g/l or Calixin 0.5 g/l or Topaz 0.25 g/l of water.
Root knot nematode (*Meloidogyne incognita*)	
Infected plants with fewer and yellowish leaves and stunted shoots. Root knot galls at the point of invasion on the roots. Reduction in fruit size and quality.	Application of Carbofuran 3 G (Furadon), Phorate, Nemacur. Steam sterilization and soil fumigation in greenhouses. Crop rotation with non-host crops.
Bacterial spot *(Xanthomonas campestris* pv. *vesicatoria)*	
Small water-soaked spots on leaves, turn necrotic with yellow borders. Heavily infected leaves may drop, resulting in severe defoliation. Corky or wart-like fruits.	Seed borne. Sprays of copper or copper + maneb. Use of clean seed and crop rotation are important in disease management.
Bacterial wilt (*Ralstonia solanacearum*)	
Wilting of lower leaves followed by a sudden wilt of plant without foliar yellowing. Vascular browning and sometimes cortical decay is evident near the soil line.	Soil borne. Fumigate seedbeds and pasteurize the planting medium for container-grown plants. Crop rotation with flooded rice. Transplanting on raised beds.
Phytophthora blight syn. Phytophthora root rot (*Phytophthora capsici*)	
Root rot. Watersoaked regions on lower stems and branches, black/brown and irregular spots on infected leaves. Sudden wilting of plant without foliar yellowing.	Soil-borne. Transplanting on raised beds and do not allow water stagnation longer than six hours. Fungicide spray may be beneficial at foliar blight stage.
Chili veinal mottle virus (ChiVMV), cucumber mosaic virus (CMV), potato virus Y (PVY)	
Generally these diseases show mosaic, mottled and/ or deformed leaves. Plants are stunted and the loss of marketable yield can be dramatic.	Aphid-transmitted. Use resistant cultivars. Reduce aphid vectors by controlling weeds, spraying insecticides, and using mesh netting to exclude aphids from the seedlings.
Pepper leaf curl virus (Pep-LCV)	
Leaves curl towards midrib, plants remain stunted, flower buds abscise and pollen development is hampered, no fruit set or development of tiny fruits with no value.	White fly transmitted. Use resistant cultivars. Check white fly by managing weeds, spraying insecticides and using mesh netting to exclude white fly from the seedlings.
Thrips (*Scirtothrips dorsalis* and *Thrips palmi*)	
Young leaves curl upward. Brown areas develop between veins of young and old leaves. Corky tissue on infested fruits.	Spray of dichlorovos (Nuvan) @ 1.0 ml/l of water. Damage can be reduced by weed control, rotating crops and using predators.
Mites (*Polyphagotarsonamus latus*)	
Leaves curl downwards and become narrow. Corky tissue develops on fruits. Mites are yellow or white, tiny and found near the mid-vein on the lower side of the leaves.	Spray Dicofol (18.5 EC) @ 2.75 ml/lit of water. Wettable sulphur @2.5 to 3.0 g/l of water. Use of tolerant cultivars, weed control and crop rotation are management practices.

16.7 References

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17

Celeriac

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17.1 Introduction and description

Celeriac (*Apium graveolens* var *rapaceum*) is a strain of celery, which is native to Europe and parts of Asia. It is found growing wild in all temperate zones. It is also known as knob celery, celery root and turnip rooted celery. The plant is similar to celery but the principal difference between them is that in celeriac the root is developed into a mass resembling a turnip, is easier to grow and has the characteristic flavour of celery. It is much better for flavouring in general cookery and for eating as a cooked vegetable. The plant produces the large beet-like root, which is used as a vegetable and spice; the stem and leaves are discarded. It does not produce roots until the season is set in. It starts to swell and becomes large in October and during the earlier months it produces only leafy growth. Originally it had a disagreeable taste and odour but in the cultivated varieties these traits have entirely vanished. The essential oil has the odour and flavour of celery. In ancient times, the plant was grown as a medicinal crop and only recently has it been used as a food plant. It is eaten as a raw salad, as a cooked vegetable and for flavouring the food.

It is a biennial plant belonging to the family Umbelliferae and resembles beet in appearance. The economic part is its enlarged root, which develops at ground level. This root has a brown skin with white interior. It is smaller than celery and has very dark green foliage. The flavour of the root tastes like a blend of celery and parsley. Celeriac is mainly used as a cooked vegetable or raw in salads but is usually boiled before use. It is used in innumerable recipes usually after blanching in boiled salt water for five minutes and cooled rapidly. It is also used as a garnish or stuffed as a major part of a meal. The leaf stalks after they are pulled off can be boiled and served like seakale. Many use celeriac in soups and stews. The seeds have medicinal properties and are used as a tonic and an aphrodisiac.

17.2 Production

17.2.1 Soil

Celeriac grows on soil that is deep, rich and moisture holding, but well drained. Although celeriac is a moisture-loving plant, in fact a semi-aquatic, it will not thrive in waterlogged soil conditions. A good well-drained sandy loam or silt loam soil is ideal for this crop. Clayey soils that are prone to water logging should be avoided. It thrives well on a soil having a pH range of 5.5–6.7.

17.2.2 Climate

It is an autumn or early winter vegetable which thrives best when the weather is relatively cool and with moderately well distributed rainfall during the growing season. It is less sensitive to heat and drought than celery, but more sensitive than most garden vegetables. It has good frost tolerance.

17.2.3 Varieties

Monarch is an excellent, high quality variety, which has very smooth, easily washable, creamy coloured roots. It is easier to grow than celery and can be grated raw over salads, cut into slices and boiled, or into strips which are fried.

17.2.4 Propagation

It is propagated through seeds. It requires a 120-day growing season. It can be cultivated in two ways either by transplantation or direct sowing.

Transplantation

Seeds are sown in early March in boxes in greenhouses or hot beds at a temperature of 20–25 °C. The required seed rate is about 200–250 gm per hectare (100,734 seeds). The seed is sown thinly to facilitate pricking out on transplanting and to encourage stocky plants. Seeds germinate in 21–25 days. When the plant has grown six to nine centimetres high, it is transplanted into the garden, usually at the beginning of June. Plants must be carefully trimmed before planting. Trimming allows sunlight to reach the soil, keep the surface dry and reduce damping off losses. Transplanting outdoors is usually done in late spring. Cool weather is desirable at planting time. Plants are spaced 20–30 cm apart in rows placed at 60 cm apart.

Direct sowing

In mild climates, it may be direct seeded. Seeds are sown about half a centimetre deep and the seed bed is kept moist until the seedlings emerge. They are planted at a spacing similar to the transplanted crop.

17.2.5 Manures and fertilizers

The crop responds well to the application of manures and fertilizers. On medium soils, it should be applied with 10–12 t of compost or farmyard manure, 200 kg of nitrogen, 60 kg of phosphorus and 50 kg of potassium per hectare. The organic manure along with 50% nitrogen and entire dose of phosphorus and potassium is

given as a basal dose and the remaining nitrogen is applied at 30 and 60 days after transplanting.

17.2.6 Intercultural practices

Regular intercultural operations like thinning, hoeing and earthing-up should be attended to. Any side shoots or suckers appearing should be removed promptly. The surface of the soil should be loosened. It is done at frequent intervals to draw soil away from the plants rather than upwards because the further they stand out of the ground, the better. From mid-November onwards the scheme is altered and the soil is drawn up towards the plant to give some protection.

17.2.7 Irrigation

Celeriac requires much water while growing. The first irrigation is given immediately after planting or the roots may not attain best size and quality. Water should be given before the first indication of wilting or any check to growth. It requires 2.5–4 cm of water each week during dry periods. During dry weather, mulching will be helpful. Black polythene sheeting has been found to retain moisture.

17.2.8 Pests and diseases

Celeriac is not seriously attacked by many insects and it is seldom that any of them cause serious losses. However, under some conditions, the tarnished plant bug and carrot rust fly cause considerable injury. The celery looper and the larva of the black swallow-tail butterfly attack celeriac, but are seldom very injurious. They can easily be controlled by available insecticides. Celeriac is susceptible to injury by several diseases including late blight, early blight, phoma root rot, black heart and root knot.

Late blight is caused by *Septoria apii* and this organism attacks only celeriac and celery. Small brown spots appear on the leaves, which later coalesce, and the entire leaf may become dry. The early blight caused by *Cercospora apii* leads to small circular yellowish brown spots, which enlarge rapidly to dark brown, surrounded by a band of yellow. Both the diseases could be controlled by soaking the seeds in hot water for 25 minutes or in formaldehyde solution for 30 minutes or in mercuric chloride solution for 20 minutes following a half-hour soak in lukewarm water to soften the fungus.

Phoma root rot caused by *Phoma apiicola* and leads to wilting and dropping of leaves. Crop rotation, burning refuse, guarding against seedbed infection and destruction of infected seedlings are recommended. Black heart is a physiological disorder that is first seen as tip burn in younger leaves and quickly spreads to all of the young foliage. Hot weather and excessive moisture leads to this disorder. Root knot is caused by a nematode, *Heterodea radicicola*, which leads to deformed roots, unfit for consumption. Steam sterilization of the seed bed before sowing will effectively control nematode infestation.

17.2.9 Harvesting

Celeriac attains its full flavour after it has received a frost. Harvestable maturity is reached when the roots have attained a diameter of five centimetres. When fully

mature, they will be around 5–12 cm in diameter depending on the growing region. The branching roots at the base are cut away and tops trimmed off. In areas with mild winters, roots can be mulched with leaves or straw left in the ground and harvested as needed. Roots may also be removed and stored in moist sand in a cool place.

17.2.10 Yield

The crop is ready for the harvest during October–November. In late November, if there are any remaining roots, they should be collected and stored in damp sand in a cool and dry place. Under normal cultivation practices a tuber yield of 15–20 t per hectare may be obtained.

17.3 Further reading

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18

Celery

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18.1 Introduction

Celery (*Apium graveolens* L.) is an important aromatic plant grown mostly for its fresh herbs as salad crop in different parts of the world. The dried fruits are also used as spice. Celery is known as Celeri in French, Sellerie in German, Apio in Spanish, Salleri in Swedish, Karafs in Arabic, Selderiji in Dutch, Sedano in Italian, Aipo in Portugese, Syelderey in Russian, Serorjini in Japanese; Chin in Chinese; Karnauli or Ajmod in India. The origin of celery and its allied varieties is not clear. Wild forms can be found in marshy areas throughout temperate Europe and Western Asia. Although the eastern Mediterranean region appears to be the most logical centre of domestication, the distribution of wild types raises some doubt (Rubatzky and Yamaguchi, 1997).

Celery was probably not under widespread cultivation till the middle ages, though ancient literature documents that celery was cultivated before 850 BC. Celery production developed in the lowlands of Italy and further spread to France and England. The first mention of its cultivation in France was reported in 1623. The present cultivated celery plants are a quite sweet, appetising and wholesome food but its wild ancestors were considered poisonous. The ancients associated celery with funerals and believed it to be a bad luck omen. In India, celery was introduced from France around AD 1930 by a trading company in Amritsar in Punjab and now is commercially grown on a large scale for seeds and spice in that area.

The wild plants were used for medicinal purposes hundreds of years before its use as a food plant. The early forms of celery having an adaptation to its marshy origins, had a tendency to produce hollow stems and petioles. During domestication, selection altered this heritable characteristic and reduced the associated bitter and strong flavour. Celery leaves and stalks have been used as salad vegetables for thousand of years in Europe and the Middle East. The seeds have also been used in traditional systems 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 flavourer in the USA and Europe.

18.1.1 Classification

In the treatise *Handbook of Herbs and Spices* Peter (2001) has given a conventional classification of spices based on degree of taste and classified celery as an aromatic vegetable because it is mainly grown for fresh herb, the leaves and petioles. In another classification of plant organs used as spice, celery has been categorised as a seed spice because seeds are used as whole seed, powdered or in the form of seed oil or oleoresins. The taxonomic classification of celery is:

Division:	*Spermatophyta*
Sub-division:	*Angiospermae*
Class:	*Magnoliospida* (Dicotyledoneae)
Sub-class:	*Rosidae*
Order:	*Apiales*
Family:	*Apiaceae*
Genus:	*Apium*
Species:	*graveolens*

On the basis of characteristic features, celery can be classified as shown below:

Foliage colour:	green or yellow/golden
Blanching habit:	early or late
Bolting behaviour:	slow or quick
Climate:	temperate or sub-tropical
Life cycle:	annual or biennial
Height:	tall, intermediate or dwarf
Season:	autumn or winter

The classification of *Apium graveolens* L. on the basis of horticultural types as given by Orton, 1984 is:

1. *Apium graveolens* var. *dulce* – blanched celery
2. *Apium graveolens* var. *rapaceum* – edible rooted celery
3. *Apium graveolens* var. *secalinum* – leafy type (smallage type)

Rubatzky and Yamaguchi (1997) have reported *A. graveolens* var. *secalinum* to be the most popular celery in Asian and Mediterranean regions. Of the above three morphotypes of celery, *Apium graveolens* var. *secalinum* (smallage type) has been reported to be commonly cultivated in India for seeds as spices and behaves annual in growth habit (Malhotra, 2006a).

18.1.2 Description

Celery is a herbaceous annual or biennial erect herb growing to a height of 60–90 cm with conspicuous branches bearing well-developed leaves on long expanded petioles. Stems are branched, angular or fistular and conspicuously jointed. Leaves are radical, pinnate, deeply divided into three segments, once or twice divided and toothed at apex. The leaflets are ovate to suborbicular, 3-lobed, 2–4.5 cm long. The flowers are small, white in colour and inflorescence is a compound umbel. Calyx teeth are obsolete; five petioles ovate, acute with tip inflexed; carpels semiterete, subpentagonal, primary ridges distinct and filiform. The fruit is a schizocarp with two mericarps, suborbicular to ellipsoid, 1–2 mm in diameter, aromatic and slightly bitter. The seed (mericarp) results from the splitting of schizocarp (fruits) and is also ribbed and much smaller than carrot seed.

In cytogenetical studies, Choudhary and Kaul (1986) observed celery as a diploid with chromosome number as 2n = 22. The flowers, although potentially self-fertile, are normally cross-pollinated by insects.

18.1.3 Production and international trade

Celery is widely distributed in Europe, America and Asia. In the Western countries, it is grown for the herb, which is consumed as salad or cooked as vegetable and ranks second only to lettuce. In the USA, the major growing states are California, Florida, Michigan and New York, whereas in Europe major producing countries are France, Germany, the UK, Hungary, Italy, Belgium and Holland. Celery is cultivated for seed as spice predominantly in India, southern France, China and Egypt. India is the major producer and exporter of celery seed in the world market, which is partly used for extraction of seed oil and oleoresins. In India, it is cultivated in Amritsar, Gurdaspur, Jalandhar and Ludhiana in Punjab, Panipat in Haryana and Saharanpur in Uttar Pradesh for production of celery seed (Vijay and Malhotra, 2002).

Celery, as seed spice, is grown on around 6000 ha with production of 5500 tonnes annually in India. Indian celery seed and extractives are exported to the USA, Canada, the UK, Kuwait, the Netherlands, Singapore, South Africa, Japan and Germany. During 2005–2006, India exported 3400 tonnes celery seed of worth $2.5 million. India is meeting 62% of world demand for celery seed. About 284 metric tonnes of celery spice powder worth Rs. 14.5 million, celery essential oil quantity of 17 metric tonnes worth Rs. 33 million and oleoresins 183 metric tonnes worth Rs. 46.4 million was also exported from India during 2005–2006. The total world production of seed oil is about 45 tonnes, of which 17 tonnes is produced from India and the remainder from Egypt, China, France, the UK and the USA.

18.2 Cultivation

Celery thrives best in climates with a long, cool growing season, especially at night and where rainfall is well distributed or irrigation is assured. Optimum production occurs when mean temperatures range between 16 °C and 21 °C with the introduction of cultivars for tolerating upper temperature ranges. Celery can be grown in some subtropical regions. Celery is sensitive to freezing temperatures but on acclimatization can tolerate light frost for a short time. Leaf celery type has been reported to be more heat tolerant than root celery or stalk celery type. Celery has a shallow root system and thus requires a highly fertile soil with good moisture-holding capacity. Though it is reported to be cultivated in a wide range of soils, peat and clay loam soils are usually well suited for production. Celery is moderately sensitive to salinity and grows best within a pH range of 6.0–6.6 in mineral soils and 5.5–6.0 in organic soils. In Ohio and Michigan celery is grown on muck soils for fresh market (Swaider, *et al.,* 1992; Rubatzky and Yamaguchi, 1997). The celery seed crop under Indian conditions in Punjab is grown on soils with an average pH of around 7.5.

Celery needs high soil fertility, usually maintained by the application of balanced commercial fertilizers. Supplemental nutrient applications averaging about 300 kg N, 75 kg P_2O_5 and 250 kg K_2O ha^{-1} are used on mineral soils. Depending upon nutrient availability and the fertility status of the soil, doses up to 220–450 kg of N, 120 kg P_2O_5 and 180 kg K_2O ha^{-1} may be used. About half the nitrogen and all

phosphorus and potassium are applied at the time of planting and the remainder used as side dressing (Kadam and Salunkhe, 2001). Areas with minor nutrient deficiencies require the application of boron, calcium and magnesium for controlling deficiency, otherwise physiological disorders such as cracked stem, black heart and leaf chlorosis may occur (Rubatzky and Yamaguchi, 1997). The application of phosphorus induced a non-significant increase in seed yield but maximum returns were obtained with the application of 200 kg N and 33 kg P_2O_5 ha^{-1} in Punjab, India (Bains *et al.,* 1977).

Seed germination and the emergence of celery plants are slow even when conditions are favourable. The celery seeds are reported to possess thermo dormancy resulting in no or slow germination at temperatures greater than 25 °C. A seed soaking treatment at 10 °C using growth regulators GA 4/7 or ethephon at 1000 ppm can overcome this dormancy (Thomas, 1990). Far red light or 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 thermo dormancy and photo dormancy existing in celery have been reviewed in detail by Desai *et al.,* (1997).

Celery crop may be raised from transplants or by direct seeding in the field. The time of sowing is determined on the basis of crop to be raised for fresh herb or seeds. In California, seed beds are sown in July or sometimes December–January. The 8–12-weeks-old seedlings are transplanted in a well-prepared field. In other parts of the USA with more severe winters, crops are started in the early spring by sowing in a greenhouse or hot beds and seedlings are dug in the autumn, rouged for off type and cold stored until planting time in spring. The tops are stored at 0 °C and 90–95% RH, maintaining moisture and good ventilation around the roots. The roots are placed in moist soil. The withered and decayed leaves should be removed when plants are transplanted at distance of about 90 cm between rows. The closer spacing results in higher seed yields. In some coastal areas of California, celery is seeded directly in the production beds. In India the celery is grown as seed spice covering a large area in the Punjab which is situated in the northern plains. It 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 maximum seed yield. Celery crops yield about 60–70 t/ha as the fresh herb whereas seed yield of 2–4 q/ha can be obtained from crops grown exclusively for the purpose, respectively. The seed crop requires fertilizer dose of 90 kg N, 40 kg P_2O_5 and 20 kg K_2O ha^{-1} for annual cultivars under semi-arid agro-climatic conditions in India (Malhotra, 2005).

The celery crop is affected by several diseases and insect pests. But insects pose comparatively less of a problem than do diseases. The main insect pests causing occasional damage are leaf minor (*Liriomyza trifolii*) and celery fly (*Euleia brercolai*) but carrot rust fly (*Psila rosae*) may cause occasional damage. The important diseases of celery include early blight (*Cercospora apii*), late blight (*Septoria apii*), Fusarium yellows (*Fusarium apii* and *F. apii f.sp. pallidum*), stem rot (*Rhizoctonia solani*), bacterial blight (*Bacterium apii*), aster yellows (virus) and celery mosaic (virus). The diseases and insect pests of celery crops have been reviewed in the texts of Thakur (2000) and Malhotra (2006a,b). Successful chemical control measures for various diseases and insects pests are available.

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). It is usually ready for harvest 90–120 days after transplanting, whereas direct seeded crop

takes about 30–40 days longer than a transplanted one. In the past, blanching was popular, but due to increasing demand for green celery and the expense involved with blanching, it is no longer a common practice.

The seed crop of celery behaves biennially in a temperate climate and annually in a tropical to sub-tropical climate. It takes five months to reach seed maturity in plains. Celery seed is usually ready to harvest from August to early September under United State conditions whereas, it is harvested in April–May in Indian plains. The harvested crop is cured in the sun before threshing. The shattering of seed is a common problem and can be avoided by timely harvesting of seed in the morning hours or by spraying poly vinyl acetate (PVA) glue on seed umbels (Desai *et al.,* 2001; George, 1999).

18.3 Post-harvest handling

Preparations for market include a series of post-harvest operations such as removal of 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 relatively long periods. Such storage conditions require a temperature of 0 °C and high RH in an atmosphere of 1–2% O_2, 4% CO_2 and with facility of ethylene removal (Kadam and Salunkhe, 1998).

The seed crop of celery is collected after harvest and allowed to cure under the sun for a period of about 7–10 days. The cured crop is transported to the threshing floor, where it is dried in a thin layer for one or two days before carrying out light threshing to separate the seeds. The shade-dried seed contains more oil content than the sun dried seed. The seed can be cleaned easily with a screening mill followed by a gravity separator. The seeds are cleaned, graded through sieving and stored in gunny bags in a cool dry place. Under Indian conditions 1.4 tonnes/ha of seed can be harvested. The fresh seed should be taken to an oil extraction unit for more recovery of volatile oil content (Malhotra, 2005).

18.4 Cultivars

The cultivars of celery are generally classified 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 18.1.

The different cultivars of celery with salient characteristics as per Tigchelaar cited by Desai *et al.,* (1997) are:

Clean Cut: open pollinated, duration 125 days from transplanting, excellent shipping quality, large heavy petioles of good length, relatively few side shoots, similar to Utah 52–70.

Florigreen: attractive, uniform, vigorous widely adopted green stalk cultivar developed

Table 18.1 Types and cultivars of celery

Golden cultivars	Green cultivars
Golden Plume	*Utah type*
Golden Self Blanching	Utah 52–70 R
Michigan Improved Golden	Utah 52–70 HK
Cornell 619	Florida 683
Golden Detroit	Utah 52–70
	Tall Green Light
	Tender crisp
	Summer Pascal type
	Summer Pascal
	Giant Pascal
	Slow Bolting type
	Slow Bolting Green No. 96
	Slow Bolting Green No. 12

Source: Swaider *et al.,* (1992).

to provide a distinct petiole type for fancy grade pack, tolerant to brown spot, wide adaptability, similar to Florida 683.

Transgreen: wide thick petioles and excellent yield potential, similar to Florida 683.

In a review Kadam and Salunkhe (1998) mentioned that all green varieties are resistant to Fusarium wilt. Emerson Pascal is resistant to early and late blights and to Fusarium wilt but has a tendency to bolt. Early blanching Sanford Superb or Newark Market is thought to have originated from Golden Self Blanching, the most extensively grown yellow variety. The important yellow or self blanching varieties are Michigan Improved Golden, Cornell 19, Supreme Golden, Golden Plume, Wonderful, Golden No. 15 and Cornell 619. The varieties from the Cornell group have thick petioles whereas Pascal varieties are resistant to Fusarium yellowing but have a bolting tendency. White Cornell 19 is susceptible to brown spot, while others such as Michigan Golden, Michigan Improved Golden and Supreme Golden are resistant to Fusarium yellowing.

The Indian Agricultural Research Institute, New Delhi, recommended Standard Bearer and Wright Gieve Giant vegetable type introductions of celery in India. Farooqui and Sreeramu (2001) in their compilation mentioned EC 99249-1 and RRL 85-1 a 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 NRCSS-A Cel –1 of celery suitable for cultivation under semi-arid conditions for high yield and essential oil content (Malhotra, 2004) and has been identified for release very recently.

18.5 Chemical structure

The chemical composition of leaves, stalks, seeds and volatile oil varies in constituents. The composition of the constituents differs considerably depending upon the age of the seed, geographical region, stage of harvesting and method of distillates. According to Chevallier (2001), the key constituents of celery seed are volatile oil (1.5–3.0%) containing 60–70% limonene, phthalides and β-salinene, coumarins,

furanocoumarins (bergapten) and flavonoids (apiin). Celery seed volatile oil as reported by Farrell (1999) 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 GC/MS analysis (Cu Jian-Qin *et al.,* 1990) are given in Table 18.2.

The chemical composition varies with the stage of the plant at harvesting. 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 celery leaves (Kavalali and Akcasu, 1985); McMurry and Irvine, 1988), respectively. The chemical constituents extracted from the roots of *Apium graveolens* var. *dulce* were 4-phthalides butylphthalide, neocnidilide, cnidilide, z-lingustilide and senkyonolide and *Apium graveolens* var. *rapaceum* contained butylphthalide and z-butylide nephthalide, cnidilide, E and Z-ligustilide, neocnidilide and senkyonolide (Gijbels *et al.,* 1985). Chowdhury and Gupta (2000) found that celery oil contained 28 compounds belonging to different categories such as terpenes, sesquiterpenes and their derivatives (Table 18.3). The compound β-selinene was the major constituent (29.23%), whereas most of the other workers have reported limonene and selinene as the major constituents. The chemical structures of both compounds are given in Fig. 18.1.

Table 18.2 Details of aroma-chemicals reported 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-Ionone	0.05
Cinnamic aldehyde	0.15
Thymol	0.17
β-Selinene	12.17
α-Selinene	2.05
Epoxycaryophyllene	0.55
η-butyl phthalide	2.56
Eudesmol	0.29
Lingustilide	2.41

Source: Cu Jian-Qin *et al.,* (1990).

Table 18.3 Constituents (%) in fruits of *Apium graveolens*

Constituent	Percentage
Terpenes	
Limonene	0.22
β-phellandrene	0.38
Alpha-pinene	0.98
β-pinene	1.02
Sesqui Terpenes	
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).

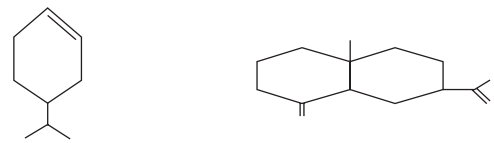

Fig. 18.1 Chemical structures of limonene and β-selinene.

18.6 Main uses in food processing

Celery is cultivated mainly for fresh herbs and seeds. Several processed products from celery fresh herb and seeds are popular in the markets of the USA, Europe and

Asian countries. The main uses of celery in the food processing industry are described here.

18.6.1 Processed products from celery leaves and petioles

The long fleshy petioles and leaves are valued for their flavour and texture and are used as salad and in the preparation of some value added products and have been documented by Pruthi (2001).

Dehydrated celery

Celery stalk and leaves are dehydrated and are commercially available in the USA and the UK markets as:

- 10 mm celery stalk dice
- leaf and stalk flakes
- stalk and leaf granules
- celery powder.

These various styles of dehydrated products are used for flavouring soups, broth base, canned tuna fish, stuffings and stewed tomatoes and as a garnish on potato salad and meat sauces.

Celery stalk products

Stalk products have been reported to retain the deepest green colour. This is frequently protected by the addition of minute amount of sodium bisulphite or sodium sulphite. Celery flakes are used in dry soup mixes, canned soups, sauces, stuffings, casserole products and vegetable specialities. Granulated or powdered celery is a good choice for canned and frozen sauces and dry mixes for bread and soups. Cross cut and diced celery is used in canned and frozen soups, relishes, vegetable specialities and salad mixes.

Processed celery juice blends

Processed celery juice blends in combination with vegetables have been prepared successfully and marketed. Organic celery and tomato; organic celery and carrot juice blends are becoming popular as nutritious drinks and have been reported to function as a cleansing drink that is good for recovery from many chronic illnesses.

Freeze-dried celery

Cross-cut slices of celery stalks are also available in freeze-dried form. The freeze-dried process is also effective in retaining original shape and crispness of celery. This product makes a crisp garnish for potato salad, casseroles, chinese dishes, gelatin salad, pickles and relishes. Overall quality for retention of nutrients is better in freeze-dried celery petioles.

Blanched celery

Blanching removes the green colour in the petioles and is accomplished by excluding light from leaf stalks while plants are still growing in the field. This process makes the leaf stalks 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 these days there is more demand for green celery owing to the presence of more natural nutrients.

Pickling celery
Celery petioles are also processed for the preparation of pickling. Such processed celery for pickling has a ready market in the USA and some European countries. The stalk celery and root celery can both be used in the processing industry for preparation of picklings. In the pickling process the tender petioles of celery are cured in dry brine and subsequently preserved by using spices and condiments or vinegar. Celery petiole pickling can also be prepared in mixing with other vegetables and mixed pickling can be prepared. Pickled celery is known as a good appetizer and adds to the palatability of different kinds of meals.

Canned celery
Tender celery petioles both blanched or 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 temperatures 115–121 °C (high pressure of 10–15 lb/inch^2) in the autoclave. The temperature and time of processing vary with size of can. Celery petioles are usually canned for later use in the off season or in combinations in canned soups, meats and culinary sausages.

18.6.2 Processed products from celery seeds

Celery is grown widely in Asia and the Mediterranean regions for seed purpose. The seed and its extractives are used as a condiment for flavouring purposes in the food industry and some pharmaceutical industries.

Whole seed
Celery seeds are very small, dark brown and emit a characteristic odour. The seeds are used as a spice in India and a condiment in the USA. The seeds give a burning sensation and are bitter. Celery seed may be used as a spice for seasoning practically any dish that calls for the flavour of celery and is particularly useful where fresh celery stalks would be impractical. Celery seed may be used in tomato and other vegetable juices, bouillons, pea soup, chicken and turkey soups, coleslaw, pickles, scrambled eggs and omelettes, chicken and tuna casseroles, salads and salad dressing, seafood chowders, sandwich spread and on cucumber, cabbage and beets. Celery seed has its importance in the food processing industry worldwide and is used in many Balkan, French, English, American and Asiatic recipes. The whole seed is the basic material for the preparation of various value added items, *viz*., oils, oleoresins for flavouring purposes in foods, beverages and perfumery and for medicinal purposes in the pharmaceutical industry. A few seeds of celery can be sprinkled over lightly boiled carrots, grilled tomatoes or salads and they are especially complementary to egg and fish dishes (Clevely *et al.,* 1997).

Celery essential oil
The volatile oil of celery is the most functionally important constituent of seed. The volatile oil content of seed varies from 2.5–3.0% and fixed oil content is 15%. The oil can also be extracted from seeds, herbs and chaff, but quality of seed oil is superior to others and is commercially more important. 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 oil also finds use in perfumery and the pharmaceutical industry. The aroma of celery seed oil is warm, spicy, fruity and persistent.

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 in 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 as being 2–2.5% depending upon the quality and quantity of seed and approximately 20–30 kg of celery oil is extracted from one hectare (Farooqui and Sreeramu, 2001). The distillation wastes are usually redistilled. Indian seeds give a better yield of oil compared to French seed.

Celery oleoresin

Celery oleoresin is one of the most valuable flavouring agents as it imparts a warm, aromatic and pleasing flavour to food products. Essentially, the celery oleoresin consists of essential oil, organically soluble resins and other related materials present in the original spice. Celery oleoresins are extensively used in processed foods, snacks, sauces, sausages, seafood, vegetable preparations and alcoholic/non-alcoholic beverages.

The oleoresin of celery seed 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 as per the ISO, as well as the standards of importing countries with their fixed maximum permissible limits for the approved solvents. Oleoresins could rightly be considered as 'liquid celery seed' which is easier to handle in the preparation of tinctures and extracts. Celery seed oleoresin is a green liquid having a volatile oil content of about 9 ml/100 g and is free flowing with a herbal, slightly lemony and bitter flavour. The Indian types of celery oleoresin have been reported to be more herbal with a pleasant lemon-like aroma and tenacious herbal undertones (Pruthi, 2001)

Celery powder

Celery seed powder is mainly used in food items for flavouring purpose such as salad dressings, soups, sausages, vegetable juices and pickles. The celery powder of seed has its importance in the food processing industry worldwide and is used in many Balkan, French, English, American and Asiatic recipes. Celery seed powder can be sprinkled over salads, soups, sausages, juices, eggs and fish dishes.

Celery powder is produced by milling or grinding the dried seeds. The loss of the characteristic aroma of celery powder occurs in the process of grinding. Therefore, to overcome the loss of volatiles, pre-chilling and reduced temperature grinding are used (Anon. 1975). An innovation for idealized grinding of spices is freeze-grinding (–70 °C) which has many advantages; increased retention of volatiles, and dispersability of the fine ground material in food preparations (Russo, 1976). The quality of ground spice deteriorates in its aroma by rapid loss of volatiles and this loss could be controlled by careful selection of packaging material. The 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.

18.6.3 Other products from celery

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 or 25% of celery seed powder or ground celery and 75% table salt (Pruthi, 2001).

Celery seed vinegar
Select a bean-preserving jar not a narrow-topped bottle. Place two tablespoons celery seeds in the jar for every 600 ml/1 pint/$2^1/_2$ cups white wine vinegar. Cover and leave in a cool dark place for 2–3 weeks, shaking the jar from time to time, until the flavour is developed. Strain the vinegar into clean bottles, label and store in a cool place away from direct sunlight. Use the vinegar in salad dressings and to sharpen herb sauces (Clevely *et al.,* 1997).

18.7 Functional properties

Celery leaf petioles and seeds are valued for their appetizing succulence, bulk vitamins and minerals. The fresh leaves and stalks contain mainly protein, fat, fibre, carbohydrate, minerals and vitamins. The nutritional composition of leaves, petioles and seeds are shown in Table 18.4. The composition varies with variety, region, part of plant and age of product. Celery seeds and extractives are known to have significance in traditional medical systems. Celery has been reported to demonstrate a number of functional properties. The key properties are as below:

- antirheumatic
- antispasmodic
- diuretic
- hypotensive
- anti-inflammatory

Table 18.4 Nutritional constituents of celery (per 100 grams)

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)	0.03	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
Sodium (mg)	–	–		160
Zn (mg)	–	–		7

Sources: Gupta *et al.* (1993), Bahl *et al.* (1982), Farrell (1999).

Due to its sedative and nerve-stimulant properties, celery has been successfully employed in curing rheumatoid arthritis (Guenther, 1950). It helps in detoxifying the body and improving the circulation of blood to the muscles and joints. The phthalides present in celery seed and oil are said to have antirheumatic properties. Prajapati *et al.,* (2003) has advocated the use of celery for curing rheumatic pain in muscles of neck and sacrum and curing dysmenorrhoea with short pains in both ovarian regions. The coumarins, (furanocoumarian, bergapten) stimulate skin tanning and are a smooth muscle relaxant. The presence of minerals such as calcium, iron, magnesium, phosphorus, potash, sodium and zinc also supports the repair of connective tissue and is thus useful for treating arthritis.

The seed oil and other fatty oils from celery seed have been reported to possess antispasmodic qualities. Celery seed oil acts as an intestinal antiseptic. The emulsion of seed oil is useful in relieving flatulence, colic pain, vomiting and is a house-hold remedy to correct gastric disorders. The presence of δ-limonene and β-selinene probably contribute towards celery antispasmodic action.

As reviewed by Chevallier (2001), a study in India found the seeds to have marked liver protective activity and extracts of the seeds may also lower blood fat levels. Chinese research indicates that oil lowers blood pressure. One phthalide, 3-n-butyl-phthalide, in celery is said to relax the smooth muscle linings of the blood vessels, thereby lowering blood pressure. Phthalide works directly by dilating vessels. The phthalides are a natural sedative also. Perhaps this sedative activity could translate into reduced stress further translating into reduced cardiopathy. Celery is therefore one of the dozens of reputed aphrodisiacs. 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, so celery contains, hypotensive, hypercholesterolemic and calcium blocker phyto chemicals (Kaufman *et al.,* 1999; Duke, 1983). As well as hypotensive properties, Kaufman *et al.,* (1999) reported that celery contains more than two dozen anti-inflammatory compounds (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 synergetically useful in gout and other types of arthritis problems.

Celery stems and seeds have long been taken for the 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 its diuretic properties, celery herb and seed is helpful in curing obstinate retention of urine (Prajapati *et al.,* 2003). Thus the consumption of organic celery juice with carrot juice is preferred for its cleansing action on the body; it is an effective treatment for cystitis, helping to disinfect the bladder and urinary tubules.

The major functional properties have been already discussed and a few authors have mentioned celery as being a stimulant and carminative, emmenagogue. It also has properties to cure headache and itching blotches with burning. The traditional and modern uses of celery as a medicine are given below (Sayre, 2001).

Traditional use as medicine

Europe, America, Asia	Leaves, stalks, root stalks as a nutritional source
European use	Roots as a folk aphrodisiac
Ancient Egyptian use	Seeds as medicine

Chinese use	Seeds as a remedy for arthritis, dizziness, gout
Indian use	Seeds as diuretic and appetizer
Modern use as medicine	
European use	Fresh herbs and seeds as folk remedy for weight loss, lowering blood pressure, relief of anxiety, insomnia, and reducing blood sugar.
Western use	Seeds and extractives as remedy for arthritis, gout, rheumatism and urinary tract problems.
American use	Stalks as remedy for high blood pressure and prevention of heart disease.
Chinese use	Seeds as remedy for arthritis, dizziness, gout, high blood pressure, insomnia, nervousness and rheumatism.
Indian use	Seeds and extractives as remedy for arthritis, urine problems and for liver protection.

The popular key preparations from celery are also given in Table 18.5.

Table 18.5 Key preparations from celery and uses in medicine

Preparation	Dose formulation	Properties as medicine	Dose	Reference
1. 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
2. Celery and carrot juice	Juice of organic celery fresh herb and carrot	Cleansing drink	One cup of juice daily	Chevallier, 2001
3. Infusion of seed	Infusion of seed	Gout and arthritis	One cup daily	Chevallier, 2001
4. Fineture of seeds	Fineture from seed	Rheumatism	30 drops 3 times a day	Chevallier, 2001
5. Celery seeds	Whole seed	Chest problems, asthma and bronchitis and urinary problems	mix 1 tsp 3 times a day with food	Chevallier, 2001
6. Powder of seeds	Powdered seed	Arthritis	mix 1 tsp 3 times a day with food	Chevallier, 2001
7. Celery salt	25% celery seed powder + 75% salt	Appetizer and improves digestion	1/2 tsp daily	Pruthi 2001
8. Celery pepper	Celery seed powder 30% + pepper powder (70%)	Appetizer and improves digestion	1/2 tsp daily	Pruthi 2001
9. Fresh herb	Fresh herbs	Anti-gout	4 celery stalks a day for more than 8 months	Kaufman *et al.,* 1999

18.7.1 Toxicity

Celery has been identified as one of the plants known for causing dermatitis due to phototoxic reactions. Rubatzky and Yamaguchi (1997) have discussed phototoxic activity in detail in their treatise and have reported that celery foliage and seed contain phthalides, terpens, psoralen, xanthotoxin, bergapten and isopimpinellin. Out of these compounds psoralen, xanthotoxin and bergapten are phototoxic causing dermatitis in humans and animals after contacting the skin in sunlight. Some individuals exhibit much greater sensitivity to psoralens than others. Normally the concentrations of these compounds in celery, parsley and other umbellifers does 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.

Apium graveolens has been listed as the potential photosensitising action crop (McGuffin *et al.,* 1997) and phototoxic reactions exhibited by the skin are generally associated with the presence of phenolic compounds such as furocoumarins or psoralens. Trumble *et al.,* (1990) reported the presence of a much higher concentration of furocoumarins than petioles. It has been further advised that celery and celery products should not be used during pregnancy unless otherwise directed by an expert qualified in the appropriate use of the substance. Therefore, celery preparations carry a warning against taking celery medicinally in pregnancy or if suffering from kidney disorder (Chevallier, 2001). The use of celery leaves, stalks and seeds has been condemned for attempted use as an illegal abortifacient. In one compilation, Sayre (2001) mentioned that celery seeds lower the potassium levels in the body. If a great deal of celery seed is consumed, the consumption of bananas and other fresh vegetables containing high amounts of potassium is needed to counterbalance this effect. Celery seeds have, therefore been suggested to be toxic if taken in excess. According to Kaufman *et al.,* (1999), drowsiness might also be a side effect of celery due to the presence of phthalides, which have the properties of natural sedatives. Calcium antagonistic properties of celery due to the presence of coumarins has also been reported. Celery has been reported to possess calcium antagonistic properties due to the presence of calcium blocker of phyto-chemical coumarins such as bergapten, at 1–520 ppm, isopimpinellin, at 4–122 ppm and xanthotoxin, at 6–183 ppm (Kaufman *et al.,* 1999).

In one of the studies Wuthrich *et al.,* (1990) reported that celery is a partly thermostable allergenic. In addition a relatively high number of cases of severe anaphylactic reactions due to ingestion of celery have been reported in Switzerland. It was further added that the thermostable allergenic components of celery allergy seems to be associated with a co-sensitization of mugwort pollen. In this context Breiteneder *et al.,* (1995) succeeded in the molecular characterization of celery and the identification of the Api g 1 gene responsible for allergen of celery.

18.8 Quality specifications

18.8.1 Specifications for whole seeds

The quality of celery seed depends mainly on:

- external appearance, which provides visual perception of quality such as colour, uniformity of size, shape and texture. Celery seeds are minute, globular, light brown seeds having paler ridges and seeds seldom exceed 1 mm in diameter.

- flavour, which is influenced by the composition of aromatic compounds: the intense flavouring qualities are due to the presence of phthalides and terpene (Rubatzky and Yamaguchi, 1997).

Agmark of India provides three grades of celery seeds, viz., special, good, fair. The BSI has laid down Indian standards for various spices but under the PFA (Protection of Food) Act, no specification has yet been provided for celery seed. The grade designations and definitions of quality of celery seed are given in Table 18.6 (Pruthi, 2001).

The minimum specific quality indices as per Farrell (1999) are seed moisture 10%, total ash 14%, acid insoluble ash 2%, volatile oil 2%, non-volatile ether extract 12%, foreign organic matter 2%. The latest contaminant tolerance limits of celery seed as prescribed by the American Spice Trade Association (ASTA) are whole insects, dead by count four, excreta, mammalian by 3 mg/ef; excreta other by 3 mg/ef; infested by weight 1%; extraneous foreign matter by weight 0.5%. The other common specifications of quality minimums for herbs and spices as per the European Spice Association have also been cited by Muggeridge *et al.,* (2001). The International Standard Organization, has also laid down standards and production specifications of celery seed as per the European Spice Association and are given here in Tables 18.7 and 18.8.

Table 18.6 Grade designations and definitions of quality of celery seeds

Grade designation	Special quality characteristics		General characteristics
	*Extraneous matter, percentage by weight (maximum)	Moisture, percentage by weight (maximum)	
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.

*Definition: extraneous matter means dust, dirt, stones, earth, chaff, stalks, stems, straw or any other foreign matter.

Table 18.7 ESA – individual product specifications for celery seed

Celery seed	Ash %	A/A %	H_2O	V/O %
(ISO)	w/w(maximum) 12	w/w (maximum) 3	%w/w(maximum) 11	v/w(minimum) 1.5

Source: European Spice Association.

Table 18.8 Cleanliness specifications for celery in Germany, the Netherlands, the 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: European Spice Association.

18.8.2 Powdered celery seed specifications

Celery powder is produced by grinding dried, cleaned and sterilized celery seed. Celery powder is made by pulverizing dry seeds and at least 95% of the ground spice shall pass through a US Standard No. 55 sieve (Farrell, 1999) 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 readily dispensable in the matrix. Some flavour may be lost by heat development during grinding. The loss can be minimized by adopting cryo-milling and freeze grinding. Celery powder is yellowish brown with an aromatic slightly camphoraceous odour and taste. The following precautions should be taken for production of celery powder.

- minimum moisture level to increase storage life
- cryo-milling or freeze grinding to minimize the volatile oil and flavour loss
- particle size as per specified mesh size to ensure free flow
- airtight and safe packaging
- ensure microbiological cleanliness

In addition to the specific celery seed powder specifications mentioned above, the whole celery seed specifications should be strictly followed.

18.8.3 Volatile oil specifications

- The volatile oil content of celery seeds averages 2.5 to 3.0% and it contains primarily 60% d-limonene, 10% d-selinene, 2.5–3.0% sedanolide, 0.5% sedanomic anhydride and a fixed oil content of 15% (Farrell, 1999).
- The aroma of celery seed oil is warm, spicy, slightly fatty, fruity, penetrating and very persistent. Its flavour gives a burning sensation and is very bitter.

The physiochemical properties of celery volatile oil are given in Table 18.9.

18.8.4 Celery oleoresin specifications

- Celery seed oleoresin should be a green liquid having a volatile content of at least 9 ml/100 gm.
- The oleoresin should have a lemon-like aroma, be tenacious and have a sweet herbal tone.
- The oleoresin should be prepared with the recommended organic solvents followed by the subsequent removal of the solvent as per importing country specifications.

Table 18.9 Physiochemical properties of volatile oil of celery

Properties	Specification values		
	Singhal *et al.,* (1997)	Bahl *et al.,* (1982)	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)	+65°53′ to 76°5′	+65°82′	+50°80′
Solubility characteristic	Saponification number 25.1–47.6	–	–
Acid value	–	15 to 40	3.5 (maximum)
Odour	–	Spicy	Persistent, spicy and typical of celery seed

18.8.5 Adulteration

Celery seed is available both in whole or in ground form. It is subject to adulteration by the addition of exhausted or spent seed (from which oil or oleoresin has been extracted), excess stems, chaff and earth or dust, etc. Ground celery is sometimes adulterated with farinaceous products, linseed meal, worthless vegetable seeds or at times even with weed seeds. Samples of celery seed are sometimes adulterated with ajowan seeds and because of a similarity in seed shape it becomes difficult to detect. Celery seed oil is also frequently adulterated with celery chaff oil or with d-limonene, the addition of which is difficult to detect. Filth, such as insect fragments, rodent droppings and fungal spores are 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. The oleoresin may be adulterated by added synthetic saturated acid. Detection of these adulterants can be achieved by sophisticated gas chromatography of the saponified extract or by thin layer chromatography coupled with HPLC. Celery seed oil contains β-selinene as one of the important components and a good quality oil should contain a minimum of 7–7.5 % β-selinene (Straus and Wolstromer, 1979). Oil containing less than 7.0% β-selinene should be suspected as being adulterated. Adulteration levels can be detected by using the specifications as explained separately for whole seed, powdered seed, volatile oil and oleoresins.

18.9 References

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19

Chives

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19.1 Introduction

The plant known as chives is a perennial of the *Liliaceae* family, *Allium* species. Chromosome number 2n = 16, 24, 32. Wild chives are widely distributed from the Arctic regions to Asia, Europe and North America (Xu and Kamelin, 2001; McCollum, 1976). There were no written records of domesticated chives from the Mediterranean region until the 16th century in Europe, whereas in East Asia chives have been domesticated since ancient times (McCollum, 1976). At present chives are cultivated as vegetables or seasoning herbs all around the world, especially in the Northern Hemisphere.

Because chives are very adaptable, tolerant of cold and hot temperatures, and grow rapidly, they can be cultivated and harvested many times throughout the year. They are also easy to propagate, either from seeds or from division of clumps all year round. Chives are grown mainly for their long, cylindrical leaves, used for culinary purposes. Their flowers can also be used for salad dressings and sometimes for decorative purposes.

Since ancient times, herbs of the *Allium* species have been considered a healthy food for their special spicy flavour. Unlike the pungent flavour of garlic and onions, the flavour of chives is milder and more delicate, and more easily acceptable to the palate. Chives also contain many vitamins and mineral nutrients (Rubatzky and Yamaguchi, 1997) as well as flavonoid compounds, which are antioxidants (Justesen, 2000). Among all *Allium* species, chives have the highest content of vitamin C and beta-carotene. Chives have green leaves that are soft in texture and are easily kept fresh and transported using drying and freezing techniques. Techniques are presently, being developed to apply chives in the processing of dairy, meat, and snack products.

19.2 Chemical composition and nutritional value

Chemically, chives are composed mainly of carbohydrates, proteins and amino acids,

as well as many other vitamins and minerals. The unique spicy flavour comes mostly from volatile sulphur and glucosides, etc. Studies into the chemistry of *Allium* flavour began in the 18th century. So far, much has been learned about *Allium* chemistry, although many questions still remain (Block, 1992). We have learned that the flavour is the result of a multifaceted interaction among many different compounds, and we have also begun to understand the factors that affect the quality and intensity of the flavour of *Allium*. The flavour substances of various *Allium* species depend on the quantitative differences in the S-alk(en)yl cysteine sulphoxides (ACSOs). The S-alk(en)yl cysteine sulphoxides (ACSOs), when hydrolyzed by the enzyme allinase, give rise to the flavour and pungency characteristic of the *Allium* plants (Randle and Lancaster, 2002). When the tissues of Allium are disrupted, the enzyme allinase hydrolyzes the flavour precursors. Sulphur compounds are found in the cytoplasm of *Allium* cells, physically separated from allinase (Lancaster and Collin, 1981).

In *Allium* species, four different ACSOs have been identified (Bernhard, 1970; Freeman and Whenham, 1975; Yoo and Pike, 1998; Randle and Lancaster, 2002). 2-methyl-2-butenal, 2-methyl-2-pentenal, methyl-propyldisulphide, and dipropyldisuphide have been found in the green leaves of chives. There is also evidence for the presence of propencyl-propyldisulphide in chives, while allyl disulphide is definitely absent (Wahlroos and Virtanen, 1965). The major thiosulfinate from chive are n-propyl groups, methyl and 1-propenl groups. The data of Table 19.2 are both qualitative and semi-quantitative (Block *et al*., 1992).

Of the thiosulfates found in chives, 77 (75)% contain the n-propyl group, 10 (12)% contain the methyl and 12% contain the 1-Propyl group. Total thiosufates is 0.19 umol/g wet (fresh), about the middle level of the edible species of *Allium*. The n-propyl group in chives is more abundant than methyl, with the methyl/propyl 1:5.8. Chives have an onion-like flavour. The pungent and stimulating flavour of chives and onions is mainly due to the propyl group, but it is the quantitative and qualitative differences in the thiosulfates that give each species its own characteristic flavour. The flavour and lachrymatory properties of chives are due to the high n-propyl content, while the flavour of onions is due to the high proportion the 1-propyl it contains.

It has been recognized that factors affecting flavour intensity and quality include genetic, ecological and cultivation techniques. Chives grown in different years, areas and different cultivars, with different cultivation techniques, may have distinct flavour intensities.

Chives also contain flavonoid glycosides, as shown in Table 19.3. The biological activities of flavonoids are mainly due to their antioxidant function. They are also known to inhibit several enzymes, including lipoxgenases and cyclo-oxygenase, etc. The green leaves of chive mainly contain kaempferol glucosides (di- and tri-glycosides), dominant as glucose and galactose. The 3-beta-D-glucosides of kaempferol, quercetin and isorthamnetin were isolated (Starke and Herrmann, 1976).

Table 19.1 Approximate chemical composition of chives (6) (7)*

% Water	Calories	CND	Pro	Fat	Fib	Ash	A	C	B1	B2	Niacin	Ca	P	K	Na	Mg	Fe	S*	Volatile o: 1*
92.0	26	3.9	2.8	0.6	0.9	0.8	6400	70	0.10	0.12	0.60	82	46	250	6	55	1.2	93	25–26

Source: Adapted from Rubatzky and Yamaguchi, 1997.
*Epmakov, 1961.

Table 19.2 Thiosulfinates and sulfines from extracts of chive as determined by Si-HPLC (concentrations in mole percent of total)

Compound no.	Compound* (response factor)**	Onions	Chives
5	n-PrSS(O)Propenyl-(E)	9–12	2.5
6,8	nPrS(O)SPropenyl-(Z<E)	10–12	16
7	EtCH=S=O	++	
9	n-PrS(O)SPr-n	4–13	58
10	MeSS(O)Propenyl-(E)	22–23	*
11	AllS(O)SMe		
12,15	MeS(O)SPropenyl-(Z,E)	25 (24)–31	1(5.5)
13	MeSS(O)Pr	1–4	5.9
14	MeS(O)SPr	1(I)–4	15(10)
16	A llSS(O)Me		
17	OSCH(CHMe)2CHSO	2–11	
18	MeS(O)Sme	0–14	1.8
	Total % MeS	28–38	13
	Total % AllS		
	Total % 1-propenylS	37–47	10 (2)
	Total % nPr	19–27	77 (75)
	Total thiosulfinates	0.14—0.35	0.19

Source: adapted from Block *et al.,* 1992.
* Chemical Abstracts names of compounds:
AllSS(O)Propenyl-(E,AllS(O), SPropenyl-(Z,E), AllS(O)SAll, n-PrSS9O)SPropenyl-(E), n-PrS(O)SPropenyl-(Z,E), EtCH=S=O, n-PrS(O)Propenyl-(E), AllS(O)SMe, MeS(O)SPropenyl(Z,E), MeSS(O)Pr, MeS(O)SPr, AllSS(O)Me, OSCH(CHME)2CHSO, MeS(O)SMe.
** Molar absorption with HPLC 254-nm UV detector relative to benzyl alcohol calibrated by NMR peak integration. Amounts given in umol/gwet9fresh) weight.

Table 19.3 m/z value of ions produced by liquid chromatography mass spectrometry of flavonoid glycosides present in chives (adapted from Justesen, 2000)

(M-H)-	Product ions of (M-H)	Product ions of aglycone	Suggested name
463	301	ND	Quercetin glucoside
477	315	300,271,151,107	Isorhamnetin glucoside
447	285	ND	Kaempferol glucoside

Source: adapted from Justesen, U. (2000).

A comparison of flavonoids from chives grown under different light conditions indicates different flavonoid response to PAR and UV-B light. Total flavonoids from the chives were 16.7 mg/10 g^{-1} f.w. The ratio of the kaempferol glucoside, quercetin glucoside and isorhamnetin glucoside was 4:1:2. Exposure of PAR flavonoids increase 30% of total flavonoids, and additional UV-B even by more than 80%. (Nitz *et al.*, 2000).

Green leaves of chive mainly contain kaempferol glycosides with di- and tri-glycosides, the 3-beta-D-glocosides of kaemferol, quercetin and isorhamnetin as by-glycosides from chive. The structures of eight anthocyanins have been determined in acidified methanolic extract of the pale-purple flowers of chives. Four of them have been identified as the anthocyanin-flavonol complexes, with the other four, anthocyanins were found. The covalent anthocyanin-flavonol complexes show intermolecular association between the anthocyanidin (cyandin) and flavonol (kaempferol) units, which influences the colour (Fossen *et al.*, 2000).

Fig. 19.1 Chive field.

19.3 Cultivation and production

19.3.1 Botany and morphology

Chives are a perennial plant cultivated as a biennial. However, the productive growth cycle is commonly completed within a year. Based on molecular data, chives belong to the subgenus *Rhizideum* (Hanelt *et al*., 1992). Bulbs are oval shaped and often clumped together. The skin of the bulbs has a grayish brown colour, with yellow or purplish tints, and the texture of carton paper. The development of elongated rhizomes and of false bulbs are advanced character states (synapomorphies), usually clustered, ovoid-cylindric, 0.5–1 cm in diameter; tunic brown or tinged with yellow, papery, laciniate, sometimes fibrous at apex. The leaves grow in clumps of 2–5, slightly shorter then scape, 2–6 mm wide, terete, fistulose, smooth or scabrous denticulate. Chives branching results from where lateral initiation occurs after the development of every two or three leaves (Poulson, 1990), and thus plants develop clusters of shoots. Scape 10–50 (60) cm, terete, covered with leaf sheaths for 1/3–1/2 its length, smooth or scabrous-denticulate (Xu and Kamelin, 2000).

After seeding, chives flower in the second year and each year afterwards. The long and thin flower scape is cylindrical in shape, hollow and smooth. Umbel subglobose, are densely flowered. The perianth is purple-red to white (or pale pink), tepal twice the length of filaments; polymorphous spices. Six needle shaped petals are of the same height. The pistil does not protrude out of the petals. (Xu and Kamelin, 2001). The flowers do not produce much pollen and seldom produce viable seeds. Chives are generally an out-crosser, and flowers are insect pollinated, but selfing frequently occurs as well (Rubatzky and Yamaguchi, 1997). Chives can be cultivated from seeds or from division.

Wild chives are confined mainly to mexerophytic habitats; meadows, forests and high mountain zones (Hanelt *et al*., 1992). Wild chives grow continuously all year round with no apparent dormant stage, and low winter temperatures only slow this down (Cheremushkina, 1985, 1992; Pistrick, 1992). Chives exhibit a monopodial growth habit, and only become sympodial after the formation of the first generative meristem. Thereafter, *Allium* plants produce renewal bulbs and flowers every year (Kamenetsky, and Rabinowitch, 2002). Temperatures play the most important role in normal scape elongation and flowering of *Allium* plants, although light conditions can markedly affect this process. Flowering usually does not occur if temperatures

are above 18°C (Rubatzky and Yamaguchi, 1997). Like other major cultivated *Allium* crops, cold exposure is required for floral induction in chives. (Poulsen, 1990).

In chives male sterility is conditioned by genetic male sterility (GMS), which is controlled by a single nuclear gene with recessive inheritance (Engelke and Tatioglu, 2000a,b,c). An alternative cytoplasmic male sterility (CMS) depends on the interaction between the cytoplasm (S) and a single nuclear fertility restoration locus (X) (Tatlioglu, 1982). Fertility of some male-sterile plants, however, can be regained under favourable environmental conditions. Hence, exposure to a constant temperature of 24 °C resulted in production of viable pollen (Tatlioglu, 1985). This temperature sensitivity is controlled by a single dominant allele (*t*) (Tatlioglu, 1987).

A. Schoenoprasum contains several closely related species, in part of polyploid nature, with partly unclear species status. In such situations, molecular markers could bring some clarification that is difficult to obtain by other means. A cladistic tree of the Schoenoprasum RAPD data has been constructed (Friesen and Blattner, 2000).

19.3.2 Culture and production

Chives are very adaptive to different environments. Tolerant of cold temperature, chives can germinate slowly when daily temperature averages 3–5 °C, while its most suitable temperature ranges from 15–20 °C. Because of its shallow root system, care must be taken to maintain soil moisture, especially to prevent flooding. Chives grow best in well-drained, fertile soil with medium acidity. Optimum growing temperatures are between 17–25 °C, cold hardy, and tolerant of high temperatures (Rubatzky and Yamaguchi, 1997), and so can be grown widely distributed throughout the world. The plants will start to flower after staying dormant for a period in cold temperatures.

Chives grow all year around and can be cultivated and harvested in batches throughout the four seasons. Propagation is usually with seed or division. For mass production, seeding in spring or autumn is suitable. Seedlings can be planted once 15 cm high. Each 20 × 10 cm pocket can accommodate 4–6 seedlings. They can be harvested in about two months when the plant reaches 30 to 50 cm in height. The first harvest will produce a relatively low yield. Chives can be harvested about once each month, and more frequently after the second harvest, to about 5–7 times per year in warm areas, and 2–4 times per year in colder climates. In cold areas, each harvest will yield more, to about 15 ton/ha. When processing, do not cut to the sheath (4 cm above ground). On average, reseeding or dividing the clumps every four years will keep the productivity high. In some areas, harvesting is done by plucking the plants by the roots rather than cutting.

It is important to strictly follow the guidelines of organic farming practice to grow chives. Select well drained, fertile sandy or clay soil; maintain the cleanliness of the field and weed promptly; use high quality organic compost with appropriate N.P.K. ratios. The crop is susceptible to many root diseases. Rotations are a key aspect for a sustainable agricultural production system, a rotation of at least five years is recommended. Although chives can be grown in all kinds of soils, the most suitable soils are sandy loams to loams with a fair content of organic matter and good soil structure. Soil pH of 6–6.5 is considered sufficient. Chives demand a high nutrient level. In the years following planting, the annual uptakes in yield are 185–200 kg/ha for nitrogen, 17–20 kg/ha for phosphorus, and 120–140 kg/ha for potassium in the most intensively fertilized treatment producing the highest yield. Black plastic mulch is effective in increasing yield, controlling weeds and maintaining soil moisture.

Selected productive cultivars or populations produced 10–20% higher yields than the less productive cultivars. The results show that chive is feasible for commercial production with improving cultivation techniques (Suojala, 2003).

Chives and related *Allium* crops are subject to a variety of diseases and attack by arthropod pests that can reduce crop yield and quality. Integrated pest management (IPM) is a sustainable approach to managing diseases and arthropod pests. IPM promotes the use of a variety of strategies and tactics, including pest-resistant varieties and biological, cultural and chemical controls, in a way that reduces costs, conserves natural resources and minimizes health and environmental risks. Decision-making is a key component of IPM programs (Binns and Nyrop, 1992). So far, monitoring programs forecasting systems for diseases (*Botrytis* leaf-blight, downy-mildew and purple-blotch) and pests (onion maggot, onion thrips, leek moth, cutworms, beet armyworm, aster leafhopper, aphids and mites) have been set up. IPM will continue to be the preferred strategy as it takes a whole-system approach as environmental problems take on greater importance. Since chives compete poorly with weeds, the use of herbicides is widespread and the economic advantages of their use have been demonstrated (Menges, 1987; Rubin, 1990). Scientific studies are starting to appear on the effects of organic production methods of weed control (Bond *et al*., 1998).

19.3.3 Post harvest and uses in food processing

Because chives are used as a vegetable or for seasoning, it is important to preserve the fresh green appearance as well as the unique aroma. After harvest, remove withered and damaged leaves. Immediately store in temperatures as low as 0 °C (32 °F), but not lower in order to prevent freezing. At 0 °C, with humidity of 95–100%, chives can be kept fresh for one to two weeks (Snowdon, 1991). The respiration rate of chives increases with temperature. At 0 °C, mg CO_2 $kg^{-1}h^{-1}$ is 22, which increases to 110 at 10 °C, and 540 at 20 °C (Peiris *et al*., 1997). In fact, when the temperature rises above 10 °C, chives will wilt quickly (Cantwell and Reid, 1993). When transporting, chives are usually packed into 1–3 kg packets, also in bunches of 10–50 g and kept moist in wax cartons at around 2–6 °C. Pre-cooling is recommended (Aharoni *et al*., 1989). In an experiment, green tops were bunched, 25–30 g per bunch, packed in perforated or non-polythene bags (20 × 25 cm) and stored at 2, 5, 10, 15 or 20 °C by Umiecka (1973). The control was kept unpacked. The tops stored better in non-perforated than in perforated bags and the longest satisfactory storage of 14–21 days was in non-perforated bags at 2 °C, but deteriorated rapidly at the higher temperatures (Thompson, 2003). Studies have been conducted on freshly harvested chives under simulated conditions of air transport from Israel to Europe, and also with an actual shipment, during which temperatures fluctuated between 4 and 15 °C (Aharoni *et al*., 1989). Packaging in sealed polyethylene-lined cartons resulted in a marked retardation of both yellowing and decay. However, sealed film packaging was applicable only if the temperature during transit and storage was well controlled, otherwise perforated polyethylene was better.

Drying is a common technique to process chives. The usual methods are heat drying at 50 °C, or freeze drying. Freeze drying is more costly, but preserves the flavour well. After drying/freeze drying, there are losses of 24–34% vitamin C, 19–21% chlorophyll, 11–18% beta-carotene, and 47–82% volatile sulphur (Lisiewka *et al*., 1998). Chive leaves for freezing contained 13.9 g dry matter, 133 mg vitamin C 4.7, beta-carotene, 121 mg, chlorophylls (a + b), 40.4 mg nitrates, and 0.19 mg

nitrites in 100 g of edible part. Blanching of the raw material before freezing reduced the level of dry by 22%, vitamin C 29%, beta carotene 20%, chlorophylls 21%, and nitrates 26%, while the nitrites increased three times. A further enhancement of losses was observed with a storage temperature at –20 °C, After 12 months storage of frozen chive, the preserved content of vitamin C ranged from 11 to 66%, beta carotene 37 to 65%, chlorophylls 65 to 75% and the nitrates 58 to 81% (Kmiecik and Lisiewska, 1999). The development of the catering business and industrial preparation of ready-to-cook food, most frequently pizza and au gratin dishes, has increased the demand for chives throughout the year. This demand can be met by preserving the vegetable as a dried or frozen product.

Chives can be used as seasoning for many dishes, or as garnish. Chives especially enhance the flavour of fish. There is a very delicious Chinese dish known simply as fish with chives. Chives can be included in many food items such as pancakes, buns, dumplings, and cookies. It can also be used in many dairy and meat products.

19.4 Varieties

There are many differences between the *Allium* species. The volatile sulphur content of different species ranges from 15 to 155 mg/10g^{-1} fresh weight (Ermakov and Arasimovich, 1961). It is easy to test the pyruvate of *Allium's* sulphur precursors, to determine the difference between the species. The difference ranges from 1–22 μmolg^{-1} fresh weight, while difficult to select through genetic breeding, are perhaps the key to improving flavours (Randle and Lancaster, 2002). The volatile sulphur content in Alliums is closely related to the soil and usable sulphur in the soil. Experiments have shown that in peat soil, where sulphur content is as high as 470 mg/10g^{-1}, the volatile sulphur content in the chives reaches 157 mg/10g^{-1}, while in clay soils with a sulphur content of 58 mg/10g^{-1} , the volatile sulphur content in the chives is only 42.8 mg/10g^{-1}. At the temperature range of 10–30 °C, volatile sulphur content increases from 42.8 mg/10g^{-1} to 130.9 mg/10g^{-1} (Ermakov and Arasinovich 1961). Also, improving cultivar, fertilization and cultivation techniques, shows that chive is feasible for commercial production. In summary, the flavour of chives is closely tied to its genetic traits, growing environment and as cultivation techniques. Chives are mildly flavoured. Chives have the highest beta-carotene and vitamin C content among all *Allium* species, and contain many antioxidants. These characteristics make Chives a superior and well appreciated vegetable and seasoning. However, there has not been much research done on chives. The understanding and improvement of chives has a lot of potential.

The formal name of Chives is *A. schoenoprasum L.* Syn. *A. sibiricum* (Kamenetsky and Fritsch, 2002), there are some other related species, including:

1. *A. schoenoprasum L. var. schoenoprasum*, also known as *A. raddeanum* Regel; *A. sibiricum* L. Leaves, leaf sheaths, and scape smooth. Fl. and fr. Jul–Sep. leaves 1 or 2, shorter than scape. Widely distributed in meadows, valleys, damp slopes; 2000–3000 m, in Xinjiang in China, India, Japan, Kazakhstan, Korea, Mongolia, Pakistan, Russia, SW Asia, Europe, and North America (Xu and Kamelin, 2000). Fl. May–June, second bloom in late summer possible (Kamenetsky and Fritsch, 2002).

2. *A. schoenoprasum* L. var. *scaberrimum* Regel, (Trudy Imp. S. –Peterburgsk. Bot. Sada 3(2): 80. 1875) Leaves, leaf sheaths, and scap scabrous-denticulate along angles, Fl. Aug. distributed in meadows, along streams; 2000–2500 m Xinjang in China, Russia, Kazakhstan, and Mongolia.
3. A. *schoenoprasum* L. var. *foliosum* Mutel. Originated in the Mediterranean region, with two to five smooth leaves (Mutel, 1834).
4. A. *schoenoprasum* L. var. *orientale* Regel. Bulb usually solitary or paired, rarely clustered (Xu and Kamelin, 2000).

A. *schoenoprasum* section contains several closely related speices, in part of polyploid nature, with partly unclear species status. It is not certain whether geographical varietals exist, probably need more work in near future to clear.

Nowadays there are a few plants commonly called 'chives' or 'green onions', but some of them are not *A. schoenoprasum*. For example, *A. cepiform*, a 'chive' (Rubatzky and Yamaguchi, 1997) from China with tender leaves named xi-xiang-cong, is possibly a cross of *A. cepa* x *A. festival*. Many people also call *A. fistulosum L. var. aespitosum* Makino 'chive'. These varietals are different in flavour from chives, and usually have only white flowers, or no flowers.

19.5 References and further reading

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20

Galanga

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20.1 Introduction

Galanga (not to be confused with the galangal, which is *Alpinia galanga*) is a perennial aromatic rhizomatous herbaceous plant belonging to the genus *Kaempferia* of the family Zingiberaceae. This genus is comprised of about 70 species. In *The Flora of British India*, Baker (1890) described 22 species, among which *K. galanga* and *K. rotunda* are of economic value and are used for flavouring food and in medicine. Rhizome and roots are aromatic and are used as spice. It is widely used in Indonesia (called 'Kenkur'), Philippines and Thailand (called 'Krachai' or 'Kachai') in flavouring a variety of dishes. In Thailand a related species, *K. parviflora* is under cultivation (Pojanagaroon *et al*., 2004). The essential oil from the rhizomes is used in perfumery and folk medicines. In Java, the rhizomes are used in seasoning rice dishes, and also pickled. The Javan beverage 'beras kentjoor' is made from the rhizomes. In many Asian countries galanga is used interchangeably with galangal (Duke, 2003). Leaves are eaten raw or after steaming, or cooked with chili (Duke, 2003). Both rhizomes and leaves are used in Asian countries for perfuming oil, vinegar, hair washes, powders, etc.

The genus is presumably native to tropical Asia and is distributed in the tropics and subtropics of Asia and Africa. It is cultivated in home gardens in India, Sri Lanka, Malaysia Moluccas (Indonesia), Philippine Islands and South East Asia.

20.1.1 Botanical notes

The plant attains a height of maximum 30 cm but often is much shorter and has fleshy, cylindrical aromatic root tubers. There are two (sometimes more) broad, round leaves that are spread horizontally over the soil. Leaves are sessile, ovate, deltoid-acuminate, thin and deep green. Petioles are short channeled; flowers irregular, bisexual, white, 6–12 from the center of the plant between the leaves, fragrant and opening successively; bracts lanceolate, green, short, calyx long as the outer bracts, short cylindrical, petals three, corolla tube 2.5 cm long, lanceolate, pure white, stamen one, perfect, filament short, arcuate, anther two celled, cells discreet. Flowering starts in June and ends in September, with peak flowering during July to August.

The underground part consists mainly of one or more prominent fairly big, vertically oriented tuberous rootstocks together with several smaller secondary tubers and a cluster of roots. The main tuber has several transverse or horizontal annular scars of scale leaves. Directly attached to these nodes are a few smaller tubers, which are also vertically oriented. Several roots arise from the rhizomes, they are either fibrous and long or short and thick. The latter roots bear at their tips tubers of various forms (oval, conical or rarely spherical). These tubers are succulent and watery and differ from the rhizomes.

A mature tuber in transverse section appears more or less circular in outline and shows a narrow light brownish border and a central well marked stele and a narrow cortex in between. A number of small vascular bundles are found scattered throughout the parenchymatous ground tissue. Leaf epidermal morphology of 12 species of Zingibearceae was compared and *K. galanga* showed the highest stomatal index (Gogoi *et al*., 2002). Variation in leaf anatomical features could be effectively used to distinguish species difference (Hussin *et al*., 2001). The somatic chromosome number of *K. galanga* is 2n = 54 (Ramachandran, 1969). Beltram and Kam (1984) reported that the Asiatic *Kaempferia* species have x = 11 and that of African species have x = 14. Pollen morphology studies revealed the absence of exine in *Kaempferia* and palynologically *Alpinia, Amomum, Zingiber* and *Kaempferia* constitute one group (Mangaly and Nayar, 1990).

Kurian and Nybe (2003) assembled a collection of 30 genotypes and evaluated them for yield and quality associated characters. They reported that all the characters except numbers of leaves showed a significant difference among the collections. They have identified two high-yielding and high-quality lines and these were released for cultivation under the name 'Kasthuri' and 'Rajani'.

Random amplified polymorphic DNA (RAPD) profiles generated from six species of *Kaempferia* species was used to analyze the degree of relationship of other genera (Vanijajiva *et al*., 2005) and also characterization of cultivars (Pojanagaroon *et al.*, 2004).

20.2 Cultivation and production

Galanga requires fertile sandy soil and a warm humid climate. It thrives well up to an elevation of about 1500 m above MSL. A well-distributed annual rainfall of 1500–3000 mm is required during the growing period and dry spells during land preparation and harvesting. The species is propagated by rhizome fragments. Mother rhizomes are superior compared to fingers. The rhizome bits are planted on beds of 1–2 m width and 25 cm height at a spacing of 40–60 cm^2 (Anonymous, 1981; Bhattacharjee, 2000). About 750 kg of seed rhizomes per hectare is required. Planting during the third week of May gave significantly higher rhizome and oil yields.

The performance of three ecotypes of *K. galanga* under 70 and 50% shading, and 10 and 20 cm tillage depths was investigated in an experiment conducted in Kerala, India during 2001–2. High rhizome yield was correlated with high P, K and Ca contents while high essential oil content was correlated with high Mg, S, Mn and Zn contents in the rhizome. Rhizome yield was higher at shallower tillage depth and higher light intensity, while essential oil yield was higher at deeper tillage depth and lower light intensity. Low light intensity increased the biosynthesis of oleoresin and essential oils in the rhizomes, as well as the contents of Ca, Mg, Mn and Zn. Cv. Thodupuzha showed the highest rhizome yield, while cv. Echippara showed the

highest essential oil and oleoresin production (Gangadharan and Menon, 2003). Ghosh and Pal (2002) studied the effect of N and K on growth, yield and oil content of *K. galanga* grown as an intercrop in *Terminalia arjuna* plantation. Sankar and Thomas (2000) reported that the effect of fertilizers has no significant effect on rhizome and oil yields.

K. galanga is a potent aromatic, medicinal plant suitable for cultivation in coconut gardens. Maheswarappa *et al*. (1998, 1999a, 2000a,b,c, 2001) studied various cultivation aspects such as influence of planting material, plant population and organic manures, dry matter accumulation in different parts as influenced by agronomic practices and nutrient content and uptake by *K. galanga.*

Planting time and type of seed material affect the growth, yield and quality of *K. galanga*. Mother rhizomes planted in May and harvested after six months gave the highest essential oil and oleoresin yields, compared to those planted in June and the mean nutrient uptake by the plants was 22.8 kg N, 28 kg P_2O_5 and 36.9 kg K_2O/ha (Rajagopalan and Gopalakrishnan, 1985a,b; Rajagopalan *et al*., 1989). Application of 50–75 kg N, 60 kg P_2O_5 and 50–75 kg K_2O is found to be beneficial for increased rhizome and oil yields. Application of farmyard manure at 30 tonnes/ha is superior to the application of nutrients through inorganic form of fertilizers and it increased the yield by 60%. A well-managed plantation yields about 4–6 tonnes of fresh rhizomes per hectare. Dry recovery varies from 23 to 28%. Leaf rot disease is found to occur during the rainy season and it can be controlled by trenching with 1% Bordeaux mixture. In Kerala, cultivation of *K. galanga* is restricted to some localized tracts and the productivity of the crop is low ranging from 2–5 tonnes of fresh rhizomes per hectare. There is an acute shortage of planting material and the absence of seed set limits the scope for breeding (Kurian *et al*., 1993).

Root Knot nematode (*Meloidogyne incognita*) is a serious problem in *Kaempferia*. A study of phyto-nematodes associated with *K. galanga* in Kerala revealed that an initial population of 200 and 1000 J2 (*M. incognita*) larvae per plant reduced the production of leaves, length and weight of rhizome (Sheela and Rajani, 1998). Effects of leaf mulches from *Azaridacta indica, Glirizidia maculata, Acacia mangium, Clerodendron infortunatum, Calotropis gigantea*, and *Chromolaena odorata* on root knot nematode and *K. galanga* was studied by Nisha and Sheela (2002). Application of *A. indica, C. odorata*, and *G. maculata* mulches at 5 kg/m^2 at 15 days before planting reduced nematode population by more than 60%, with mulches from A. *indica* being the most effective. Mulches from *A. indica* and *C. odorata* resulted in the lowest gall index. All treatments improved *K. galanga* yield and yield components. The highest rhizome yield (5.6 kg per plot) was obtained with *A. indica* mulches. Occurrence of leaf rot disease during the rainy season was noticed (Anonymous, 2003b). *Pseudomonas solanacearum* causing bacterial wilt of *K. galanga* from Kerala, India, was reported (Dake and Manoj, 1995).

The crop matures in about 6–7 months after planting. The aerial portion dries off on maturity. The rhizomes are dug out, cleaned and washed to remove soil and are dried in the sun. The essential oil is extracted by steam distillation of sliced and dried rhizomes. The oil yield varies with season and maturity stage of the rhizome.

20.3 Tissue culture studies

Various reports are available on tissue culture studies in *K. galanga* and related

species. Vincent *et al*. (1992c) reported micropropagation of *K. galanga*. High frequency single step *in vitro* protocols for rapid propagation from the rhizome buds were also established (Geetha *et al*., 1997; Geetha, 2002; Jose *et al*., 2002; Swapna *et al*. 2004). A rapid clonal propagation system for *K. galanga*, has been developed for large-scale propagation and *ex situ* conservation (Shirin *et al*., 2000). LaiKeng and WengHing (2004) reported *in vitro* propagation of *Zingiberaceae* species with medicinal properties. Plant regeneration from callus derived from rhizome bud explants and somatic embryogenesis had also been reported (Vincent *et al*., 1991, 1992a,b; Lakshmi and Mythili, 2003). Mostly MS medium supplemented with cytokinin like benzyladenine (BA) and auxins such as indole butyric acid (IBA) or α-naphthalene acetic acid was used for *in vitro* responses. After executing proper acclimatization protocol, *in vitro* plantlets could be successfully planted in the field with a high percentage of survival. The micropropagated plants could not be used on a commercial level as they produce sufficient quantity of rhizome only after three seasons of growth in the nursery. In order to reduce this time gap, efforts were made at the Tissue Culture Facility of Centre for Medicinal Plants Research to develop *in vitro* microrhizome technology in *K. galanga* in culture media supplemented with higher levels of sucrose (Geetha *et al*., 2005). The microrhizome derived plants exhibited superiority over normal micropropagated plants in rhizome formation after planting out. Chirangivi *et. al*. (2005) also reported microrhizome induction in this species. *In vitro* conservation by slow growth methods were developed for medium-term conservation of this important medicinal plant (Geetha, 2002).

20.4 Functional properties

Galanga is pharmaceutically a very active plant and many biological properties have been reported (Table 20.1). As in the case of other zingiberaceous plants like ginger, greater galangal, etc., galanga also shows potent antitumour activities and antimutagenic activities. Vimala *et al*. (1999) reported seven zingiberaceous genera having such activities, and *Kaempferia* is a potent one that inhibited Epstein-Barr Virus (EBV) activation induced by TPA (12-o-tetradecanoylphorbol-3-acetate). The above authors concluded that the naturally occurring non-toxic compounds inhibited the EBV activation. *K. galanga* extract exhibited amoebicidal activity against *Acanthamoeba* (DanMy *et al*., 1998).

Essential oil from the root induced gutathione-s-transferase activities in the stomach, liver and small intestine of mice. Ethanol extract of dried rhizome showed antispasmodic activity vs. histamine-induced contraction and barium-induced contraction in guinea pigs. An ethanol-water extract indicated smooth muscle stimulant activity. Water extract of dried rhizomes exhibited antitumour activity. Rhizome and root oils showed antibacterial activity against *Escherichia coli, Staphylococcus aureus* and antifungal activity against *Alternaria, Colletotrichum, Fusarium*, etc. (Thomas *et al*. 1996; Arembawela *et al*., 1999a,b).

K. galanga extracts showed strong lipoxygenase inhibitory activities of more than 80% at 0.1 mg/ml (Ling *et al*., 1998). The hypolepidemic action of the ethanoic extract of *K. galanga* was observed *in vitro*. The oral administration of the extract was effective in lowering the total cholesterol, triglycerides and phospholipid levels in serum and tissues (Achuthan and Padikkala, 1997). Extract of *K. galanga* exhibited marked larvicidal effect against *Culex quinquefasciatus* (Pitasawat *et al*., 1998) and

Table 20.1 Biological actions of important components

Component	Biological property
Borneol	Analgesic, antiacetylcholine, antibacterial, antibronchitic, antifeedent, anti-inflammatory, antiotitic, antipyretic, antispasmodic, antimicrobial, CNS-stimulant, hepatoprotectant, irritant, myorelaxant, sedative, tranquilizer.
Camphene	Antioxidant, expectorant, hypocholesterolemic, spasmogenic, insecticidal
Carene	Antiseptic, irritant.
Kaempferol	Antiallergic, antiaggregant, anticancer, antifertility, antiimplantation, antilymphocytic, antihistamine, antioxidant, antispasmodic, serotonin suppressor, aromatase inhibitor, antitumour, glucosyl transferase inhibitor, antihepatotoxic, anti-inflammatory, lipoperoxidase inhibitor, antimetastatic, antimutagenic, antimyocardiatic, ATP-ase inhibitor, CAMP-phosphodiesterase inhibitor, carcinogenic, (at/V 40000 ppm), catechol-o-methyltrasferase inhibitor, COX-2 inhibitor, De-iodinase inhibitor, lipoxygenase inhibitor, metalloproteinase inhibitor, NADH-oxidase inhibitor, NO-inhibitor, ornithine decarboxylase inhibitor, P450 inhibitor, phospholipase inhibitor, protein kinase-c-inhibitor, tyrosinase inhibitor, protein tyrosinase kinase inhibitor, quinone reductase inducer, lopoisomerase I and II inhibitor, xanthine oxidase inhibitor, vasodialator.

Sources Duke, 2003; Duke and Du Cellier, 1993.

the hexane fraction exhibited high mosquito larvicidal effect and also repellent activity for adult mosquito (Taesotikul *et al*., 1999). Xue and Chen (2002) have shown that *cis*- and *trans*-ethyl p-methoxy-cinnamate inhibit EBV *in vitro* and also has an inhibitory effect in TPA assays or croton oil-induced ear edema, ODC activity in mouse epidermis and papilloma indicating a relatively strong anti-carcinogenic potential of ethyl-p-methoxy cinnamate.

Ethyl cinnamate (EC) inhibited the contractions induced by high K^+ and Phenylephrine (PE) in a concentration-dependent manner. The relaxant effect against PE-induced contractions was greater in the presence of endothelium. This inhibition effect of EC is believed to involve the inhibition of Ca^{++} influx into vascular cells and release of NO and prostacyclin from the endothelial cells (Othaman *et al*., 2002). This explains the traditional use of galanga for treating hypertension. Chloroform extract inhibited vascular smooth muscle contraction by the inhibition of Ca^{2+} influx and Ca^{2+} sensitivity of contractile elements (Mustafa *et al*., 1996).

The hexane fraction of *K. galanga* rhizome is categorized as a non-irritant both in animal and human volunteer studies (Choochote *et al*., 1999; Kanjanpothi *et al*., 2004). Acute toxicity studies using alcoholic extracts of the rhizome on mice and rabbits, indicated that oral administration of 5 g/kg and 10 g/kg of crude extract was non-toxic (Kanjanapothi *et al*., 2004). *K. galanga* demonstrates less toxicity, but it is considered as an effective botanical insecticide with high larvicidal activity and a protective effect against mosquitoes (Choochote *et al*., 1999).

20.5 Chemistry

K. galanga rhizome contains about 2.5 to 4% essential oil. The main components of the oil are ethyl cinnamate (25%), ethyl-p-methoxycinnamate (30%) and p-methoxycinnamic acid and a monoterpene ketone compound, 3-carene-5-one (Kiuchi

et al., 1987). The first three compounds are reported to have larvicidal activity (Kiuchi *et al*., 1988). The other constituents are camphene, δ-3-carene, p-methoxy styrene, γ-pinene, β-myrcene, p-cymene, 1,8-cineole, isomyrcene, camphor, α-terpineol, p-cymene-8-ol, eucarvone, δ-cadinene, hexadecane, heptadecane, limonene, octanol, tetradecane, 2-3-dehydro benzofuran, vanillin-p-methoxy phenol, caravacrol, carveol, myrtenol, β-cymene, p-methoxybenzaldehyde, β-cadinene, carcine, m-anisaldehyde, quinasoline-4-phenyl-3-oxide, sandaracopimaradiene-9-ol-1-one, sandaracopimaradiene-1, 9-diol, 6-acetoxy sandaracopimaradiene-9-ol-1-one (and its isomers) etc. (Arembewela and Silva, 1999; Arembewela *et al.*, 2000). The leaves contain kaempferol, quercetin, cyanidin and delphinidin. The camphor present has been characterized as ethyl-p-methoxy-trans-cinnamate (Rastogi and Mehrotra, 1998).

The composition of essential oil of rhizome of *K. galanga* growing in Malaysia has been investigated by capillary GC, GC-MS and IH-NMR (Wong *et al*., 1992). The major components of a Malaysian sample of the oil are ethyl-*trans*-p-methoxy-cinnamate (51.6%), ethyl cinnamate, (16.5%), pentadecane (9.0%), delta-car-zone (3.3%), borneol (2.7%) and 1,8-cineole (5.7%). It also contains monoterpene ketone, 3 caren-5 ene. The oil has been reported to possess insecticidal activity which is attributed to ethyl-*trans*-p-methoxy-cinnamate and ethyl-cinnamate. The rhizome is also reported to display cytotoxic properties.

20.6 Uses

K. galanga is cultivated for its aromatic rhizomes and also as an ornamental and has a long history of medicinal use. The rhizome is chewed and ingested. It is used as a flavouring for rice. The rhizomes are considered stimulatory, expectorant, carminative and diuretic. They are used in the preparation of gargles and administered with honey in cough and chest afflictions. In the Philippines, a decoction of the rhizomes is used for dyspepsia, headache and malaria. The juice of the plant is an ingredient in the preparation of some tonic preparations. The rhizomes and roots are used for flavouring food and in medicine in South East Asia (CSIR, 1959). The rhizome is mixed with oil as a cicatrizant applying it to boils and furuncles (Duke, 2003). Bown (2001) cited a mix of four ginger relatives (*Alpinia, Curcuma, Kaempferia and Zingiber*) called 'awas empas', a Jamu remedy for headaches, stiff joints and urinary tract infection.

Kaempferia is indicated for amebiasis, bruise, cancer, childbirth, cholera, cough, dandruff, dyspepsia, enterosis, fever, furuncle, headache, inflammation, lameness, lice, lumbago, malaria, myosin, ophthalmia, pain, parasite, rheumatism, rhinosis, scabies, sore-throat, swellings, toothache and tumor (Duke, 2003). 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. The powdered rhizome mixed with honey is given as an expectorant. The leaves are used in locations and poultices for sore eyes, rheumatism and fever. 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 the treatment of abdominal pain, vomiting, diarrhoea and toothache with the functions of promoting vital energy circulation and alleviating pain. In *Ayurveda*, the Indian traditional system of medicine, *Kaempferia* rhizomes are made use of in at least 61 formulations that are used in treating a variety of illness.

In Java, the rhizomes are used widely in seasoning many dishes, especially rice dishes. Rhizomes are also pickled or used to make 'beras', a sweet, spicy beverage. Another beverage, 'berao kentjoor' is made from the roots. Dried rhizomes are also added to curry powder. In many Asian countries galanga and galangal are used interchangeably, leaves and rhizomes may be used in curries, eaten raw or steamed, or cooked with chilli. Leaves of the narrow-leaved variety are eaten and both types are used in lalabs. Asians employ the rhizomes and leaves as a perfume in cosmetics, hair washes and powders. Rarely it is used as a hallucinogen (Duke, 2003).

In Malaysia, the rhizome is used for chills in elephants. In Sri Lanka, rhizome mixed with oil is used externally for healing of wounds and applied to warm rheumatic regions. The powdered rhizome is mixed with honey and given for coughs and pectoral ailments. Dried rhizome is used as a cardiotonic in Thailand. In Papua New Guinea the rhizome is used orally as an abortifacient. (Arambewela and Silva, 1999). The essential oil is used in flavoring curries, in perfumery and also for medicinal purposes (Bhattacharjee, 2000).

20.7 *K. rotunda*

K. rotunda L. (Indian crocus) is found scattered thoughout India in most localities. It is cultivated occasionally as a garden plant. The tuber is used in about 21 medicinal preparations in Ayurveda. It is a perennial herb having a tuberous rhizome. Leaves are simple, ligulate, few, erect, lanceolate, acute, variegated, green above and tinged with purple below, up to 45 cm long and 10 cm wide, petiole short, channeled, leaf base sheathing, flowers on a short crowned spike, flowers are bractolate, bisexual and trivenous and having the typical Zingiberaceous floral structure. Propagation is though rhizomes.

Tubers are acrid, thermogenic, aromatic, stomachic, anti-inflammatory, sialagogue, and emetic. They are useful in vitiated conditions of *Vata* and *Kapha*, gastropathy, dropsy, inflammations, wound, ulcer, blood clot, tumors and cancerous swellings (Warrier *et al*., 1995). The fresh bruised tubers are in popular use in many parts of India and applied to bruises to reduce swelling. The decoction is also applied to wounds with coagulated blood and with any purulent matter.

20.8 References and further reading

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21

Galangal

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21.1 Introduction

Alpinia galanga (L.) Sw. (Zingiberaceae) is commonly known by various names as galangal, greater galangal, Java galangal and Siamese ginger (English). The related species are *A. officinarum* Hance and *A. calcarata* Rosc., which are known as lesser galangal. All the three species have more or less similar properties and uses and hence in trade practically no distinction is made among them. Data on production, consumption and trade individually are not reliable because traders make no distinction between *A. galanga, A. calcarata* and *A. officinarum;* all the three are used as the source plants for the Ayurvedic raw drug *'raasna'*. India is a major supplier along with Thailand and Indonesia (Scheffer and Jansen, 1999), however, its volatile oil attracts more international interest because of its high medicinal value (http://www.indianspices.com).

Galangal is a native of Indonesia though the exact origin is not known, but has become naturalized in many parts of South and South-East Asian countries. Oldest reports about its use and existence are from Southern China and Java. It is of frequent occurrence in the sub-Himalayan region of Bihar, West Bengal and Assam. 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 southwest India, and is cultivated throughout the Western Ghats (Warrier *et al.,* 1994). India exports galangal in different forms (http://www.indianspices.com). 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 tons 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) and Gupta and Tandon (2004).

21.1.1 Botanical notes

A. galanga (L.) Sw is a perennial, robust, tillering, rhizomatous herb; grows 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 (pseudostem) 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. Inflorescence terminal, erect, many flowered, racemose, 10–30 × 5–7 cm, pubescent; bracts ovate, up to 2 cm long, each subtending a cincinnus of 2–6 greenish white flowers; bracteoles similar to the bracts but smaller; flowers fragrant, 3–4 cm long, yellow-white. Fruit 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. Much variability may exist as the species occur naturally in many countries under varying agroecological situations, however, information is lacking. Cultivars with pink to red rhizomes and with yellow-white rhizomes are known. The pseudostems of white cultivars reach about 3 m in height, and the rhizomes 8–10 cm in diameter. The red cultivars that 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).

21.1.2 Chemical notes

Tewari *et al.* (1999) reviewed the chemical composition of *Alpinia spp*. Galangal rhizome on analysis yielded (per 100 g): moisture – 14 g, total ash – 9 g, matter soluble in 80% ethanol – 49 g, matter soluble in water – 19 g, total sugar – 9 g, total nitrogen – 3 g, total protein – 16 g, essential oil content – 0.2–1.5% (dry wt.). Fresh rhizomes on steam distillation yield about 0.1% of oil, having a peculiar strong and spicy odour. Earlier investigations indicated camphor, 1, 8-cineole (20–30%), methyl cinnamate (48%) and probably d-pinene, as the oil components. Scheffer *et al.* (1981) analysed a sample from Indonesia and reported 1,8-cineole (47.3%), β-pinene (11.5%), α-pinene (7.1%), α-thujene (6.2%), terpinen-4-ol (6.0%), α-terpineol, limonene (4.3% each) and many compounds in lesser concentrations. De Pooter *et al.* (1985) analysed a sample from Malaysia and reported (E)-β-farnasene (18.2%), β-bisabolene (16.2%), α-bergamontene (10.7%), and α-pinene (10.2%) as the important components. Charles *et al.* (1992) reported that a sample from the USA yielded 52.3% myrcene, 17.15 (Z)-β-ocimene, 9.0% α-pinene as the major components. The root contains a volatile oil (0.5 to 1.0%), resin, glalangol, kaemferid, galangin, alpinin, etc. The active principles are the volatile oil and acrid resin. Galangin has been obtained synthetically. The essential oil obtained by hydrodistillation of fresh flowers contains sabinene, limonene, 1,8-cineole, p-cymen-8-ol, patchoulene, (E)-methyl cinnamate, (z)-allylcinnamate, α-gurjunene and β-caryophyllene (Syamasundetr *et al*. 1999). Chaudhury (1961), Nair *et al*. (1962), Barik *et al*. (1987) and Kumar *et al*. (1990) also reported chemical studies on *Alpinia.*

The volatile constituents of the rhizomes and leaves of *A. galanga* from the lower Himalayan region of India were analysed by GC and GC/MS. The main constituents identified in the rhizome were 1,8-ciniole, fenchyl acetate and β-pinene. The leaf oil

contained 1,8-ciniole, β-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 *A. galanga* 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. The essential oil of *A. galanga* leaf is rich in 1,8-cineole (28.3%), camphor (15.6%), beta-pinene (5.0%), (E)-methyl cinnamate (4.6%), bornyl acetate (4.3%) and guaiol (3.5%). The stem essential oil contains 1,8-cineole (31.1%), camphor (11.0%), (E)-methyl cinnamate (7.4%), guaiol (4.9%), bornyl acetate (3.6%), β-pinene (3.3%) and α-terpineol (3.3%). 1,8-cineole (28.4%), α-fenchyl acetate (18.4%), camphor (7.7%), (E)-methyl cinnamate (4.2%) and guaiol (3.3%) are the main constituents of the rhizome essential oil. The root essential oil contains α-fenchyl acetate (40.9%), 1,8-cineole (9.4%), borneol (6.3%), bornyl acetate (5.4%) and elemol (3.1%).

21.2 Production

A. galanga 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 soils rich in organic matter and with good drainage are preferred. Wild or semi-wild types occur in old clearings, thickets and forests. In the tropics, galangal occurs up to an altitude of 1200 m. Rhizomes (a rhizome piece with an aerial shoot, known as slips) 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 galanga rhizome emerge about one week after planting. About four weeks after planting 3–4 leaves develop. Rhizomes develop quickly and reach their best harvest quality in three months after planting. If left too long they become too fibrous and large clumps will hamper harvesting. Seeds rarely reach maturity. Often trenches are dug to drain the field after rainfall, as rhizomes do not develop under waterlogged conditions. Usually planted along the borders of gardens, in rows at distances of 0.5–1 m square. Weeding and subsequent earthing up are carried out respectively 1–2 months after planting.

Harvesting for use as spice is done usually three months after planting (during late summer or early autumn) for market purposes. Whole plants are pulled out, shoots cut off and rhizomes washed and cleaned. Rhizomes more than four months old turn woody, fibrous and spongy and lose their value as spice. 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 fibrous. No reliable data is available on the yield (Scheffer and Jansen, 1999). Harvested rhizomes are washed, trimmed, dried and marketed fresh or dried after packing (Scheffer and Jansen, 1999). Dried product is ground before use. Ground rhizomes are not traded in bulk as they may be adulterated. Essential oil is also a product.

21.3 Molecular pharmacology

Dried rhizomes of *A. galanga* 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 the therapeutic effectiveness of the galangal (Table 21.1)

21.4 Functional properties

Rhizome is bitter, acrid, thermogenic, aromatic, nervine tonic, stimulant, carminative, stomachic, disinfectant, aphrodisiac, expectorant, bronchodilator, febrifuge, anti-inflammatory and tonic (Warrier *et al.,* 1994). Galangal has a wide range of applications in traditional medicine. Rhizomes show antibacterial, antifungal, antiprotozoal and expectorant activities (Scheffer and Jansen, 1999). Galangal's anti-bacterial effect acts against germs, such as *Streptococci, Staphylococcus* and coliform bacteria. This

Table 21.1 Attributed biological properties of the chemical components of galangal

Compound	Attributed properties
Borneol	Analgesic, antibronchitic, acetylcholine anatagonist, antiinflammatory, antipyretic, antispasmodic, CNS-stimulant, CNS-toxic (at high doses), hepatoprotectant, myorelaxant, sedative.
1,8-cineole	Anesthetic, antiacetylcholinesterase, antiallergic, antibacterial, antibrochitic, anticarcenogenic, antiinflammatory, antirheumatic, antirhinitic, antiseptic, antispasmodic, antitussive, candidicide, ascaicide, carcinogenic (high and constant use), CNS-stimulant, convulsant, decongestant, expectorant, myorelaxant, P-450 inducer, neurotoxic, rubifacient, sedative, testosterone-hydroxylase inducer.
Eugenol	Analgesic, anticancer, anticonvulsant, antiedemic, 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, vasodialator, vermifuge.
Galangin	Antiaflatoxic, antiinflammatory, antimutagenic, anticancer, antioxidant, antiviral, aromatase inhibitor, cycloxygenase inhibitor, COX-2 inhibitor, hepatoprotective, NO inhibitor, quinone reductase inducer, topo-isomerase –1-inhibitor, tyrosinase inhibitor.
Camphor	Analgesic, anesthetic, antiseptic, antispasmodic, fungicide, anticancer, decongestant, expectorant, antiemetic, carminative.
β-bisabolene	Abortifacient, antirhinoviral, antiulcer, stomachic.
Myrcene	Analgesic, anesthetic, antibacterial, anticonvulsant, antimutagenic, anti-nitrosaminic, antioxidant, antipyretic, antispasmodic, irritant, aldose-reductase inhibitor.
Quercetin	Analgesic, antiaggregant, antiinflammatory, antileukemic, antileukotriene, antilipoperoxidant, antimelanomic, antimutagenic, antinitrosaminic, antioxidant, antiperoxidant, antitumour, apoptotic, COX-2 inhibitor, cyclooxygenase inhibitor, hepatoprotective, lipoxygenase inhibitor, mast-cell stabilizer, ornithine decarboxylase inhibitor, P-450 inhibitor, protein kinse-C-inhibitor, topoisomerase I and II inhibitor, tyrosine kinase inhibitor, NADH-Oxidase inhibitor, hypoglycemic, quinone reductase inhibitor.

Source: Collected from various sources, mainly from Duke (2003), *Martindale, the Extra Pharmacopoeia* (2002).

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. It is included in several compound preparations. The reddish-brown powder is used as a snuff in catarrh. Young rhizome is a spice and is used to flavour various dishes in Malaysia, Thailand, Indonesia and China. In the Indian traditional medicine, Ayurveda, *A. galanga* (known as '*Raasna*') is used in over 62 formulations that are used for curing a variety of ailments. Two of the related species, *A. calcarata* and *A. officinarum* are also used as sources of the raw drug *Raasna*.

Antifungal activity of *A. galanga* was reported by Haraguchi *et al.* (1996). They have isolated an antimicrobial diterpene (diterpene 1) and found that his compound synergistically enhanced the antifungal activity of quercetin and chalcone against *Candida albicans.* Its antifugal activity was reversed by unsaturated fatty acids. Protoplasts of *C. albicans* were lysed by diterpene 1. These results suggest that the antifungal activity of this compound is due to a change of membrane permeability arising from membrane lipid alteration. The ethanolic extract of *A. galanga* rhizome exhibited hypolipidemic activity *in vitro.* The 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. The study suggests that galangal is useful in various lipid disorders especially atherosclerosis (Achuthan and Padikkala, 1997). The USDA database lists 387 distinct activities for *A. galanga.*

Galangin and kaempferol, the flavanols present in the rhizome, are known to possess tyrosinase-inhibitory activity as well as COX-inhibitory activity. These activities are probably due to their ability to chelate copper (and also other divalent cations) in the enzyme. Galangin inhibits monophenolase activity, and both galangin and kaempferol inhibit diphenolase. Galangin also possesses (so also quercitin, another flavonol present in the rhizome) antioxidant and radical scavenging activities, and hence can modulate enzyme activities and suppress the genotoxicity of chemicals (Duke, 2003). 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 betamethazone. Antihypertensive activity of galangal was shown by its ability to inhibit angiotension converting enzyme (ACE) by water, ethanolic and acetone extracts to the level of 29, 42, and 31% respectively (Somanadhan *et al*. 1999).

Among the many compounds reported, 1-acetoxychavicol acetate, a component of newly dried rhizomes, is active against dermatophytes, and together with another compound, 1-acetoxyeugenol acetate, exhibits anti-tumor activity in mice. The same compounds isolated from roots showed anti-ulcer activity in rats. Oil shows potential insecticide property. Galangal root, root oil and root oleoresin are given the regulatory status 'generally regarded as safe' (GRAS) in the USA (Scheffer and Jansen, 1999).

Itokawa *et al.* (1987) isolated the phenylpropanoids, 1-acetoxychavicol acetate and 1-acetoxyeugenol acetate, both showing antitumour activity against sarcoma 180 ascites in mice. Sadique *et al.* (1989) reported that *A. galanga* extract showed sheep

RBC membrane stabilizing activity. Al-Yahaya *et al.* (1990) demonstrated that ethanolic extract of galangal has potent gastric antisecretary, antiulcer and cryoprotective properties. Duke (2003) has listed the application of *A. galanga* and *A. officinarum* as herbal medicines in the treatment of various ailments.

A novel composition of aromatic and terpinoid compounds present in *A. galanga* showed synergistic effects with respect to immunomodulation, and effectively suppressed hypersensitivity reactions. These compounds are used for preparing medicaments for the treatment or prevention of allergic reactions and such conditions as asthma, allergic rhinitis, anaphylaxis and autoimmune disorders like ulcerative colitis, rheumatoid arthritis, as well as for the alleviation of pain (Weidner *et al.,* 2002). The constituents isolated from the seeds of *A. galanga* are reported to exhibit anti-ulcer activities (Mitsui *et al.,* 1976a,b). 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 the nuclear ribosomal DNA internal transcribed spacer (nrDNA ITS) region sequences and the molecular markers are used to distinguish the drug at DNA level.

21.5 *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 is known to have been in use for over a thousand years in these regions. The Arabs formerly were known to feed their horses on this plant to make them fiery (Grieve, 1931). 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 northeast India. Alcoholic extract of the rhizome contains tannins, phlobaphenes; chloroform extract showed the presence of flavones such as kaempferide, galangin and alpinin (Sastry 1961). Ray and Majumdar (1975) reported the isolation of a flavonoid possessing antifungal activity. The decoction of the rhizome revealed antinflammatory activity against carragenin-induced rat paw edema (Sharma and Singh 1980). Kaleysa Raj (1975) reported anthelmintic activity against human *Ascaris lum bricoides*.

A. officinarum is a very valued medicinal plant and has been in use traditionally. Its rhizome has an essential oil that is warm and spicy. It has been in use in chronic enteritis, 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 that of *A. galanga.* The therapeutic effects when used in traditional medicines might be mainly due to the contents of quercetin, galangin and kaempferol.

21.6 *Alpinia calcarata* (lesser galangal)

A. calcarata is also known as lesser galangal and its properties and uses are similar to those of *A. galanga*. In the Ayurvedic medicines *A. calcarata* has virtually taken the place of *A. galanga* mainly due to the non-availability of the latter. The traders do not make any distinction among the three species; all of them are traded as the raw drug '*raasna'*. 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. Major constituent is 1,8-cineole (Tewari *et al*. 1999).

21.7 References and further reading

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22

Leek and shallot

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22.1 Introduction

Leek (*Allium ampeloprosum* L.) (Synon. *A. porrum* L.; *A. ampeloprasum* var *porrum* (L.) Gay) is an important crop of the family Alliaceae which exhibits morphological differences with onions. It is larger than the onion. The leaf blades are flattened rather than radial. The leaf base of leek stores some reserves but does not thicken into a bulb. Leek has a milder and more delicate flavour than onion, though a coarser texture. When tender, it is eaten raw. It is also cooked with other vegetables or used as a flavouring in soups and stews. Leeks are mainly grown in northern Europe and less frequently in India, the United States, and Canada. Leeks are especially important in northern European countries such as Belgium, Denmark, and the Netherlands (Warade and Shinde, 1998). A non-bulb forming biennial is grown for its blanched stem and leaves. In India and Sri Lanka, it thrives well at higher altitudes but moist localities are adverse to its cultivation. Commercial cultivation is not followed in India and wherever it grows, it is on a home scale, mainly in the kitchen garden as a favourite vegetable.

Shallots (*Allium ascalonicum* L. Syn.; *A. cepa* L. var *ascalonicum* Backer) are a perennial crop that is grown as an annual for its cluster of small bulbs or cloves. They have a delicate onion-like flavour and may be grown for their dry bulbs or used in the same manner as green onions. Leeks and shallots are indeed valuable, not only as spices for flavouring dishes, but also as medicinal plants of importance. This chapter deals briefly with leeks and shallots. The chapter contains the following sections on leek and shallot; description, botany, origin and distribution; chemical composition; cultivation and production; uses in food industry/processing; functional properties and quality issues.

22.2 Leek

22.2.1 Description, botany, origin and distribution

Description

Leeks known botanically as *Allium ampeloprosum* L. (Synon. *A. porrum* L.; A. *ampeloprasum* var *porrum* (L.) Gay) are related to garlic and bear a resemblance to onions, shallots and scallions. Common names in different languages are as follows: Leek (English); jiu cong (Chinese); poireau, porreau (French); Porree (German); porro, porretta (Italian); liiki (Japanese); luk porej (Russian); ajo porro, apuerro (Spanish). Leeks look like large scallions, having a very small bulb and a long white cylindrical stalk of superimposed layers that flows into green, tightly wrapped, flat leaves. Cultivated leeks are usually about 30 cm in length and 5.0–8.5 cm in diameter, and feature a fragrant flavour that is reminiscent of shallots but sweeter and more subtle. Wild Leeks, known as 'ramps' are much smaller in size, but have a stronger, more intense flavour (Anon. 2005a). With a more delicate and sweeter flavour than onions, leeks add a subtle touch to recipes without overpowering the other flavours that are present.

Botany

Leeks (*Allium ampeloprasum*) are members of the Alliaceae family. Other members of the family include onion and garlic. The leek plant is a robust herbaceous biennial that has been cultivated for centuries but has not been found wild. Leek plants resemble large onion plants with flat leaves. Unlike onion and garlic, leeks do not form bulbs or produce cloves. Leeks are made up of sheaths of basal leaves that can be 15–25 cm long and 5 cm in diameter. The taste of leeks is milder than those of onion and garlic. The leek is a tall, hardy, biennial with white, narrowly ovoid bulbs and broad leaves. It resembles the green onion but is larger. Leek, is a tetraploid (2n = 32). It differs mainly in its lesser tendency to form bulbs. Many cultivars selected for long, white, edible bases and green tops, winter hardiness, and resistance to bolting are available for cultivation. These cultivars differ from one another mainly in length and diameter of the sheath part, leaf spacing, breadth and colour of leaf blades, vigour and bolting, and resistance to cold.

Resistance to cold is of special importance where leeks are to be harvested throughout the winter, while slowness to bolt permits a prolonged harvest period in the spring (McCollum, 1976). Rather than forming a tight bulb such as the onion, the leek produces a long cylinder of bundled leaf sheaths which are generally blanched by pushing soil around them (trenching). They are often sold as small seedlings in flats which are started early in greenhouses, to be set out as weather permits. Once established in the garden leeks are hardy; many varieties can be left in the ground during the winter to be harvested as needed (Anon., 2005a).

Origin and distribution

Randy Baker (1991) reported that the leek originated in Middle Asia, with secondary centres of development and distribution in Western Asia and the Mediterranean countries. The leek has been cultivated in Western Europe since the middle ages and found its way to North America with early settlers from Europe. Leeks have been cultivated from very early times (Silvertand, 1996). The garden leek was a popular vegetable in the ancient Near East when the Egyptians built their pyramids, for example, that of

Cheops in 2500 BC. Leek was an important vegetable for the Greeks and Romans, and its use later spread throughout medieval Europe. Leeks have enjoyed a long and rich history, one that can trace its heritage back through antiquity.

Leeks were valued most by the ancient Egyptians, Greeks and Romans and were especially revered for their beneficial effect upon the throat. The Greek philosopher Aristotle credited the clear voice of the partridge to a diet of leeks, while the Roman emperor Nero supposedly ate leeks every day to make his voice stronger. The Romans are thought to have introduced leeks to the United Kingdom, where they were able to flourish because they could withstand cold weather. Leeks have attained an esteemed status in Wales, where they serve as the country's national emblem and the Welsh wear it on St David's Day. According to legend, Saint David ordered his Welsh soldiers to identify themselves by wearing the vegetable on their helmets in an ancient battle against the Saxons that took place in a leek field. Today, leeks are an important vegetable in many northern European cuisines and are grown in many European countries (Anon. 2005a).

22.2.2 Chemical composition

The nutritional composition of leek is given in Table 22.1. Compared to onion, leek contains more proteins and minerals on a fresh weight as well as dry weight basis. The energy value of 100 g of the edible portion of leek is also higher than that of onion (van der Meer and Hanelt, 1990). The composition of alliums was reviewed by Fenwick and Hanley (1990). The major storage tissues of leek are the leaf sheaths, which are normally 1–2% lower in dry matter (DM) than those of bulb onion; about 11% DM. DM constituents are 70–85% storage carbohydrates (mostly fructans), 10–20% proteins and about 1% lipids and ash. The flavour compounds in alliums are sulphur-containing non-protein amino acids, with a common general structure of cysteine sulphoxide, but with differences in their chemical R groups between the major allium crops. Besides methyl, leek contains mainly propyl as the R group.

Table 22.1 Chemical composition of leek (per 100 g fresh weight)

Constituent	Content
Water (g)	90
Protein (g)	2
Fat (g)	0.3
Carbohydrates (g)	5.0
Minerals (g)	1.5
Sodium (mg)	5
Potassium (mg)	250
Calcium (mg)	60
Iron (mg)	1
Phosphorous (mg)	30
Vitamins	
β-carotene (μg)	600
Thiamine (B_1)	120
Nicotinic acid	500
Pyridoxine (B_6)	250
Ascorbic acid (vitamin C)	25

Source: van der Meer and Hanelt, 1990.

Effect of nutrition on chemical composition

Compost is widely used to increase soil fertility, usually practised by incorporating the compost into the upper soil layer. This study questions the rationale behind this practice. Compost was applied as a mulch and compared with compost worked into the soil in a growth experiment with leek ('Siegfried Frost'). Each of the eight combinations of variables (application method, compost type, and soil type) was repeated three times with 20 leeks in each replicate. Significantly higher yields were obtained with compost applied as a mulch. Here, the yield averaged 78 g fresh weight per leek, compared to 59 g per leek from plots with compost incorporated. Compost mulching also resulted in significantly higher-quality leeks, including more first-class leeks, longer and thicker shafts, and a generally better appearance. The advantage of placing the compost on the soil surface rather than thoroughly mixing it with the soil can be attributed to a higher availability of plant nutrients (Reeh and Jensen 2002).

Staugaitis and Viskelis (2001) investigated the effects of N rates (0, 60, 120, 180, 240 and 340 kg/ha) on the yield, quality and storability of leek cultivars Rival and Pandora in 1996–99 at the Lithuanian Institute of Horticulture. N fertilizers increased the above ground mass of leek and marketable yield. N at 300 kg/ha increased the biomass by 2.2 times and marketable yield by 1.8 times, and yield was over 40 t/ha in all years. Leek yield increased with increasing N rate, and the yield in the control increased only by 1–2 t/ha. N increased the contents of N, K and nitrates, and reduced the contents of sugars, vitamin C (ascorbic acid), dry soluble compounds and dry matter. The best storability was obtained with N at 180 kg/ha. N content at 180 kg N/ha was 158 kg/ha, while that at 300 kg N/ha was 193 kg/ha. Analysis of N balance showed that it is optimum to use 180 kg N/ha. About 30% of accumulated N stays in the crop residue. At 180 and 240 kg N/ha, approximately 50 kg N/ha was left with the plant residues. The effects of N were similar in both cultivars.

Brunsgaard *et al*. (1997) compared the effect of a range of N levels on leek quality in dietary experiments with rats. Protein content increased with N applications, while during the autumn the protein content tended to fall; the total biological food value rose over time from September to November. In a three-year study where leeks received N at 100, 200 or 300 kg/ha, P_2O_5 at 70, 140 or 210 kg/ha and K_2O at 140, 240 or 360 kg/ha applied in various proportions before and after planting, it was found that in years with high rainfall the optimum results were obtained with high N, medium P_2O_5 and low K_2O rates, whereas in years with low rainfall the best results were obtained with the lowest rate of all three nutrients. N and P reduced leek vitamin C content whereas K increased it. The leek sugar content rose with rising NPK rates. Increasing the number of top dressings augmented the vitamin C content but reduced that of sugar (Kolota 1973).

Bloem *et al.* (2004) reported that onion (*Allium cepa*) and garlic (*Allium sativum*) were among the earliest cultivated crops and have been popular in folk medicine for centuries. Alliins (cysteine sulfoxides) are the characteristic sulfur (S) containing secondary metabolites of *Allium* species like onions, shallot, garlic, leek and chives and they have taste and sharpness that are criteria for pharmaceutical quality. The influence of the S nutritional status on the content of secondary S containing metabolites was shown for different *Allium* species. It was the aim of this study to investigate the influence of the S and nitrogen (N) supply on the alliin content of onion cv. Stuttgarter Riese and garlic cv. Thermidrome and to evaluate the significance for crop quality. In a greenhouse experiment, three levels of N and S were applied in factorial combinations of 0, 50 and 250 mg S pot^{-1} and 250, 500 and 1000 mg N pot^{-1}. Eight plants were

grown in a Mitscherlich pot containing 8 kg sand. Leaves and bulbs were sampled twice during the growth period to follow up translocation processes. The first sampling was carried out when leaves were developed, but bulb growth had not yet started and the second one during main bulb growth. An increasing S supply was related to an increasing alliin content in leaves and bulbs of both crops, whereas nitrogen fertilization had only a minor influence. The alliin content in bulbs could be doubled by S fertilization. A translocation of alliin from leaves to bulbs was found so that time of harvest has a strong influence on the alliin content. At the beginning of plant development high alliin contents were found in leaves, while with bulb development they were translocated into this plant organ. The results show that the potential health benefits of *Allium* species could be distinctly improved by S fertilization.

Brunsgaard *et al.* (1997) stated that leeks were cultivated under conditions differing in level of N supply (100, 160, 220 or 280 kg/ha), level of water supply (normal or low) and time of harvest (September, October or November). The protein content of the leeks increased progressively from 90 to 163 g/kg DM with N supply. This increase in protein was associated with a reduction of all essential amino acids (g/16g N: lysine 5.60, methionine 1.42 and threonine 3.40) and subsequently, a significant reduction of the biological value. Protein and energy digestibilities increased with level of N supply. Leeks harvested in September (protein 160 g/kg DM, biological value 82.8%) had a higher ($P < 0.05$) protein content, but had at the same time the lowest ($P < 0.05$) biological value as compared to leeks harvested in October (protein 128 g/kg DM, biological value 89.7%) or November (protein 125 g/kg DM, biological value 90.5%). This was due to a lower content of essential amino acids (g/16g N) in leeks harvested in September as compared to leeks of later harvest. Only small differences between the two levels of water supply were observed in the composition of the leeks. The content of non-starch polysaccharides (NSP) was high in all samples of leek (approximately 240–280 g/kg DM) and appeared to be unaffected by the growth conditions applied in the investigation. Soluble NSP constituted approximately half of the total NSP.

Effect of method of growing and age of seedling on chemical composition

Kunicki (1993) reported that in a three-year trial, leek cultivar Argenta transplants 11, 13 or 15 weeks old were planted in a mid-July after an early potato crop at a depth of 6, 12, or 18 cm and spacing of 40×15 cm. The length and time for which the field was used for these two crops amounted to an average of 212 days. The marketable yield of leeks grown as an aftercrop was 17.1–33.6 t/ha. Transplant age had no effect on the crop height or quality. With increasing depth of planting, the pseudostem and its blanched part increased in length, but the DM and vitamin C (ascorbic acid) contents decreased.

Kaniszewski *et al.* (1989) reported that in field experiments conducted from 1985 to 1987, the effects of four growing methods, viz. (i) traditional planting at a depth of 5 cm, (ii) planting as above followed by earthing-up, (iii) planting into 15 cm deep furrows, levelled during the growing season, and (iv) planting into 20 cm deep holes, were investigated using the cultivars Alaska, Darkal, Jolant and Nebraska. Planting into 20 cm deep holes reduced the yield, compared with the other three treatments which gave similar yields. Earthing-up, planting into furrows or into 20 cm deep holes increased the length and weight of the blanched part of the shaft, compared with traditional planting. Laboratory trials showed that blanched shafts contained more DM and total sugars, and less vitamin C, reducing sugars and nitrates than

green shafts. Length and weight of the whole shaft and of its blanched part, as well as the chemical composition, were also affected by the cultivar.

22.3 Cultivation and production

22.3.1 Cultivars

Basically, there are four groups of leeks based on season of maturity: (i) summer leek; (ii) autumn leek; (iii) autumn and winter leek; and (iv) winter leek. Leek cultivars differ significantly in growth habit which affects the final product. They vary from long, green narrow-leaf types with long slender white stems to long wide leaf types with thicker shorter white stems and blue green leaves (Randy Baker, 1991). Leek is a slow-growing monocotyledonous species. Leek cultivars differ from one another mainly in such characteristics as length and diameter of the sheath part, leaf spacing, breadth and colour of the leaf-blades, vigour, ease of bolting, and resistance to cold. Vigorous types are best for summer production; resistance to cold is of special importance where leeks are to be harvested throughout the winter, while slowness to bolt permits a prolonged harvest period in the spring.

Turkish and Bulgarian types have long, thin pseudostems, whereas those from Western Europe have shorter, thicker ones. Leek is mainly grown for the fresh market and varieties of different earliness are demanded. Varieties with good storability are available but cheap imports from Holland during winter dominate the market. Breeding material includes types of different stalk lengths. Medium-stalked types with a large leaf mass give high total yield and are thus desired by the food industry. The fresh market prefers long-stalked types with a small leaf mass. The thickness of the stalk is also important for the economic outcome and thick stalks are often more crispy. Plants with blue-green leaves which are much keeled have better winter hardiness than plants with light-green, flat leaves. Plants should have an upright growth habit and no bulb formation. A higher dry matter content favours the cooking characteristics but can reduce crispness.

Resistance against rust (*Puccinia allii*) is an important breeding objective (Leijon and Olsson, 1999). Leek breeders look for varietal homogeneity, high yield, long shaft, correct leaf colour, no bulb formation, resistance to cold (in winter types) and diseases and suitability for mechanical harvesting. Leek cultivars with dark blue-green foliage have a higher content of chlorophyll and covering of wax than those with pale green foliage; they survive a minimum temperature of –5 °C and the leaves contain more sugars (glucose, fructose and disaccharides) for conversion during the winter. The wax layer protects leaves from attack by various insect vectors of viruses; the cultivar Castelstar (dark green) showed only 21% incidence of leek yellow stripe virus in comparison with 45% in the pale green cultivar Otina (Benoit and Ceustermans, 1990).

In Germany new leek cultivars for harvesting from September to April must be suitable for mechanical harvesting and trimming, and industrial handling. They should produce higher yields of improved quality (length, vigour, form) than existing cultivars, be cold resistant (down to –20 °C) and resistant or tolerant to yellow stripe virus. Of the new cultivars, Kamusch is good for harvesting in early autumn and Kilima slightly later. The most commonly grown late autumn cultivar Elefant is short and compact but is easily damaged during mechanical trimming. The late-winter (harvested March/April) cultivar Poros gives a 30% higher yield than Elefant, but both cultivars suffer badly from yellow stripe virus (Kampe, 1978).

In the Netherlands, Albana, Alba, Alma-902, Jolant, SG 446 and SG 448 were considered better. Albana has the highest yields and crops early. Alba and Jolant have dark-coloured leaves and an upright habit. Alma-902 and SG 446 are not so high yielding but leaf colour and habit are favourable (Aalbersberg, 1985).

Twenty leek cultivars were assessed for possible production in coastal British Columbia. Among the early leeks (harvested in October), Colonna, Longa, Odin and Kilima all yielded over 40 t/ha of trimmed, marketable leeks. Their marketability when harvested in January ranged from 0–16 t/ha. Among the winter-hardy cultivars, marketable yields in October and January were not significantly different but there was a marked increase for the April harvest. Cultivar Goliath, for example, yielded 23 t/ha in October and January and 33 t/ha in April. Other good winter-hardy cultivars include Siberia, Artico and Derrick (Maurer, 1982).

22.3.2 Climatic requirement

Leek is a relatively long-season crop requiring about 120 days from seeding to harvest. It is generally more cold-tolerant than the onion in its early development, but it can be damaged at harvest by frost (Swiader *et al*., 1994). Most leek cultivars will grow at least reasonably well wherever the crop is produced. The main reason for this great adaptability of the leek is that it neither forms bulbs nor enters a rest period, as does the common onion, but continues growth and can be harvested over a long period of time. The leek is also more adaptable because it has greater cold-resistance than the onion. Like onion, however, the leek is induced to bolt by low winter temperatures; as bolting plants are not desirable for market, prolonged periods of low temperature markedly affect planting dates and production periods. According to Decoteau (2000) leeks will grow in any region that can produce onions and tend to be more frost and freeze tolerant than onions. The tendency of leeks to bulb is an undesirable characteristic that appears to be temperature controlled (with bulbing occurring between 18–20 °C).

22.3.3 Soils

Leek is grown on practically all soil types, the most important requirement being a loose texture. On peat soil, yields are usually high but quality is poor. Sandy clay soils are most suitable for leek cultivation. Harvesting is difficult in heavy soils in autumn and winter. Deep ploughing is a prerequisite for the development of long white shafts. In the Netherlands it is found that phreatic water levels of 40 cm or less below the soil surface result in poor yields; optimal levels are 80 to 90 cm. Randy Baker (1991) reported that leeks may be grown successfully on a wide range of soil types but deep top soil is preferred for vigorous plant growth and above average yields. Soil pH 6.5–7.0 is most desirable. Coarse sands should be avoided because sand particles under the leaf sheaths are not palatable to the consumer. The soil should be prepared with green manure ploughdown or farmyard manure to enhance organic content and provide nutrients and extra moisture-holding ability for the crop.

22.3.4 Season of sowing/planting

The objective in leek culture is the production of shoots of marketable size before the leek plants bolt. In temperate Europe, premature bolting may be a problem of very early plantings and normal bolting occurs in late-spring-harvested crops. In most

countries, leek plants are transplanted after a nursery period of about 12 weeks. This method permits the rejection of weak-growing plants. However, in the UK direct drilling is more frequent.

Leeks are always started from seed and need a fairly long growing season to reach a marketable size. In cool climates, leeks are usually planted as early in the spring as possible, and are frequently transplanted from hotbeds or cold frames into the open as soon as the soil becomes warm. Such early plantings are often ready by late summer; successively later plantings may be harvested into the autumn, winter and early spring. These later plantings are direct-seeded or grown from transplants. When the false stems are to be blanched, the leeks are transplanted into trenches, where the soil can be gradually banked against them. Leeks are sufficiently cold-resistant that even in cool climates harvesting can continue throughout the winter, when many other greens are off the market. Unlike onions or garlic, leeks have no definite maturity dates and, as markets usually accept a wide range of sheath sizes, single plantings may be harvested over a considerable period (Wurr *et al*., 1999; Jones and Mann, 1963).

22.3.5 Seedling raising

Seed germination depends upon temperature, with 18–22 °C being the optimum. In India, seeds are sown during August to October in the nursery beds, and seedlings are ready to plant when they attain a height of 15 cm. About 5–7 kg of seeds are sufficient to raise seedlings for planting one hectare of land. A method of magnetic separation of leek seeds of low germination from commercial seed lots was described by Krishnan and Barlage (1986).

22.3.6 Seed quality and priming

Adequate seed cleaning and grading and following this, the selection of large and uniform seedlings at transplanting for improved crop uniformity in leek is very important. A further improvement in germination performance and field uniformity can be achieved by seed priming, in which controlled hydration of seeds permits pregermination metabolic events to take place without radicle emergence. The process engineering of leek seeds was developed, comprising osmotic priming, washing, fluidized-bed drying (heated air is blown up from underneath through a layer of seeds to promote rapid drying while they are floating in the air) and film coating; this has been proven feasible (Bujalski *et al*., 1991). The superiority of the processed seeds is usually reflected in improved germination, rapid and uniform emergence in the field and improved early plant growth compared with untreated seed.

22.3.7 Plant density

The optimum plant density for leeks depends on the size grade required at harvest, the date of planting or sowing, which influences the potential yield and the intended harvest date. Mean width and length increase as the crop grows, and increase as plant density decreases. For leeks of 20 mm minimum diameter and 150 mm minimum length, a planting density of about 30 plants m^{-2} is optimal for early production. However, to produce large leeks, densities of 20–25 plants m^{-2} are used. Leeks grown at a high plant density appear more elongated than those grown at low density, i.e.,

the pseudostems have a higher length to breadth ratio. Also the degree of blanching increases with density, especially for plants from the centre of beds (Brewster, 1994).

22.3.8 Planting

Leek seeds are generally sown directly into fields at rates of 10–15 seeds per 30 cm row. Emerging seedlings are thinned to 10 cm apart. Transplanting of leeks is also done and is often necessary for obtaining mid-summer through to autumn harvests. Leeks are also sown directly or transplanted into trenches 15 cm wide and 15 cm deep. As the leek plant grows, the trench is filled in. This results in the formation of long white stems, a desirable characteristic for marketing leeks. The deeper the leeks are trenched or hilled, the longer the tender white portion of the leaf stem becomes (Decoteau, 2000).

The crop can be established more cheaply than transplanting by direct sowing into beds in the spring. The viability and vigour of leek seed is highly variable and high-quality seed is important for direct sowing. Besides being cheaper, direct sowing tends to result in crops with less dirt in the leaf axils and with fewer bent pseudostems, but the length of blanched sheath tends to be shorter than transplanted crops, and direct-sown crops are more prone to bulbiness. In Bulgaria, leeks are grown from transplants, sown mid-March and mid-April, and also by direct seeding at 8 kg seed/ha without thinning. Direct seeding results in a higher total yield and is considerably cheaper. For both methods the earlier sowing date produced considerably higher yields and larger plants (Milanov, 1972).

22.3.9 Manuring and fertilization

Because the leek is larger than the onion, its requirements for manure and fertilizer are higher. A crop of 30 t/ha removes 100 kg of nitrogen, 60 kg of P_2O_5, and 130 kg of potash from the soil. The diameter and length of bulbs are increased by nitrogen fertilization (McCollum, 1976). Kaniszewski (1986) reported the highest yield of leeks with a preplanting application of 200 kg of N/ha under irrigated and non-irrigated conditions in dry years. In wet years, split application of 600 kg of N recorded maximum yields. Randy Baker (1991) stated that leeks require about 200–250 kg N (nitrogen) per hectare, preferably in three instalments – one-third pre-plant incorporated, one-third as a side dressing, and one-third as a top dressing when the leaves are dry. Phosphate requirements of leeks are not very substantial and applications of 50–100 kg P_2O_5 per hectare are adequate. Potash requirements are also low and 150–200 kg K_2O per hectare as sulfate of potash are adequate.

22.3.10 Irrigation and mulching

Uninterrupted growth is required for quality leeks and irrigation is often necessary in areas where moisture stress occurs. Randy Baker (1991) reported that, depending on weather conditions, a post-planting irrigation is desirable to ensure rapid establishment. Further irrigation will be necessary if rainfall is deficient during the hot summer days when rapid growth should take place. In Belgium two-year trials with leek cultivars Proka and Catalina, sown in December and harvested in July, grown either in the open or under polyethylene or PVC tunnels 4 m wide and 1.7 m high or 8 m wide and

3.5 m high, the best results were obtained with 4 × 1.7 m polyethylene tunnels (Benoit and Ceustermans 1978).

Trials with leeks (cv. Santina) showed that a white polyethylene soil mulch resulted in higher yields than a black one, but did not result in any improvement compared with bare ground. Bolting in spring curtails the period of marketability of over-wintered leek crops. There is then a gap of about three months before spring planted crops reach marketable size. Early harvests can be advanced by using transparent crop covers. Trials have shown that mulches of polyethylene film with 500 × 1 cm diameter perforations/m^2 or with non-woven polypropylene fabrics, can advance harvests. The films are laid over the crop at transplanting, which is usually in late March or early April, following a January sowing in glasshouses. As the crop grows, these light films are raised by the foliage and 'float' on top of the canopy of leaves. Rainfall penetrates the perforations. Mean temperatures are raised by 1–2 °C under these mulches resulting in faster growth. The mulches are removed about seven weeks after transplanting. In European conditions marketable yields of 14 t/ha were achieved by late June, and 31–40 t/ha by late July, with an advancement of 7–9 days over the unprotected crops.

22.3.11 Blanching

Blanching is done by covering the plants to a certain height with soil to improve the quality of the crop. For this purpose, plants are sunk up to their centre leaves in trenches or pits that are heavily manured to earth up soil as they grow. Care should be taken not to earth up soil early when the plants are young.

22.3.12 Weed control

There are no registered chemicals for weed control. Alternatives that can be useful are stale-seedbed technique pre-planting, selecting fields with a low weed population (crop rotation), and using row spacing that can be easily cultivated. If the size of the crop warrants, special row crop tillage equipment is a good acquisition (Randy Baker, 1991). In two-year experiments carried out in Bulgaria, the best results in leek seedling production were obtained with Ramrod at 7 kg/ha, applied pre-emergence, and Afalon at 1 kg/ha sprayed post-emergence at the 2–3 leaf stage. Afalon alone was sufficient to control annual dicotyledonous weeds. Prometryne at 3 kg/ha applied post-transplanting, destroyed both dicotyledonous and monocotyledonous weeds. Satisfactory results were obtained with Afalon at 2 kg/ha plus Butisan at 10 l/ha, the former being effective mainly against the dicotyledons and monocotyledons. All these herbicides were well tolerated by the leeks (Velev and Ivanov, 1973).

22.3.13 Intercropping

Baumann *et al.* (2000) reported that intercropping of leek and celery in a row-by-row replacement design considerably shortened the critical period for weed control in the intercrop, compared with the leek pure stand. The relative soil cover of weeds that emerged at the end of the critical period was reduced by 41% in the intercrop. In another experiment, the biomass of *Senecio vulgaris*, which was planted 20 days after crop establishment, was reduced by 58% in the intercrop and the number of seedlings which emerged as offspring was reduced by 98%, all reductions compared with the pure stand of leek. The relative yield total of the intercrop exceeded that of

the pure stand by 10%, probably as a result of an optimized exploitation of the resources. The quality of the leek, however, was reduced.

Leek rust (*Puccinia allii*) is now also difficult to control. Experiments in which no insecticides or fungicides were applied were carried out to assess the effects on thrips populations and infection by leek rust when leek crops were undersown with subterranean clover (*Trifolium subterraneum*). To evaluate the economic aspects of this approach, both the quality and quantity of the leeks produced in the two systems were compared. Undersowing leeks with clover drastically reduced thrips infestations, which was reflected in improved quality of leeks at harvest. Leek rust incidence was also reduced slightly by undersowing with clover and the quality of the leeks at harvest was also better. Although the quality of the leeks was improved when the crop was undersown with clover, the quantity of crop produced was reduced considerably as a result of plant competition.

Legutowska and Tomczyk (1999) studied differences in the development of thrips on leek monocropped and leek intercropped with white clover. Intercropping reduced the number of thrips. Chemical analysis of leek sampled from both crops were conducted to estimate the contents of some nutritional substances (carbohydrates, vitamin C (ascorbic acid) and nitrogen), and content of dry matter was also estimated. The chemical analyses were performed separately for the white part of the leek and for the green part (leaves). Leek intercropped with clover was richer in vitamin C than monocropped leek. Analysis of monocropped leek indicated rather higher reducing sugars and more soluble sugars. Analysis of initial colonization by *Thrips tabaci* adults in leek interplanted with clover indicated that colonization rates in the intercropped leek plants were lower in comparison with the leek monocrop. Seventy per cent of the newly established thrips adults were found on the monocrop leek plants. After cutting the clover around leek plants, the thrips suppression persisted. This supports the conclusion that attractiveness or nutritional quality of the leek plant for *Thrips tabaci* is reduced as a direct result of the interaction of leek with clover (Belder Eden and Elderson, 1998).

22.3.14 Protected cultivation

The large number of leek cultivars now available make it possible to sow the crop in Western Europe from December to June. Harvesting takes place from June to May in the next year. The cultivation of leek in Europe is divided, according to the time of harvest, into three main periods, i.e., summer, autumn and winter. The ‘winter’ leek can be harvested until early April and kept in cold stores for a few weeks. Profitable yields of early leeks grown under protected cultivation (June/July) were increased by double feeding with Nitrophoska Permanent (15:5:20:2) combined with irrigation and temperatures of 18–20 °C compared with 12–14 °C or 14–16 °C. Earlier crops, by 2–3 weeks, could be obtained by forcing under perforated plastic (500–700 holes/m^2) removed at the end of May before earthing up (Will, 1979).

22.3.15 Diseases

White-tip

White-tip disease, the most important leek disease in Europe during the winter, is caused by *Phytophthora porri* Foister. Infected leaves show papery-white local lesions, sometimes surrounded by dark-green watersoaked zones. Sporangia can develop in

wet lesions and may release 10–30 zoospores, while zoospores are formed when the leaves dry up and may survive for a long period. Harvest losses may be severe; in some cases, total crop loss is reported. De clereq and Bockstaele (2002) reported that a two-step chemical control method can be used. During September and October maneb is used preventively; when the first symptoms are visible, more systemic products such as benalaxyl or metalaxyl are used curatively.

White rot (Sclerotium caplvorum)
Randy Baker (1991) reported that this soil-borne fungal disease can be devastating if present in farm soils. The fungus survives as sclerotia in the soil for long periods. Leeks should be grown on land that has not grown an onion family crop recently. Sanitation through cleaning of field equipment and disposing of cull leeks away from production areas is important in preventing the spread of this disease. The first signs are yellowing and dying back of the leaves beginning at the tips and progressing downwards. Young plants wilt and collapse and are easily dislodged from the soil, revealing a dense white mass of mycelium in which minute black sclerotia are embedded. Cool, wet growing seasons favour the development of white rot.

Leek rust
Leek rust (*Puccinia porri* G. Wint., syn. *P. allii* F. Rudolphi) causes severe damage on European leeks. As the crop is now cultivated all year round, the uredo stage of leek rust is present throughout the year. During winter, low temperatures inhibit the formation of uredosori (the bodies that produce urdeospores, one of a possible five types of rust spores). As soon as the temperature increases in spring, the epidemic of leek rust starts again. The disease develops most frequently under conditions of high humidity and low rainfall, while immersion of the spores in water reduces their viability. The highest infection efficiency occurs at 100% relative humidity (RH) at 10–15 °C and temperatures above 24 °C and below 10 °C inhibit infection. The economic threshold for leek rust is low, as all leaves are prone to damage and leaf removal is not practical. A regular spray schedule with protectant fungicides (e.g. maneb or zineb) should give adequate protection (Schwartz and Mohan, 1995). Spraying fenpropimorph, either alone or in mixtures with maneb, provides a good control. Compounds of the triazole group – tebuconazole and epoxiconazole – are also effective; treatments with propiconazole resulted in outstanding control.

22.3.16 Insect Pests

Leek moth
Kristen Callow (2003) reported that the leek moth (or onion leaf miner), *Acrolepiopsis assectella* Zeller (Lepidoptera: Acrolepidae), a pest of *Allium* native to Europe, was first positively identified in Eastern Ontario in 1993. The distribution of the pest includes Asia, Africa, Europe and Canada. The leek moth is considered a serious pest in some parts of Europe, with levels of infestation up to 40% in areas where the insect has several generations per year. Where generations are limited to 1–2 per year, the pest is sporadic and causes little economic damage. Surveys conducted in 2001 by the Canadian Food Inspection Agency (CFIA) indicated that the insect is present and established in a localized area in Eastern Ontario and Western Quebec. Leek is the preferred host of the pest, though other *Allium* crops can be attacked. The larvae will

tunnel mines in the leaf tissue, sometimes causing distortion, and are reported to occasionally attack the bulb and stems. Damage to the leaves of leek can make them unmarketable. Symptoms include mining and perforations.

On leek, larvae prefer to feed on the youngest leaves, but can consume leaves more than two months old. They bore through the folded leaves towards the centre of the plant, causing a series of pinholes on the inner leaves. Larval mines in the central leaves become longitudinal grooves in the mature plant. It is reported that pyrethroids and Bt products are effective tools for the management of infestations. Insecticides are rarely required in the United Kingdom. Cultural controls including crop rotation, delayed planting, removal of old and infested leaves, destroying any obvious pupae or larvae, early harvesting (to avoid damage by last generation larvae and population build-up), positioning susceptible crops away from infested areas and destruction of plant debris following harvest may be effective in reducing populations below damaging levels. German literature suggests covering leeks with netting prior to female activity and cutting off all outer leaves before the winter leaves appear in late season may reduce damage to leek. In Europe, a number of predators, parasites and pathogens are known to attack the larvae and pupae of the leek moth.

Thrips

The major pest of leeks is the thrips (*Thrips tabaci* Lindeman). These 2 mm long insects hide between the inner leaf blades, where they feed on cell fluids. The green leaves lose their colour as the empty surface cells form thousands of highly visible grey spots. The economic damage due to quality loss is serious. Thrips damage is most severe when plants are water-stressed in hot, dry weather. In these conditions leaf expansion is slow and the increase in thrips number is fast. At 30 °C, it takes only 11 days for the insect to develop from egg to adult (Edelson and Magaro, 1988). Given the low damage threshold for thrips, much research has been done to control this pest efficiently. The better chemical products are carbamates, including methiocarb and furathiocarb. Some phosphorous compounds, such as acephate and malathion, and also pyrethrins, may have protective effects. Novel pesticides for use against thrips are being tested.

In the Netherlands, seed-coatings with fipronil were effective in protecting seedlings, with no apparent phytotoxicity (Ester *et al*., 1997). Another approach suitable for 'organic' production is intercropping with legumes or other plants to discourage thrips from feeding in large numbers on leeks. Belder and Elderson (1998) studied the feasibility of intercropping in pot and field experiments in the Netherlands. Intercropping with clover led to reduced thrips populations on leeks, even when the legume was trimmed. Theunissen and Schelling (1998) reported that intercropping leeks with clover (*Trifolium fragiferum*), either throughout the field or in between rows, suppressed both larval and adult thrips populations.

22.3.17 Harvesting and yield potential

When harvested by hand the plants are usually lifted mechanically, then the roots are cut, the outer damaged and senescent leaves are removed, the remaining leaves shortened, and the plants are packed into boxes. When mechanical harvesting is used, hand cleaning and machine washing are necessary. The crop is ready to harvest once the blanched basal portion of the leaves is at least 1.25 cm in diameter. However, because the plant does not form a bulb, there is no rush to harvest, and growers often

wait until the plants reach 5 cm in diameter to pick the crop. Similar to onions and garlic, growers may undercut the plants to facilitate harvest. After the plants are removed from the soil, the roots are cut off, along with all but 5 cm of the green leaf blade, leaving mostly the white sheath of overlapped leaves. The plants can also be left in the ground over the winter but should be harvested in the spring before growth resumes.

Leek yield potential is dependent on plant population. Row spacings of 60 cm and plant spacings of 10 cm will give a stand in excess of 160,000 plants per hectare. If we consider a harvestable crop at 80% of original stand, approximately 3600 cartons containing 12 bunches of leeks will be marketed. Similarly, a row spacing of 91 cm and plant spacing of 15 cm will give a stand in excess of 70,000 plants with harvestable crop yield of approximately 1600 cartons containing 12 bunches (Randy Baker, 1991).

22.4 Uses in food industry/processing

The leaves and long white blanched stems are cooked. The sharp flavour of leeks often disappears upon boiling, leaving behind a very mild, pleasant-tasting product. They can also be cut into thin slices and be added to salads, which gives a mild onion flavour with a delightful sweetness (Anon., 2005c). The thick leaf bases and slightly developed bulb are eaten as a cooked vegetable or raw with or without attached leaves. The green leaves are edible and have a pungent odour and acrid taste. They are used more for flavouring in salads and cooked dishes. A favourite dish for many gardeners is leek soup (Stephens, 1994). They are used primarily for flavouring soups and stews in place of onions. Because of their symbolism in Wales they have come to be used extensively in that country's cuisines (Anon., 2005b).

22.5 Functional properties

22.5.1 Nutritional value

The leek does not offer a great deal of nutrient value besides bulk and a pleasant taste. The overall vitamin and mineral content corresponds roughly to that of onion. Nutrition facts for leeks are furnished in Table 22.2 (Anon., 2005b). Table 22.3 shows the nutrients for which leek is either an excellent, very good or good source. Next to the nutrient name the following information is furnished: the amount of the nutrient that is included in the noted serving of this food; the % daily value (DV) that amount represents (this DV is calculated for a 25–50-year-old healthy woman); the nutrient density rating; and the food's World's Healthiest Foods Rating. Underneath the chart is a table that summarizes how the ratings are devised (Anon., 2005a).

22.5.2 Health benefits

Leeks, like garlic and onions, belong to a vegetable family called the *Allium* vegetables. Since leek is related to garlic and onions, it contains many of the same beneficial compounds found in these well-researched, health-promoting vegetables (Anon., 2005a).

Table 22.2 Nutritional facts for leek (serving size 1 leek, i.e., 124 g)

Amount for serving		
Calories 38		Calories from Fat 2
		% Daily value *
Total fat 0 g		0%
Saturated fat 0 g		0%
Cholesterol 0 mg		0%
Sodium 12 mg		1%
Total carbohydrates 9 g		3%
Dietary fibre 1 g		5%
Sugars		
Protein 1 g		
Vitamin A	1% Vitamin C	9%
Calcium	4% Iron	8%

* Per cent daily values are based on a 2000 calorie diet. Our daily values may be higher or lower depending on our calorie needs.
Source: Anon., 2005b.

Table 22.3 Nutrients in leeks, boiled (0.5 cup serving; 16.12 calories)

Nutrient	Amount	Daily value (DV) (%)	Nutrient density	World's healthiest foods rating
Manganese	0.13 mg	6.5	7.3	Very good
Vitamin C	2.18 mg	3.6	4.1	Good
Iron	0.57 mg	3.2	3.5	Good
Folate	12.64 meg	3.2	3.5	Good
Vitamin B6 (pyridoxine)	0.06 mg	3.0	3.3	Good

World's healthiest food rating rule	
Excellent	DV> = 75% OR Density> = 7.6 AND DV> = 10%
Very good	DV> = 50% OR Density> = 3.4 AND DV> = 5%
Good	DV> = 25% OR Density> = 1.5 AND DV> =2.5 %

Source: Anon., 2005a.

Lower LDL cholesterol while raising HDL cholesterol

A high intake of *Allium* vegetables has been shown to reduce total cholesterol and low-density lipoprotein (LDL) or 'bad' cholesterol levels, while at the same time raising high-density lipoprotein (HDL) or 'good' cholesterol levels. This can be very important for preventing the development or progression of the blood vessel plaques that occur in atherosclerosis and diabetic heart disease. If these plaques grow too large or rupture, the result can be a heart attack or stroke. *Allium* vegetables have also been shown to lower high blood pressure, another risk factor for heart attack and stroke (Anon., 2005a).

Protection from cancer

Regular consumption of *Allium* vegetables, as little as two or more times a week, is associated with a reduced risk of prostate and colon cancer. The research focused on colon cancer suggests that several of the compounds found in these foods are able to protect colon cells from cancer-causing toxins, while also stopping the growth and spread of any cancer cells that do happen to develop. Although leeks contain many of

the same compounds as those active in fresh garlic and onions, they contain them in smaller amounts. For this reason, larger amounts of leeks may need to be eaten to obtain the benefits provided by its *Allium* family cousins. Fortunately, the mild, sweet taste of leeks makes this easy (Anon., 2005a).

Stabilize blood sugar levels
In addition to their unique properties as *Allium* family vegetables, leeks also emerged from a food ranking system as a very good source of manganese and a good source of vitamin B6, vitamin C, folate, and iron. This particular combination of nutrients would make leeks particularly helpful in stabilizing blood sugar, since they not only slow the absorption of sugars from the intestinal tract, but help ensure that they are properly metabolized in the body (Anon., 2005a).

22.6 Quality issues

Leeks of good quality have fresh green tops and well-blanched stems or shanks. In order to attain 15–20 cm or more of white shank, a common practice is to plant the young transplants in a shallow trench 10–15 cm deep and as the plants grow the rows are cultivated and gradually hilled to promote more white stalk development. The greater the length of white shank, usually the more premium is the product. Wilting and yellowing of the top will downgrade the quality. Bruised tops are unimportant if they can be trimmed without spoiling the appearance. Crooked stems and bulbous bases are not desirable characteristics and should be avoided in order to maintain a premium pack (Randy Baker, 1991).

Leeks must be grown to a certain size before they are marketable. The criteria of marketability vary from outlet to outlet, and various specifications for marketable size have been used in scientific studies on the crop. Currently, leek of pseudostem diameter greater than 20 mm and length greater than 150 mm, including a 50 mm 'flag' of green leaf at the top, meet UK supermarket specifications. Such leeks should have an average fresh weight of about 160 g. In some past studies, all leeks of diameter greater than 12.5 mm have been classed as marketable, and in some more traditional markets large leeks, greater than 40 mm diameter, are required. In fact, the leek is a variable crop and some grading into different sizes is essential to satisfy the requirements for uniformity demanded by most outlets.

22.6.1 Post-harvest handling

Following lifting, the outer leaves are removed, the remaining leaves are shortened and the plants are washed or brushed, graded for length and diameter and packed into boxes. Leeks are sometimes sold loose and sometimes pre-packed in trays with plastic covers or in plastic bags. The requirements for pre-packing leeks include uniform lengths of the white portion of the pseudostem. New products, such as 'baby leeks', are also appearing in European markets. In Europe about 90% of the leek crop is sold on the fresh market and 10% is processed by the industry. Some processed leeks are used for freezing, some are freeze-dried and some are used to prepare ready-cooked dishes.

Decoteau (2000) reported that harvested leeks are cooled by hydrocooling, icing, or vacuum cooling to preserve freshness. If vacuum cooling is used, the leeks are often wrapped in ventilated polyethylene to prevent desiccation. Leeks held at near 6 °C and about 90% relative humidity can be stored for two to three months.

22.6.2 Marketing

Markets usually accept a wide range of stalk sizes. The standard method of packaging leeks is three uniform sized stalks per bunch and twelve bunches per box. The grower usually selects bunches to give a uniform grade standard in a box. Physical size of leek is not important but bigger stalks command better prices than smaller stalks. Wholesalers prefer bunches that are uniform within the bunch and uniform throughout the box (Randy Baker, 1991).

22.6.3 Selection and storage

Leeks are available throughout the year although they are in greater supply from the autumn through to the early part of spring. Fresh leeks should be stored unwashed and untrimmed in the refrigerator, where they will keep fresh for between one and two weeks. Wrapping them loosely in a plastic bag will help them to retain moisture. Cooked leeks are highly perishable, and even when kept in the refrigerator, will stay fresh for only about two days. Leeks may be frozen after being blanched for two to three minutes, although they will lose some of their desirable taste and texture qualities. Leeks will keep in the freezer for about three months (Anon., 2005a). Goffings and Herregods (1989) reported that freshly harvested, unwashed leeks stored at 0 °C and 94–95% relative humidity in atmosphere containing 2% oxygen, 2% carbon dioxide, and 96% N_2 had improved quality.

Grazegorzewska and Bakowski (1996) reported that storage of a total of 22 leek cultivars in different types of crate was studied in several experiments between 1978 and 1993 in Poland. The leeks were stored in universal (U-type), half-size universal or specially designed leek crates at 0 or –1.5 °C. The specially designed crates were 600 × 400 × 435 mm in size and held 5 kg of leeks stored vertically. The quality of leeks was similar following storage in the universal and specially designed crates. Storage was better at –1.5 °C than at 0 °C. Goffings and Herregods (1989) reported that freshly harvested unwashed leeks (cv. Castlestar) were stored at 0 °C and 94–96% RH in atmosphere containing 2% O_2, 2% CO_2 and 0 or 5% CO (the remainder being N_2) or in normal air, or at –1 °C in an atmosphere containing 2% O_2, and 2% CO_2 and stem and leaf colour, stem firmness and mould development were monitored after eight weeks of storage when leeks were restored to a temperature of 7 °C for two weeks. Storage in a modified atmosphere (2% O_2 + 2% CO_2) improved leek quality compared with storage in unmodified air. Addition of 5% CO further reduced the incidence of storage moulds. Leeks maintained in modified atmosphere storage for up to eight weeks had a shelf-life of up to two weeks. Storage at –1 °C further improved leek preservation but slow defrosting before handling was also necessary.

22.7 Shallot

22.7.1 Description, botany, origin and distribution

Description

Shallots (*Allium ascalonicum* L.; Synon. *A. cepa* L. var *ascalonicum* Backer) are a perennial crop that is grown as an annual for its cluster of small bulbs or cloves. They have a delicate onion-like flavor and may be grown for their dry bulbs or used in the same manner as green onions (Swiader *et al*., 1994). Botanically speaking, they are

a form of bulbing multiplying onion, differentiated by their smaller size. Originally, they were named for a plant found by the Crusaders, but they bear no botanical relationship to that plant. Most shallots in the market today are not even the same shallot so beloved by the French. Instead, they are varieties developed by crossing common onions with Welsh onions or other multipliers – a primary aim of the plant breeders was to create varieties that could be readily reproduced.

Some authorities differentiate shallots from other multipliers by the colour of their skins. The Ontario Ministry of Agriculture and Food, for example, identifies shallots as those with red skins (or scales) and true multipliers as those with yellow or brown skins. Most shallots do not flower or produce seed, although breeders have developed some new varieties that can be grown from seed. In practical terms, shallots are small, layered multipliers with a special taste that falls somewhere between onion and garlic. They are propagated in the ground the same way as other bulbing multipliers, and each bulb produces from four to twelve baby bulbs in a bunch, joined at the base by a membrane. In most varieties, each bulb is split into two large cloves that may or may not share a common wrapper. Shallots are favoured by chefs for their distinctive flavour, described as a blend of onion and garlic (Brook Eliot, 2003).

Botany

The term shallot refers to the vegetatively propagated forms of *Allium cepa* var. *ascalonicum*, which were included in *aggregatum* group of the species. Shallots of this type appear to have been derived by selection from a naturally occurring variant within *Allium cepa*. Several cultivars are actually derived from *A. cepa* × *Allium fistolosum* crosses (e.g. Delta Giant). These should not be confused with the shallots of the *Allium cepa aggregatum* group. Allium cepa shallots are distinguished from natural bulb onion by their habit of multiplying vegetatively by laterals and growth – a single shallot bulb usually contains several initial shoots. The bulb can be planted (Currah and Proctor, 1990), and several leafy shoots will grow out from it. Each shoot then rapidly produces a small bulb, forming a cluster that remains attached to the original base plate (Vadivelu and Muthukrishnan, 1982). The bulbs can be separated and the process repeated in the next growing season.

Morphologically, a shallot bulb (synonyms: set, bulblet, bulbil) is very similar to the bulb of the common onion. A mature bulb consist of a compressed stem axis or basal plate, storage leaf-bases of the outer leaves, which have lost their blades, and bladeless 'true scales'. In the centre of each bulb there are a few leaf buds that under favourable conditions sprout when dormancy ends. Unlike the modern bulb onion, a typical shallot bulb contains a number of laterals in the axils of the inner leaves. All sets formed from a single propagule usually remain attached to the original basal plate, thus forming a cluster of sets (Currah and Proctor, 1990). The foliage and the inflorescence of shallots are usually smaller than those of the bulb onion. However, root morphology, the unifacial, hollow, slightly flattened tubular leaves, the hollow scape, the terminal inflorescence and the flowers are similar to those of the common onion.

Origin and distribution

In the tropics, shallots are often grown in areas where onion culture is difficult because the climate is humid and bulb onion is susceptible to leaf diseases that shallot can withstand. Shallot has a very short growing season of only two to three months, which allows it to be grown between other crops or during a short-day season. In the lowland tropics, lack of a distinct cool period can prevent onion from

flowering; under such conditions, growing of shallot is advantageous. Thompson and Kelly (1957) reported that shallot is believed to have come from Western Asia. It is a perennial and seldom produces seeds, but the bulb when planted divides into a number of cloves, which remain attached at the bottom. It has been in cultivation for a long time. It is mentioned and figured in nearly all old works on botany. It is sometimes grown for the dry bulb but usually for the young plant which is used in the same way as green onions. On a global scale, shallot is a minor Alliaceous crop. However, in South East Asia – for example, Indonesia, Sri Lanka and Thailand – as well as in some African countries, such as Uganda, Ethiopia and Ivory Coast, where onion seed is hard to produce, where onion culture is difficult and also where the growing season is too short for the production of bulb onion, the vegetatively propagated shallot is cultivated as an important substitute for bulb onion (Currah and Proctor, 1990; Grubben, 1994).

Some tropical clones of shallot flower more readily than those from temperate climates (Currah and Proctor, 1990). In many South-East Asian countries and elsewhere, the green shallot inflorescences are harvested just after the scape reaches its final length (with the green spathe still closed), and the edible floral buds are used as salad onions. Additional advantages of tropical and sub-tropical shallots are tolerance to the hot and humid tropical climate, better tolerance to pests and diseases, and longer storage life than standard short-day onions. Many of these genotypes are also preferred to bulb onions by consumers for their good culinary qualities, such as high pungency (Grubben, 1994).

22.7.2 Chemical composition

Shallots may contain more fat and soluble solids, including sugars, than bulb onions (Currah and Proctor, 1990). Standards for quality grades of shallot bulbs were issued by the U.S. Department of Agriculture (USDA) (Anon., 1946). Shallot bulbs are usually smaller and more highly flavoured than those of the single-hearted bulb onion. Shallots contain higher levels of fats and soluble solids, including sugars, than bulb onion (16–33% vs. 7–15% dry weight, respectively) (Currah and Proctor, 1990) which, together with sulphur-containing compounds, make shallot an essential component in gourmet cooking.

The dry matter of shallot consists of 70–85% carbohydrates, mainly fructans, glucose, fructose and sucrose. As in the bulb onion, cell-wall components, such as cellulose and pectins, contribute 10–15% of the carbohydrate fraction. The red shallot contains anthocyanins (glucosides of cyanidin) (Joslym and Peterson, 1958) and the yellow colour is largely of the flavonol Quercetin (Kuroda and Umeda, 1951). Shallots contain water: 79.8, calories: 72, protein: 2.5, fat: 0.1, carbohydrate: 16.8, fibre: 0.7, ash: 0.9 g/100 g fresh weight of root and calcium: 37, phosphorous: 60, Iron: 1.2, sodium: 12, potassium: 334, vitamin A: 0, thiamine: 0.06, riboflavin: 0.02, niacin: 0.2, vitamin C: 8 mg/100 g fresh weight of root (Anon., 2005c).

22.8 Cultivation and production

22.8.1 Cultivars

Improved lines of multiplier onion have been bred in India at Tamil Nadu Agricultural University, Coimbatore, by crossing with bulb onion CO-1 to CO-4 series of cultivars

developed there (Currah and Proctor, 1990). In cultivars where flowering can be induced, a cool period of 40 days at 14 °C is required. Although seeds can be produced from some lines, vegetative multiplication is usually practised. The CO cultivars are pink or red in colour. The multiplier onion splits into several daughter bulbs, ranging in number from 4–8 to 8–10. Both multiplier onion and tropical shallots take only 60–75 days to multiply and die down again, and the bulbs can be stored for considerable periods (over five months). Individual clusters of multiplier onion in the CO series have an average weight range of 25–85 g. Other cultivars reported from Indonesia are Ampenan, Cloja, Bima, Kuning, Bauji, Balijo, Suminep, Bawang Lambung, Betawi Cipanos, and Hajakuning.

22.8.2 Climatic requirement

An average temperature of 25–32 °C is optimum during the growing period of shallots. Jenkins (1954) reported that high temperature favoured bulbing. Plants grown at temperatures of 21 °C and higher all formed bulbs, but larger bulbs were produced with a 15-hour photoperiod than with a 10-hour photoperiod. When the temperature was lower than 21 °C, no bulbs were formed regardless of day length.

22.8.3 Soils

Shallots can be grown in all types of soil with a pH of more than 5.6, however, well-drained alluvial soils are preferred for better growth and development. Kusumo and Muhadjir (1987) grew shallots traditionally after the harvest of the rice crop on raised beds of subsoil obtained from the deep furrows. However, seed origin and soil type had no significant effect on yields.

22.8.4 Propagation

With a few exceptions, shallot is currently propagated vegetatively in most parts of the world. Vegetative propagation has until now been dominant in shallot culture throughout the world (Currah and Proctor, 1990). When grown from sets, the shallot-growing season is relatively short, thus enabling production where onions from seed cannot produce economic yields of commercially acceptable sized bulbs. However, when grown from seed, hybrid shallots with strong heterosis have a fast growth rate and bear high yields after 3–4 months of growth. In more temperate lands, similar practices are used in Europe, the USA and Argentina, where sets are transplanted on raised beds at 25–40 plants m^{-2}. Yields of vegetatively propagated shallots range between 5 and 30 t ha^{-1} in Indonesia (Subijanto, 1988).

In Israel, 100% of the shallot grown commercially is propagated from seed. Krontal *et al*. (1998) were the first to provide a scientific description of seedling development, based on material derived from a nameless Thai landrace, Israeli Genebank accession no. 66–1004. Seeds of tropical shallot are smaller than those of bulb onion, the 1000-seed weights being on average roughly 2–3 g and 3–4 g, respectively. Seeds have no dormancy and readily germinate when moisture is available. The black seed-coat is crinkled and the seed is irregular in shape, like that of onion.

22.8.5 Planting

Three shallot crops are grown a year, the major seasons being April to August,

January to March, and September to December (Currah and Proctor, 1990). A few plantings are made in August, although the bulk of the crop is planted during October with little planting until January (Jenkins, 1954). Sinnadurai (1973) described the growing system for shallots in the coastal area of Ghana. The shallots are planted on raised beds in sandy soils 7 cm apart. About four tons of bulbils are required for planting one hectare. In Indonesia, 900–1000 kg of planting material is needed for one hectare. Plants are spaced 15×15 cm to 20×20 cm according to the cultivar. At planting, the tops are cut if the bulbs are dormant (Currah and Proctor, 1990).

A plant will produce from two to fifteen bulblets per cluster. The crop is propagated by dividing the bulb clusters and planting individual bulblets, or cloves, 5 cm deep, 10 cm apart, in rows 30–60 cm apart. Warm temperatures and long photoperiods favour bulbing.

22.8.6 Manuring and fertilization

The shallot crop is given a basal dressing of fertilizers or mixed fertilizers 10–15 days after planting and is then fertilized at two-week intervals until two weeks before harvest with 200 kg of urea per hectare on each occasion. Muhadjir and Kusumo (1986) planted cultivars Ampenan and Medan with a basal dressing consisting of 100 kg each of N and P_2O_5 and 0, 50, and 100 kg of K_2O per hectare. The highest yields were obtained with 100 kg each of N and P_2O_5, and 50 kg K_2O. The quantity of nitrogen used had no influence on growth, leaf colour, susceptibility to bolting, or number of bulbs, however, increasing nitrogen had an adverse effect on the uniformity of leaf canopy at maturity, and 60 kg of nitrogen is recommended.

22.8.7 Culture

Shallots should be grown in the same way as onions. Plants that are not heavily cut will proceed to form many bulbs attached together forming a clump. Shallot bulbs often develop on top of the ground. Do not cover them with soil (Lane Greer and George Kuepper, 1999).

22.8.8 Diseases and insect pests

The main diseases and pests are: anthracnose (*Colletotrichum gloeosporoides*), basal rot (*Fusarium oxysporum*), downy mildew (*Peronospora destructor*), moulds (*Aspergillus niger, Penicillium corymbiferum, Penicillium cyclopium*), neck rot (*Botrytis allii*), onion blast (*Botrytis squamosa*), pink root (*Pyrenochaeta terrestris*), purple blotch (*Alternaria porri*), smudge (*Colletotrichum circinans*), white rot (*Sclerotium cepivorum*, *Sclerotium rolfsii*), nematodes (*Ditylenchus dipsaci*), thrips (*Thrips tabaci*), beet armyworm (*Spodoptera exigua*) and other *Spodoptera* sp. caterpillars, as well as a number of virus diseases (Currah and Proctor, 1990; Grubben, 1994; Kuruppu, 1999).

Loss of shallot yield from pests and diseases is common all over the world, and chemical treatment is the major means currently used to reduce damage (Anon., 1986; Suhardi, 1996). However, good agricultural practices can be used to partially control losses. Practices that are essential for high-quality long-keeping yields include crop rotation, drip irrigation (which is preferred over sprinkler irrigation to maintain low air humidity), proper spacing to allow free passage of air so as to reduce the

relative humidity of the air, proper harvesting and curing practices, well-ventilated or cold storage and proper sanitation. No information is published on resistance/tolerance to pests in shallots, but some landraces show better field tolerance to some foliage diseases than the bulb onion, and differences in resistance between cultivars are noticeable (Currah and Proctor, 1990).

Shallots are susceptible to a number of air-borne and soil-borne fungi, as well as to insects, nematodes, bacteria and viruses. Tolerance/resistance to purple blotch was reported for red shallot and the bulb onion 'Red Creole' from Ethiopia (Currah and Proctor, 1990). A variety of diseases caused by organisms like *Alternaria porri* and *Colletotrichum* species can be controlled with sprays of maneb. To control *Spodoptera* species as well as thrips, twice-weekly sprays of monocrotophos or other insecticides are used (Warade and Shinde, 1998).

22.8.9 Harvest and market preparation

Lane Greer and George Kuepper (1999) reported that autumn-planted shallots mature in nine months, but the clusters will be smaller. Expect to harvest 5–7 kg of shallots for each kg planted. This is roughly equivalent to 8–12 shallots for every shallot set planted. Shallots are ready for harvest when the leaves begin to fall over and bulb size is over 2.5 cm in diameter. Bulb maturity can be accelerated by withholding irrigation water or by undercutting the root system. Bulbs for storage may be harvested when 50% or more of the tops have fallen over, but the bulbs must cure and dry thoroughly before being stored. Bulbs intended for immediate use can be harvested when 15–25% of the tops are down. Thick-necked bulbs should be used immediately, as they do not store well. Shallots will keep for about eight months if stored in a cool, dry place. A single shallot bulb contains several shoot initials that resemble those of doubled onions and each bulblet is covered with one to three protective skins. Dormancy lasts between two and a half and four months (Sinnadurai and Amuti, 1971; Currah and Proctor, 1990).

Harvest takes place when 70–80% of the leaves have turned yellow, i.e., 65–70 days after planting in the lowlands and 80–100 days after planting in highland areas. The shallots are pulled by hand after they have obtained a diameter of at least 0.5–0.6 cm. The outer skin is peeled off and the roots are trimmed, after which they are washed and tied into 1-kg bunches. For dry bulb production, shallots are dried for 5–14 days in the field and covered by plastic if it rains (Warade and Shinde, 1998). According to Thompson and Kelly (1957) and Swiader *et al*. (1994) when shallots are grown for their dry bulbs, the harvest and handling is similar to that used for onions. Shallots grown for green onions are pulled when their tops are 15–20 cm long and after they have obtained a minimum diameter of 0.62 cm.

Barrels containing 20 dozen (240) bunches were the standard containers for many years. Shallots are also packed in 1-bushed and $1^1/_3$ bushed crates which hold five or eight dozen bunches (60 or 96). The bunches must be packed with crushed ice since they heat and spoil rapidly unless iced.

22.9 Uses in food industry/processing

The Indonesian shallot variety Sumenap is said to have a high fat content. In Ethiopia, small local red shallots grown in the highlands are highly valued in the traditional

wat sauce to accompany Injera bread made from wheat flour (Currah and Proctor, 1990). Shallots are also used in certain sauces. Shallots are often considered the gourmet member of the onion family. They have a mild, delicate but distinctive flavour and can either be grown for use as green onions, or for the clusters of small bulbs that are used like garlic or onions (Lane Greer and George Kuepper, 1999).

22.10 Quality issues

22.10.1 Storage

Dry shallot bulbs are sold either fresh or from storage. Shallot clones vary considerably in storage life, with a range of 2–9 months, and storage temperature and genetic traits are the main factors that influence storage life (Currah and Proctor, 1990; Grubben, 1994). In Thailand, high N level in the stored bulbs was found to be associated with short keeping, with premature harvest when carried out before leaf wilting and with poor post-harvest handling (Ruaysoongnern, 1994). Storage diseases, early sprouting and shrivelling seem to be the main limiting factors for long keeping of shallots in tropical and sub-tropical countries. Bulb onions and most shallots store well at low (–0 °C) and high (roughly 25–30 °C) temperatures (Krontal *et al.*, 2000). However, shallots can be stored for long periods, over five months, under ambient conditions in the tropics (Currah and Proctor, 1990). Storage in shade heaps in the field or in open sheds under ambient conditions is common in the tropics, in Israel and in other places.

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23

Lemon balm

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23.1 Introduction

Lemon balm (*Melissa officinalis* L.) belongs to the family Labiatae (Mint family) is an aromatic and fairly hardy perennial sub-shrub. Some vernacular names are balm, common balm, blue balm, dropsy plant, honey plant, Herzkraut, citronelle, cytria, cedronella, badarendjabouya, alahana, mallisa, ogulotu, kovanotu, seiyo-yama-hakka, sweet balm, limouna, limounneta, franjmeshk, toronjil, tronjan, turungan, melisso, melliss, sidrunmeliss, Melissenblatter, Melissenkraut and Melissa (WHO, 2002).

Melissa officinalis L. consists of three subspecies, *subsp. officinalis*, *subsp. inodora* and *subsp. altissim*a (Mill, 1982; Craker and Simon, 1992). Among them, *Melissa officinalis subsp. officinalis* is commonly used and has the characteristic lemony taste and smell. *Melissa* is a Latin derivation of the Greek word for honeybee and *officinalis* is the indication of its medicinal nature. It has been used in Mediterranean region and Europe since the Middle Ages for several purposes such as regulating sleep, appetite and digestion, reducing anxiety and a pain relief. Lemon balm has a documented medicinal history extending back to 50–80 BC (Kennedy *et al*., 2003). *The London Dispensary* (1696) stated, 'An essence of Balm, given in Canary wine, every morning will renew youth, strengthen the brain, relieve languishing nature and prevent baldness'.

In Victorian times lemon balm was used as a symbolic plant for transmitting messages between lovers. It was a symbol of sympathy and used to make soothing medicines. In ancient times, it was also believed to drive away evil from a house when it was grown in front of the door. Today, lemon balm naturally grows in various parts of the world, including the eastern Mediterranean region, western Asia and northern Africa (Simon *et al*., 1984). It was brought to America from Europe by colonists and started to grow in their gardens. Today, it is one of the more widely cultivated medicinal and aromatic plants in much of Europe and northern America.

Morphological features of lemon balm such as plant height, stem and leaf size show a variation depending mainly upon genotype, environment or cultural applications (Sari and Ceylan, 2002). In general, lemon balm can grow up to 1.5 m height and

spread 0.5–1.0 m across. It is characterised by square stems, lemon-scented and scalloped edge leaves, and flowers that mature from white or yellow to pale blue. The green leaves, which give off a fragrant lemon smell when bruised, are about egg or heart shaped and 2–8 cm in length and arranged in opposing pairs on the stems. Upper leaves are usually bigger than lower leaves. Veins in the leaves can be easily seen. The small flowers (0.5–1.5 cm size) are produced all summer long. They grow in loose, small branches from the axils of the leaves on the stems (Fig. 23.1).

Lemon balm is a cross-pollinating species, and has complete perfect flowers with very short-stalked epidermal glands. The flowers consist of five fused sepals, five petals, two or four stamens and four lobed ovaries forming 1–4 nutlets. The seeds are very small about 1–1.5 mm long, ovate, dark brown or black in colour. The weight of 1000 seeds is 0.5–0.7 g. A long storage period causes a reduction in germination vigour. Seeds stored for five years may no longer germinate. Lemon balm has a hairy root system with many lateral roots, which makes the plant more adaptable to different environmental conditions. The upper parts of the plant die off at the start of winter, but new shoots re-emerge from the roots at the beginning of spring.

23.2 Chemical composition

Much work on chemical composition in both essential oil and different parts of lemon balm has been reported. Essential oil rate in drug herb changes between 0.02–0.30%, which is quite low compared to other member of the family Labiatae (Sari and Ceylan, 2002; Saglam *et al*., 2004). That is why the production cost and price of essential oil is very high in the market. The main constituents of the essential oil are citral (geranial and neral), citronellal, linalool, geraniol, β-pinene, α-pinene, β-caryophyllene and β-caryophyllene oxide, comprising about 96% of the oil ingredients. Carnat *et al*. (1998) explored the chemical composition of essential oil of lemon balm, and found that major components are citral (neral + geranial) representing

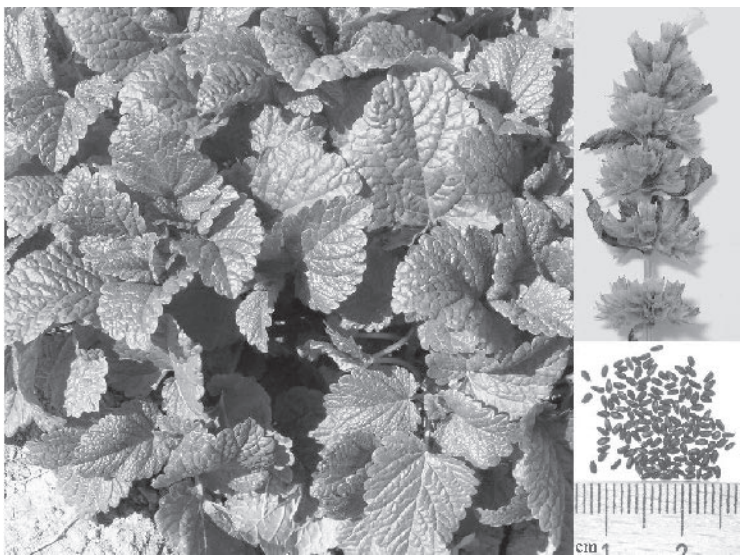

Fig. 23.1 Parts of the lemon balm plant (*Melissa officinalis* L.).

48% of the essential oil, followed by citronellal with 39.47% and Hc β-caryophyllene with 2.37%.

In another investigation, the percentages of the main constitutes found by Sari and Ceylan (2002) are as follows: α-pinene (2.86), β-pinene (11.73%), linalool (2.74%), citronella (5.86%), borneol (0.62%), neral (12.22%) and geraniol (38.13%). In addition, fresh herb of lemon balm contains total phenolics (2253 mg/100 mg), L-Ascorbic acid (53.2 mg/100 mg) and carotenoids (46.3 mg/100 mg) (Capecka *et al*., 2005). Ivanova *et al*. (2005) found that lemon balm extract contains on average 1370.09 μM total phenols and has an antioxidant capacity of 4.06 TEAC (Trolox equivalent antioxidant capacity).

As the essential oils show complex structures, essential oil rate or its chemical composition of lemon balm is strongly affected by several factors such as light intensity, nutrient, temperature, cultural practices, genotype, plant part, age, harvesting time, etc. For example, essential oil rate and tannin contents increase with increasing light intensity from 1000 to 1500 lux (Manukyan, 2004). Similarly, the nutrient applied to lemon balm also has a significant effect on average essential oil rate. Another investigation showed that lemon balm under saline conditions tends to decrease its essential oil ratio whereas it increases under drought conditions (Ozturk *et al*., 2004). On the other hand, essential rate does not change significantly with plant density and plant source used for propagation (Saglam *et al*., 2004). However, both essential oil content and its components very much depend upon the harvest cut height of lemon balm (Mrlianova *et al*., 2002). Average essential oil content in the top third part is 0.39% whereas it is 0.14% in the whole aerial part. Thus, there is an ontogenetic variation for essential oil in balm leaves (Hose *et al*., 1997). Caryophyllene oxide content as a main constituent also changes depending on age and environment (Meyer and Spiteller, 1996). Poor soils without fertilisation increase Caryophyllene oxide content. It is also important to mention that the collection period of the plant material changes product quality criteria such as essential oil content and components.

23.3 Cultivation and production

There has been a growing demand for plant based medicines, health products, pharmaceuticals, food ingredients, cosmetics, etc. Lemon balm is one of those plants and is used in several areas. In some countries such as Turkey, Syria and the Kingdom of Jordan, many medicinal and aromatic plants including lemon balm are collected from the flora. Cultivation of these species should alleviate the pressure on the wild populations and avoid their extinction. Therefore, apart from protection of biodiversity, its cultivation is commercially attractive to companies, as providing standard raw material in terms of quality and supply. Today lemon balm is widely cultivated in Europe and the United States, but also grows wild along paths and roadsides.

The plant prefers sandy and loamy fertile soils, well drained and at pH range 5 to 7. It grows well in full sun, but it also grows well in partial shade. When the plants grow in semi-shade, they produced larger leaves and habitat than those grown in sunny condition. Lemon balm can rapidly grow at temperature range 15 to 35 °C and requires 500–600 mm precipitation well distributed throughout the growing season, otherwise it should be irrigated. It is sensitive especially to drought in the establishment year. Once it develops a deep root system, its water requirement lessens. However, it

should be well irrigated in arid and semi-arid regions for obtaining high green herb yield. The average life of a plant is ten years, but economic life length is about five years.

Lemon balm can be propagated from seeds, stem cuttings and root division. The seeds (8–10 kg/ha) are very small, thus should be covered with a fine layer of soil in the spring or early autumn. Seed germination is slow, taking between two or four weeks. Therefore, probably, obtaining seedlings from seeds is preferable to direct seeding in the field for successful propagation. In addition, the use of seedlings as a propagation method produces a better herb yield compared to root division with a single shoot (Saglam *et al*., 2004). For seedling production, 50–80 g seeds are sown in 12–15 m^2 of a pre-prepared seedbed (Ilisulu, 1992). These produced seedlings will be enough to transplant 0.1 ha area. Transplanting time of seedlings to the field is autumn or spring. However, instead of propagation from seeds or seedling, vegetative propagation such as stem cutting or root division could be an easier and faster method to establish a lemon balm plantation (Davis, 1997). In another method for expanding a lemon balm plantation, a long stem, which is still attached to the parent plant, is buried in moist soil by allowing a few inches of the tip to remain above the surface. In a few weeks, the buried stem develops new roots and the new plant can be separated from its parent.

Although plant density changes depending growing conditions, both 30 × 30 cm and 40 × 20 cm plant densities give satisfactory results (Ceylan *et al*., 1994; Saglam *et al*., 2004). In the establishment year, application of a sufficient amount of phosphorus, potassium and nitrogen is recommended according to soil analysis. For example, Saglam *et al*., (2004) obtained a good result with side dressing application of 80 kg/ha P_2O_2 and 60 kg/ha N in the first year. In consecutive years, additional mineral nitrogen may be applied after cuts. Recently, however, as organic production gains more attention, organic manure or fertilisers may be preferred.

Weed control is one of the important cultural practices in lemon balm, as presence of weeds in the fresh or dried herb will reduce quality. Herbicides for weed control could be applied, but avoiding chemical residues on the plants because vegetative parts of lemon balm can be directly used for medicinal and aromatic purposes (Zuin and Vilegas, 2000). Therefore, organic control methods for weeds, diseases and insects should be preferred if they are available.

Lemon balm as a perennial plant can be harvested twice or three times a season just before blooming. Harvesting after complete flowering causes a reduction in herba quality. Plants are cut at 8–10 cm above ground in the morning after the dew has evaporated. The fresh herba is immediately dried in shade at 20–35 °C after harvest; otherwise the drug herba colour turns to dark brownish. Moreover, bruising the leaves during harvest should be avoided, because it causes the dry herb colour to become also dark brownish and, consequently reduces quality. Harvested and dried herba should be stored in dry places with good ventilation.

The fresh or dry herba yield varies depending upon genotype, growing conditions and cultivation practices. After transplanting seedling to the field, the first year can be considered as an establishment year; therefore high herba yield should not be expected and the yield increases after first year. The second and third years are production years, and between 5000 and 10,000 kg/ha dry herb yield can be obtained in a season (Saglam *et al*., 2004).

Although it is difficult to determine the size of the world market for lemon balm, as specific trade statistics are not available, most commercial production takes place

in Europe and east Mediterranean countries such as Germany, Italy, France, Ireland, England, Greece, Turkey, Bulgaria, Poland, Egypt and Syria. According to the *Essential Oils Market Information Booklet* published by IENICA (2004), the world production of lemon balm oil is estimated at a value <£100,000. Reported prices range from $7.00 to $10.00 per pound for certified organic lemon balm (Sturdivant and Blakley, 1999).

23.4 Main uses

Lemon balm in food processing has a wide range of uses such as tea, herb, flavurant or culinary. It has been used in hot tea blends, as a fresh and dry herb in Europe and Mediterranean countries (Bozan, 1995; Zeybek, 1995). Today, its leaves are also used in iced tea or other cold drinks. Fresh or dried lemon balm leaves can be often used as a food ingredient to green salads, sandwiches, pasta, marinades, sauces, staffings, soups, egg dishes, meat dishes, roast chicken, jams, vinegar, etc. It is reported to be used in many other dishes, even in desserts (cheesecake), biscuits and some alcoholic beverages such as liqueurs and wine (Rogers, 1998). For instance, fish or chicken can be cooked over a bed of lemon balm leaves. Its chopped fresh leaves also go well with plain yoghurt and sprinkle with any kind of fresh berries. If one prefers using fresh herba the leaves can be frozen for later use, but avoid freezing leaves while they are wet. Chopping with a knife causes bruises and discolours the leaves, so tearing leaves into small pieces may be preferred. Moreover, adding essential oil or extract of lemon balm into vegetable oils such as sunflower, rapeseed oil, etc., may contribute to oil quality components. For example, it was found that the ethanol extract of lemon balm improves the oxidation stability of sunflower oil (Marinova and Yanishlieva, 1997) and addition of 1.5% w/w to a salad portion increases the antioxidant capacity 150% (Ninfali *et al*., 2005).

From the earliest of times in the Mediterranean region people have used lemon balm to encourage a new swarm of bees to stay in a new hive by rubbing the inside of the hive with the leaves (Lesley, 1994; Square, 1998). Although the flowers and its smell attract honeybees, it is said that lemon balm has a repellent effect on some insects as it contains citronella oil. Some investigations revealed that lemon balm could also be used in animal feed for several purposes. For example, the herb mixture containing lemon balm is also suggested for use in animal feed instead of fodder antibiotics (Urbanczyk *et al*., 2002). Moreover, it was found that feeding calves with a mixture of nettle, tutsan, lemon balm, camomile, marigold and small plantain enhanced glucose and total protein content and lowered cholesterol content in the blood serum of calves (Bombik *et al*., 2002). Some varieties are also suitable for ornamental use, especially as border plants in gardens. Leaves and stems with flowers can be dried and used in potpourri or as room fresheners. Its essential oil smells pleasant and is used by the perfume or cosmetic industry. Fresh lemon balm shoots and leaves can even be used in natural cosmetics. As result of its therapeutic effect, lemon balm is used in hydrosols, which is considered the homeopathy of aromatherapy (Rose, 2002).

23.5 Functional/health benefits

Lemon balm has a wide range of uses for medicinal, antimicrobial, antioxidant

purposes and as a functional food. A moderate amount of investigation on lemon balm has been carried out to determine its medicinal effects such as antiviral, antibacterial, antifungal, antitumour and sedative effects. For example, Sousa *et al.* (2004) indicates that the essential oil of lemon balm as an antitumoural agent has a potential for cancer treatments or prevention. The volatile oil of Lemon balm may also be used as an anti-virus agent and contains an anti-Herpes simplex virus type 2 (HSV-2) substance (Allahverdiyev *et al.*, 2004).

The antimicrobial properties of plants have been investigated by a number of researchers world wide and the antimicrobial activity tests of lemon balm show that the most powerful scavenging compounds are monoterpene aldehydes and ketones (neral/geranial, citronellal, isomenthone, and menthone) and mono- and sesquiterpene hydrocarbons (E-caryophyllene) (Mimica-Dukic *et al.*, 2004). Lemon balm, among other members of the family Labiatae, was found to be the most effective plant against five food spoilage yeasts (Araujo *et al.*, 2003). The essential oil of lemon balm at 500 μg/ml completely inhibits all these yeast species and the fungitoxic effect is attributed to citral (58.3%), which is the main component of the oil. It also inhibits growth of some antibiotic resistant bacteria such as *Staphylococcus aureus*, *Salmonella choleraesuis* and *Klebsiella pneumoniae* (Nascimento *et al.*, 2000).

One of the potential remedies for stress-related disorders is accepted to be consumption of the functional food, which contains a number of herbal extracts (Hamer *et al.*, 2005). Lemon balm has been known as a mild sedative since the Middle Ages. Lemon balm extract is of value in the management of mild to moderate Alzheimer's disease (Perry *et al.*, 1999; Akhondzadeh *et al.*, 2003). It also affects mood changes during acute psychological stress (Little *et al.*, 2003). These behavioural consequences may be attributed to some active components of the dry herba or its essential oil (Kennedy *et al.*, 2002) although further work is required to substantiate efficacy in human subjects. There is no reported side effect of topical lemon balm, but allergic reactions should be always taken into account. Consumption as tea, fresh herba or capsule may reduce alertness and impair mental function (Kennedy *et al.*, 2002). Therefore anyone engaged in a job requiring alertness or driving should avoid using lemon balm beforehand. As a result, potential side effects of lemon balm should be considered and the patients should consult their physician before taking this herb.

Lemon balm has traditionally been used as a folk medicine for centuries and dates back at least 2000 years. It is used in tea for insomnia, fevers, migraine, headache, stomach disorders, gastric complaints, hysteria, chronic bronchial catarrh, nervous debility, toothache, earache, high blood pressure and indigestion (Herodez *et al.*, 2003; Uzun *et al.*, 2004). The essential oil is used in aromatherapy for relaxation, depression, melancholy, and nervous tension (Horrigan, 2005). Externally in salve, it is believed to relieve symptoms of rheumatism, nerve pains, sores, acne and painful swellings such as insect bites and stings. Dzik *et al.* (2004) conducted an investigation on the effect of lemon balm on experimental burn wound healing in pigs. The experiment showed that lemon balm is an ideal dressing in the treatment of burn in terms of relief of pain, a lower incidence of hypertrophic scar and post-burn contracture, with low cost and easy availability.

Synthetic antioxidants have been widely used in food products by adding them to fats in order to retard the oxidation process, which extends shelf life of those food products. However, the use of some synthetic antioxidants is prohibited in several countries, as there are concerns on their possible adverse effects on human health

(Herodez *et al*., 2003). Therefore, recently, the food industry has sought natural antioxidants because there have been some concerns about the safety and toxicity of synthetic antioxidants. Many herbs are excellent sources of natural antioxidants, and their consumption in the diet may thus contribute to the daily antioxidant intake. Lemon balm possesses a remarkable antioxidative activity of its phenolic antioxidants such as rosmarinic and caffeic acids (Labuda *et al*., 2002). This very high concentration of antioxidants may contribute to the total intake of plant antioxidants in a normal diet, and is suggested to be a better source of dietary antioxidants than many other foods such as fruit, cereals and vegetables (Dragland *et al*., 2003). A study also showed that lemon balm extract decreases serum cholesterol and lipid levels in the hyperlipidemic animals (Bolkent *et al*., 2005). This suggests that extract of lemon balm as a preventive agent could be used for hyperlipidemia disease.

Lemon balm also has a potential use in agriculture with its allelopathic effect and content of allelochemicals. It was stated that powder of lemon balm inhibits the germination and growth of some weed seeds such as *Amaranthus caudatus*, *Digitaria sanguinalis* and *Lactuca sativa* (Kato-Noguchi, 2003). This allelopathic nature of

Table 23.1 Some standards and specifications of raw material and essential oil of lemon balm

Harvest and raw material specifications	
Harvesting period	At the end of buttoning period and appearance of the first flower (June–October), in sunny, warm and non-windy days
Harvesting method	By cutting the plants and defoliating them as soon as possible (it is very difficult to defoliate the withered plants)
Processing	Naturally or artificially drying at maximum 35 °C. Steam-distillation of essential oil
Storage	Dry, clean rooms
Component specifications	
Essential oil content	0.02–0.2%
Main components	Citronellal (30–40%), citrale (20–30%)
Other components	Methyl-citronellate, (+)-ocimene, citronellol, nerol, geraniol, β-caryophyllene, germacrene D
Quality of the essential oil	
Source	Bulgaria
Appearance	Transparent liquid
Colour	Light yellow to yellow-brown, red brown
Aroma	Citric-rose, fresh with lemon smell
Relative density at 20 °C	0.870–0.906
Optical rotation	–3° to –11°
Refraction number at 20 °C	1.460–1.480
Ester number	20 to 46
Ester number after acetylation	150 to 220
Acid number	Not more than 2.5
Chemical composition (gas chromatography)	Linallol, citronellal, citral, β-caryophyllene
Source	United Kingdom
Appearance	Clear mobile oil
Aroma	Characteristics green, lemon scented aroma
Colour	Pale yellow
Optical rotation	–20 ± 2°
Refractive index at 20 °C	1.4854 ± 0.005
Specific gravity at 20 °C	0.888 ± 0.005

Source: IENICA, 2004.

lemon balm may therefore have a potential as a weed control in organic agriculture systems where only organic substances are allowed. However, for commercial use, much work should be carried out. Moreover, lemon balm extract also has an insecticidal activity and causes a significant reduction in the growth of the cotton worm (*Spodoptera littoralis*) larvae population (Pavela, 2004).

23.6 Quality issues

Medicinal and aromatic plants such as lemon balm are traditionally harvested from flora or cultivated field, dried and then stored until required for use. The quality of lemon balm has traditionally been based on appearance. However, efficacy of the raw material in many herbs varies, dependent on species and even different parts of the same species, not to mention cultural applications and harvesting time. In addition, value-added products, where plant appearance has been destroyed, make impossible visual assessments for species identification. These processed products can range from ground-dried raw material to liquid or solid extracts or capsules including a formulation, sometimes containing more than one herb. As such products cannot be detected with organoleptic techniques, chromatographic techniques such gas chromatography or HPLC can be employed for identification. Setting chemical quality standards has progressed slowly because of a lack of conclusive clinical evidence for the activity of specific compounds, multiple active constituents, synergistic effects, and the reluctance of some health authorities to agree on recognition of medicinal herbs as valid therapeutic agents (Wills *et al*., 2000). Some standards and specifications for raw material and essential oil of lemon balm are presented in Table 23.1.

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24

Lemongrass

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24.1 Introduction

Lemongrass is a tropical perennial plant which yields aromatic oil. The name lemongrass is derived from the typical lemon-like odour of the essential oil present in the shoot. The lemongrass oil of commerce is popularly known as Cochin oil in world trade, since 90% of it is shipped from Cochin port. The state of Kerala in India had the monopoly in the production and export of lemongrass oil. The annual world production of lemongrass oil is around 1000 t from an area of 16,000 ha. In India it is cultivated in about 4000 ha and the annual production is around 250 t. The crop is extensively cultivated in the poor, marginal and waste lands and also along the bunds as live mulch. The well ramified root system of the plant helps in soil and water conservation.

24.2 Species and varieties

Lemongrass belongs to the family *Graminae* (Poaceae) and the genus *Cymbopogon*. Generally, three species are identified (Gupta, 1969; Chandra and Narayanan, 1971).

24.2.1 *Cymbopogon flexuosus* (Nees ex Steud) Wats. (2n = 20, 40)

It is known as East Indian, Cochin or Malabar grass. *C. flexuosus* is a tufted robust perennial grass of about 2 m height. The leaves are linear and lanceolate. It flowers freely. The inflorescence is very large and highly branched terminal drooping panicle bearing paired spikes on tertiary branches. The 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. Under this species two varieties or types are identified based on the colour of the stem.

C. flexuosus var. flexuosus (red grass)

The stem and leaf sheath are reddish or purple in colour. It is recognized as the true

lemongrass and is commercially cultivated. The essential oil contains more than 75–80% citral, exhibits good solubility in alcohol and hence is superior in quality (Guenther, 1950).

C. flexuosus var. albescens
This white grass is characterized by the white colour of the stem. The plant is normally seen wild. The essential oil contains less than 65–70% citral, exhibits poor alcohol solubility and is hence considered inferior in quality.

24.2.2 *Cymbopogon citratus* (DC) Stapf. (2n = 40, 60)

Known as West Indian or American lemongrass, it is a stemless perennial grass with numerous stiff tillers arising from short rhizomatous rootstock, making large tussocks. It seldom flowers under cultivation. Leaf blade is narrow, linear, glaucous, drooping with scabrous margin, ligule truncate, inflorescence rarely produced, a large loose panicle; spathe bracts long and narrow, sessile spikelets, awnless, linear, lanceolate. The essential oil contains 74–76% citral and exhibits poor alcohol solubility.

24.2.3 *Cymbopogon pendulus* (Nees ex Steud) Wats

Jammu lemongrass is white stemmed and dwarf in nature. The plant is frost resistant and suited to sub-Himalayan areas of North India. The essential oil contains around 75–80% citral and exhibits medium solubility in alcohol (Joy *et al*., 2001).

24.3 Origin and distribution

Lemongrass is distributed in Africa, the Indian subcontinent, South America, Australia, Europe and North America. In India, it grows wild in all regions extending from sea level to an altitude of 4200 m. Several species are endemic to India. East Indian lemongrass grows wild in India and is cultivated well in Kerala, Assam, Maharashtra and Uttarpradesh. It is also distributed in Guatemala and China. West Indian lemongrass 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, Guatemala, Brazil, Congo, Tanzania, India, Thailand, Bangladesh, Madagaskar and China. Jammu lemongrass is mostly confined to North Indian states such as Jammu and Kashmir, Sikkim, Assam, Bengal and Madhya Pradesh (Handa and Kaul, 2001). Lemongrass is cultivated on a large scale at Chinnar wildlife sanctuary in the Western Ghats of India (Nair and Jayakumar, 1999).

24.4 Cultivation and processing

24.4.1 Climate

C. flexuosus and *C. citratus* flourish in the sunny, warm, humid conditions of the tropics. In Kerala, lemongrass grows well between 900 and 1250 m above mean sea level. Both species produce the highest oil yield per tonne of herbage where the rainfall averages 2500–3000 mm annually. *C. citratus* is more drought tolerant (Weiss,

1997). In areas where rainfall is poor, it can be grown with supplemental irrigation. Day temperatures of 25–30 °C are considered optimum for maximum oil production, with no extremely low night temperature. Short periods above 30 °C have little general effect on plants, but severely reduce oil content.

24.4.2 Soil

Lemongrass flourishes in a wide variety of soil ranging from rich loam to poor laterite. In sandy loam and red soils, it requires good manuring. Calcareous and water-logged soils are unsuitable for its cultivation (Farooqi and Sreeramu, 2001). Both species can be grown on a range of soils and it appears that good drainage is the most important factor. Plants growing in sandy soils have higher leaf oil yield and citral content. Although *C. flexuosus* flourishes in well drained sandy loams in India, it is grown in almost all types of land available from very light sandy soil to upland laterites. Soils of pH 5.5 to 7.5 are utilized. *C. citratus* is more commonly grown on soils with higher acidity than *C. flexuosus*. In India, the highest herb and oil yields per hectare 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).

24.4.3 Cultivated varieties

Lemongrass varieties released for cultivation are Sugandhi, Pragati, Praman, RRL-16, CKP-25, RRL-39, Kavery, Krishna, SD-68, GRL-1 (Farooqi and Sreeramu, 2001) and SB-9 (Patra *et al*., 1999).

- Sugandhi (OD-19): released from the Aromatic and Medicinal Plants Research Station (AMPRS), Odakkali, Kerala, India. A red stemmed variety adapted to a wide range of soil and climatic conditions and most popular in India. The plant grows to a height of 1–1.75 m with profuse tillering, yielding 35–40 t/ha/year herb containing 0.3% oil (125 kg/ha) with 80–85% citral under rain-fed condition (Joy *et al*., 2001).
- Pragati (LS-48): evolved through clonal selection from OD-19 at Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, India. It is tall growing with dark purple leaf sheath, adapted to North Indian Plains and Tarai belt of subtropical and tropical climate. Average oil content is 0.63% with 86% citral (Sharma *et al*., 1987).
- Praman (Clone 29): evolved through clonal selection at CIMAP, Lucknow and belongs to species C. *pendulus*. It is a tetraploid type with profuse tillering. Leaves are erect and medium in size. Oil yield is 227 kg/ha/annum with 82% citral content (Anon., 1988).
- RRL-16: evolved from *C. pendulus* and released for cultivation from Regional Research Laboratory (RRL), Jammu, India. Average yield of herb is 15 to 20 t/ha/annum giving 100 to 110 kg oil. Oil content varies from 0.6 to 0.8% and citral content is 80% (Anon., 1983).
- SD-68: developed by SC Datta using ionizing radiation, yielded up to 375 kg of oil/ha/year with a citral content of 90–92% (Nair, 1977).

- RRL-39: released from RRL, Jammu.
- Kavery and Krishna: released from CIMAP Regional Station, Bangalore, India.
- Chirharit: a high yielding variety, developed by systematic breeding for genetic improvement at Pantnagar, Chirharit, India. It is frost resistant and the essential oil contains 81% citral (Patra *et al*., 2001).

Lemongrass germplasm, consisting of about 406 accessions, is maintained at AMPRS, Odakkali. There are 17 other types in the germplasm in which the major constituent of the oil is not citral.

24.4.4 Propagation

Lemongrass is generally propagated through seeds. Seed is mixed with dry river sand in the ratio of 1:3 and sown in the field at the rate of 20 to 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, which requires 3–4 kg seeds/ha, is ideal for uniform stand and better growth of the plants. Small plantations of lemongrass can be established by planting of slips.

C. flexuosus is propagated through seeds while *C. citratus* is propagated through division of clumps (Anon., 1981). Hussain and co-workers (1988) reported that propagation through vegetative means from selected clones was considered better as seed propagation tended to cause considerable genetic heterogeneity resulting in deterioration of yield and oil quality and clonal proliferation played a very important role in the propagation of lemongrass.

24.4.5 Nursery

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 and February are usually sown in the nursery during April and May. Germination is very poor if sown after October. For one hectare of land, 1000 m^2 nursery has to be raised. The area is made to fine tilth by repeated ploughing. Beds of 1–1.5 m width and convenient length are prepared. The seeds are uniformly broadcasted on the beds at 3–4 kg/ha and covered with a thin layer of soil. The seed bed is irrigated frequently. Seeds germinate in 5–7 days.

24.4.6 Transplanting

The seedlings raised in the nursery beds are transplanted in the field at 6–7 leaf stage; 50–70-day old seedlings 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).

24.4.7 Manuring

Spent lemongrass compost at 10 t /ha and wood ash at 2 t/ha, which are obtained as 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/annum. Under rainfed conditions at Kerala, application of 100 kg N in three to four split

doses was found to be optimum though a response up to 200 kg was recorded. The application of 50 kg/ha each of P_2O_5 and K_2O as a basal dose gave encouraging 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 a top dressing during the growing season as an optimum dose. It also responds well to the application of copper, iron, calcium and sulphur. It was reported from CIMAP, Lucknow that a lower dose of 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). Soluble nitrogen fraction and total carbohydrate content increased essential oil content. Pattern of formation of citral in *C. flexuosus* oil revealed that the constituents increased up to reproductive phase and then declined; it again increased after post-reproductive phase of the plant. Optimum application of fertilizers increased the citral content of the oil (Ghosh and Chatterjee, 1991). Excess fertilizer application is undesirable as it promotes more vegetative growth and oil with less citral content (Joy *et al*., 2001).

24.4.8 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 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).

24.4.9 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. Once the crop is well established, it can compete with weeds. Generally, 2–3 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). They also suggested spraying paraquat at 2–2.5 l/ha in 500 l of water immediately after cutting the grass as an excellent method of weed control. Under rain-fed conditions, the field gives a dried appearance during the summer months of Dec.–May. The dry grass and stubble of the crop is set on fire in May, prior to the onset of monsoon. This practice kills the termites attacking crop stubbles and also helps to rejuvenate the old clumps.

24.4.10 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 AMPRS, Odakkali indicated poor tillering, lean and lanky growth and reduced oil yield when the crop is grown as an intercrop in coconut gardens; the oil content was also found to be reduced by 20%. In contrast, intercropping in a 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 four to five years of 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 the cost of plantation establishment. Pratibha and Korwar (2003) suggested lemongrass for crop diversification in semi-arid regions.

24.4.11 Plant protection

Pests and their management

Few pests are reported in this crop. Infestation by the spindle bug (*Clovia bipunctata*) has been observed at Odakkali and severe damage by a stem boring caterpillar of *Chilotrea* sp. under North Indian conditions has been reported. Spraying malathion (0.2%) can control the insects. Nematodes like *Tylenchorhynchus vulgaris*, *Rotylenchulus reniformis*, *Helicotylenchus* spp. and *Pratylenchus* spp. have also been found to infect the grass.

Diseases and their management

The common diseases and their causal agents are given in Table 24.1. These leaf diseases can be managed by prophylactic sprays of dithane Z-78 at 3g/l thrice, at intervals of 15 days. *Helminthosporium cymbopogi* caused very serious disease in the lowlands of Guatemala. Brown top 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 of lemongrass causing elongated, stripe-like, dark brown lesions on both sides of leaf surfaces have been described. The causal organism is *Puccinia nakanishikii* (Koike and Molinar, 1999). Root segments of lemongrass were heavily infested with multiple vesicular arbuscular mycorrhiza (VAM). Moreover, brown septate hyphae of non-mycorrhizal fungus also co-existed with VAM in 50% of root segments (Hussain and Ali, 1995). Burning of stubble in summer is practised in some areas to ward off pests, diseases and weeds.

Table 24.1 Common diseases of lemongrass and their causal agents

Disease	Causal organism
Little leaf (malformation of inflorescence)	*Balensia sclerotica* (Pat) Hohnel
Leaf spot (eye spot)	*Helminthosporium saccharii, H. leucostylum, Drechslera victoria* and *D. helm*
Leaf spot	*Curvularia andropogonia* (CLS)
Leaf spot	*C. veruciformis, C. trifolii* and *Collitotrichum graminicola*
Leaf spot and clump rot	*Fusarium equiseti* and *F. verticillium*
Leaf blight	*Curvularia andropogonia* (CLB)
Leaf blight	*Rhyzoctonia solani*
Grey blight	*Pestalotiopsis magniferae*
Smut	*Tolyposporium christensenni* and *Ustilago andropogonia*
Root rot	*Botrydiplodia theobromae*

24.4.12 Harvesting of the herb

Harvesting is done by cutting the grass 10 cm above ground level, with the help of sickles. The number of harvests in a year depends on the climatological factors such as temperature, rainfall and humidity and level of soil fertility. Generally the crop thrives best in humid condition (Handa and Kaul, 1997). Cutting can begin as soon as the night dew has evaporated from the plants, as wet grass left for later distillation quickly ferments. Sunny days are preferable, since cloudy and misty conditions tend to depress leaf oil content.

Chandra *et al*. (1970) have suggested first harvest at 75 days after planting, second at 120–130 days after first harvest and the third at 150–160 days after second harvest. However, Nair *et al*. (1979) and Shiva (1998) have suggested that first harvest can be taken at 90 days after planting and subsequent harvest at 50–55 days interval up to 5–6 years from the same crop. During the first year of planting, three cuttings are obtained and subsequently 5–6 cuttings per year (Subramanyam and Gajanana, 2001). The harvesting season begins in May and continues till the end of January. A herbage yield of 10–15 t/ha/harvest may be obtained.

24.4.13 Seed collection

Lemongrass kept for seed purpose is not cut as the yield of seeds from plants subjected to regular harvesting is very low. Generally, the plant flowers during November–December in plains and mature seeds are collected during January–February. A healthy plant produces 10–20 g of seeds. The whole inflorescence is cut and dried in the sun and seeds are collected by threshing against the floor or beating with sticks. Fresh seeds are recommended for use in raising a plantation since the seeds lose viability beyond six months of storage. Seed germination is very poor till May, increases up to July and thereafter decreases. Germination is meagre beyond October (Thomas, 1995).

24.4.14 Processing

Distillation

Lemongrass oil is collected by steam distillation of the herbage. 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.
2. Hydro and 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 fired 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: in this method, no water is added to the still. Instead, saturated or superheated steam is introduced through open or perforated steam coils below the charge.

The distillate on cooling separates out into a layer of oil, floating over the bulk of water. For obtaining 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 oil recovery. Drying in the sun reduces oil recovery but has little effect on oil composition. Generally, Clevenger apparatus is used for distilling small quantities (up to 1.0 kg) of the herb in the laboratory. Large-field-scale distillation units are fabricated to distil 500 kg or more of the herb at a time. On an average, the herbage of *C. flexuosus* contains 0.2–0.4% oil and the oil yield is 100–125 kg/ha/year.

Oil of lemongrass is a viscous liquid, yellow to dark yellow or dark amber in colour turning red on prolonged storage. The presence of water imparts a turbid appearance. Whole oil is mainly used as a source of citral. Differentiation of lemongrass oils into West Indian and East Indian in trade has no geographical significance as oils from both species are produced in both 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 fresher, lighter and sweeter.

Morphological characters like plant height, number of tillers/plant and number of leaves/plant is significantly correlated with essential oil yield/plant. Maximum elimicin content as a major chemical constituent of oil had also been observed at flowering stage. Among the physiological characteristics, a significant correlation was observed between essential oil content and crop growth rate ($r = 0.6018$) as well as net assimilation rate ($r = 0.9474$). The oil of lemongrass is chemically reactive. The terpene mixture undergoes a series of complex reactions when exposed to air and sunlight. It is slowly converted into a dark coloured viscous resinous substance on keeping. However, if stored in aluminium or stainless steel vessels insulated from air, water and light, the quality of the oil is stable for long periods of time.

Solvent extraction

Distillation being a high temperature process, yields an oil with a burnt note. Also it is devoid of volatile fractions. An oil of softer note is yielded by solvent extraction. However, the process is more expensive than steam distillation. Lemongrass oil can be extracted by the following methods using different solvents.

1 Maceration: this involves macerating the dried plant material in the presence of a non-polar solvent like hexane, filtering and concentrating the extract to recover the solvent.
2 Percolation: in this method, the solvent is made to percolate through a column of the dried plant material. The percolate is later subjected to distillation to obtain the oil and recover the solvent. *Soxhlet extraction* is a method of continuous percolation using special equipment. The plant material is packed in a porous container placed inside an extraction vessel. The solvent is introduced slowly and continuously into the container. The extract is siphoned into a recovery vessel where the solvent is distilled off. The solvent thus recovered is added back into the porous container. The process is repeated in a cyclic manner such that extraction of the material and recovery of the solvent proceeds simultaneously using a limited quantity of solvent.

Spent grass

The residue obtained after extraction of the oil is called spent grass. It can be used as cattle feed fresh or after ensilaging. It can be used for mulching or manuring crops as

such or after composting. In some plantations in India, the spent lemongrass after drying is used as a fuel for distillation. It is also a cheap packing material.

24.5 Physiology and Biochemistry

A quick and non-destructive method of leaf area estimation has been worked out by Joy and Thomas (1990). A direct relationship between chlorophyll (influencing primary metabolism) and odour bearing constituents (secondary metabolites) was noted (Sharma *et al*., 1988). Maffei *et al*. (1988) suggested that lemongrass may possess a C_4 photosynthetic mechanism. The differential oil and citral synthesis in specific genotypes over diverse seasons may be due to physiological homeostasis as production of essential oil is the criterion of the homeostatic features of bioenergetic balance as well as developmental feed back mechanism (Sharma *et al*., 1988). Application of Well Bloom, a tricontanol containing growth regulator, had no significant effect on oil yield and citral content though a favourable effect on herbage yield was recorded (Sankar and Thomas, 1990). Repeated application of 10–100 ppm of IAA, IBA, NAA or GA_3 increased oil content significantly though herbage yield and citral content were not affected. It was suggested that these growth substances influenced the enzymes of carbohydrate metabolism which in turn ensured high demand of hexoses required for essential oil synthesis (Anon., 1983).

Synthesis of terpenoids in plants takes place in secretory cells in leaves. It has been claimed that the precursors of essential oils are obtained by the degradation of carbohydrate and proteins. Ghosh and Chatterjee (1976) highlighted the phenomenon of decrease in total and protein nitrogen in the plant concomitant with the increase in essential oil content as evidence of the above hypothesis. Steps involved in the biosynthesis of monoterpenes were reviewed by Akhila and Nigam (1983). Activities of mevalonate kinase and phosphomevalonate kinase in lemongrass leaves were reported by Lalitha and Sharma (1986) who suggested the possibility of mevalonoid route to citral synthesis. Verma *et al*. (1987) suggested the presence of a geraniol citral enzyme complex controlled by independent genes which have no competitive influence on each other in lemongrass. Singh *et al*. (1989) have shown that young expanding leaves are biogenetically more active and that the leaf age and the leaf position are important factors for the amount and composition of the essential oil. Singh and Luthra (1987) reported that the ability to synthesise oil and citral from ^{14}C-sucrose by lemongrass leaves decreased greatly long before full expansion. Soluble acid invertase was the major enzyme in sucrose breakdown.

In order specifically to locate the sites of citral accumulation, the Schiff's reagent that stains aldehydes has been used. Using this technique, single oil accumulating cells were detected in the abaxial side of leaf mesophyll, commonly adjacent to the non-photosynthetic tissue and between vascular bundles. The cell walls of these cells are lignified (Lewinson *et al*., 1997).

24.6 Chemical composition

24.6.1 Herb

The spent grass on an average contains N 0.74%, P 0.07%, K 2.12%, Ca 0.36%, Mg

0.15%, S 0.19%, Fe 126.73 ppm, Mn 155.82 ppm, Zn 35.51 ppm and Cu 56.64 ppm (Joy, 2003).

24.6.2 Essential oil

East Indian lemongrass oil contains 75–85% of aldehydes consisting largely of citral. Other constituents in the oil are linalool (1.34%), geraniol (5.00%), citronellol, nerol (2.20%), 1,8 cineole, citronellal (0.37%), linalyl acetate, geranyl acetate (1.95%), α-pinene (0.24%), limonene (2.42%), caryophyllene, β-pinene, β-thujene, myrcene (0.46%), β-ocimene (0.06%), terpenolene (0.05%), methyl heptanone (1.50%) and α-terpineol (0.24%) (Weiss, 1997; Ranade, 2004).

The essential oil of *C. citratus* contains approximately α-pinene (0.13%), β-pinene, delta-3-catrene (0.16%), myrcene (12.75%), dipentene (0.23%), β-phellandrene (0.07%), β-cymene (0.2%), methyl heptanene (2.62%), citronellal (0.73%), β-elemene (1.33%), β-caryophyllene (0.18%), citronellyl acetate (0.96%), geranyl acetate (3.00%), citral b (0.18%), citral a (41.82%), geraniol (1.85%), elemol (1.2%) and β-caryophyllene oxide (0.61%) (Saleem *et al*., 2003a,b).

The average composition of *C. pendulus* oil is reported to be pinene (0.19%), camphene (0.01%), β-pinene (0.16%), car-3-ene (0.04%), myrcene (0.04%), dipentene (0.35%), phellandrene (0.3%), p-cymene (0.36%), methyl heptanone (1.05%), citronellal (0.49%), linalool (3.07%), β-elemene (0.7%), β-caryophyllene (2.15%), citronellyl acetate (0.72%), geraniol acetate (3.58%), citral b (32.27%), citral a (43.29%), geraniol (2.6%), elemol (2.29%) and β-caryophyllene oxide (1.56%) (Shahi *et al*., 1997; Sharma *et al*, 2002). The chemical structures of important constituents of lemongrass essential oil are given in Fig. 24.1 and a gas chromatogram of the oil in Fig. 24.2.

The two isomers of citral constitute the bulk of lemongrass oil. Citral is separated from the oil by fractional distillation and used as a starting material for the synthesis of a number of industrially important products. Citral has a citrus flavour. Geraniol, linalool and citronellol are the most important acyclic terpene alcohols that can be separated from lemongrass oil and used as flavour and fragrance substances. In flavour compositions, geraniol is used in small quantities to accentuate citrus notes. Nerol is used for bouquetting citrus flavours. Citronellol too is added for bouquetting purposes to citrus compositions. Pinene is an important starting material in the fragrance and flavour industry.

24.6.3 Oleoresin

A total extract of lemongrass comprising the volatile and non-volatile components imparting flavour and aroma to the product can be prepared by subjecting the herb to extraction with a suitable solvent or a mixture of solvents. The oleoresin that results will be a concentrated wholesome product with better storage characteristics.

24.7 Uses in food processing

24.7.1 Herb

Herbal teas

Dried lemongrass leaves are widely used as a lemon flavour ingredient in herbal teas,

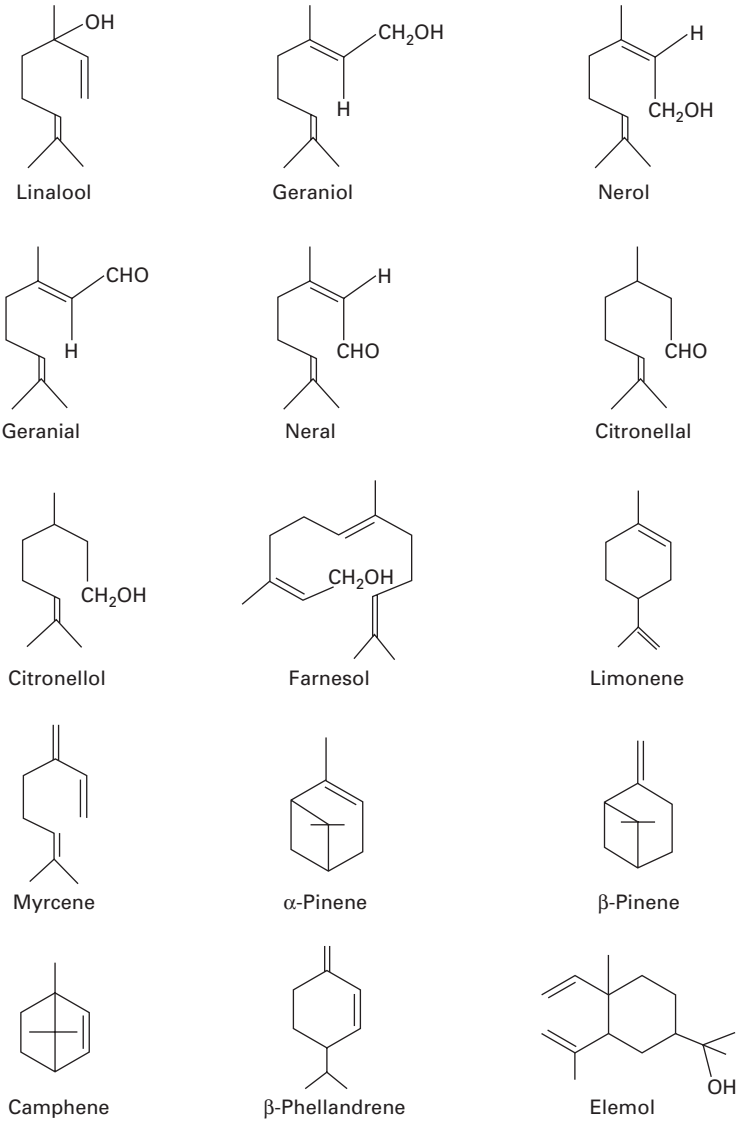

Fig. 24.1 Chemical structures of important constituents of lemongrass essential oil.

prepared either by decoction or infusion of 2–3 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 with 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.

Food flavouring

Lemongrass is commonly used in Asian cooking. When Thai food was embraced in the US, lemongrass became a household name. A little experimentation with this delightfully fragrant herb is all it takes to realize that it can be used in many more ways than just in Asian dishes. 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

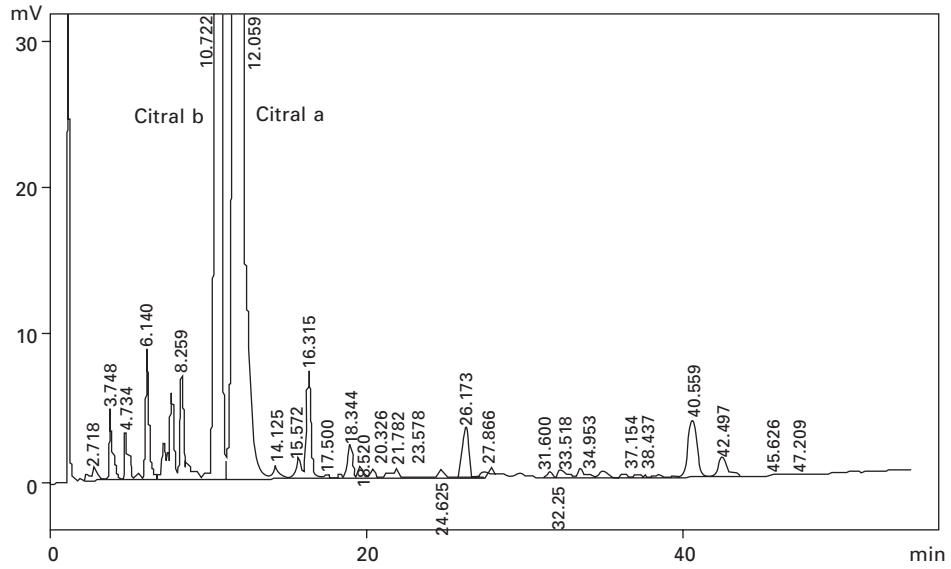

Fig. 24.2 Gas chromatogram of lemongrass oil (*Cymbopogon flexosus*).

home made soda by mixing it with seltzer. A blend of lemongrass, garlic, ginger and oil will be stable in the freezer during winter. This paste can be fried until fragrant and then cooked down with a can of coconut milk (strain to remove tough lemongrass fibres) for a delicious sauce for noodles, vegetables or seafood dishes. Some Thai recipes using lemongrass are given below.

Spiced carrot soup with ginger and lemongrass

Ingredients	*Approximate measure*
1. Carrots, scrubbed and chopped	2 small sized
2. Leek, coarsely chopped	2–3 small sized
3. Onion, diced	2–3 small sized
4. Celery, diced	1 or 2
5. Ginger, minced	half inch piece
6. Lemongrass	2–3 stalks
7. Honey	1 tbsp
8. Curry	1 tbsp
9. Cloves, garlic, minced	2 of each
10. Oil	2 tbsp
11. Water	1 cup
12. Lemon juice	half lemon
13. Salt and pepper	to taste

Method

Sauté leeks, carrots and celery in oil till translucent. Add garlic, curry and ginger. Sauté for several minutes. Add water and bring to the boil. Add honey, lemongrass, outer leaves removed and inner core minced, salt and pepper. Simmer until vegetables are tender. Puree until smooth. Add lemon juice and adjust seasoning. It can be served hot or cold as a garnish with thinned yogurt or crème fraiche and parsley or cilantro.

Lemongrass coconut rice

Ingredients	*Approximate measure*
1. Long grain rice	1 cup
2. Lemongrass	2–3 stalk
3. Coconut milk	3/4 cup
4. Bay leaves	2
5. Turmeric	half tsp
6. Salt	to taste

Method
Wash rice under cold water. Bruise lemongrass by banging it with a heavy knife handle or skillet. Put all ingredients into a saucepan. Slowly bring to a boil, stirring occasionally. Lower the heat and cover. Simmer for 25 minutes or until all liquid is absorbed. Let sit for 5 minutes with the cover on and then fluff with a fork. Remove bay leaves and serve.

Vegetarian Pad Thai

Ingredients	*Approximate measure*
1. Rice noodles	8 oz.
2. Fresh bean sprouts	half cup
3. Peanuts (chopped)	half dry roasted
4. Lemongrass	two stalks
5. Cilantro	seven sprigs
6. Cloves garlic	four
7. Jalapeno	one stemmed and seeded
8. Carrot (diced small)	one medium size
9. Egg	two
10. Peanut oil	1/4 cup
11. Green onions (thinly sliced)	four
12. Sugar	two tbsp.
13. Lemon juice	three tbsp
14. Catsup	two tbsp.
15. Thai fish sauce (nam pla)	two tbsp
16. Lime	one

Method
The creamy coconut and lemongrass base is loaded with chunks of white meat chicken.

Other Thai lemongrass preparations are listed below.

Tom Yum Koong – Thai traditional jumbo shrimp soups with lemongrass, lime leaf, mushrooms, chilies paste and lime juice. Garnished with cilantro.
Tom Ka Kai – sliced chicken breast cooked in coconut milk with mushrooms, galangal, lemongrass, lime leaf and chilies paste. Garnished with cilantro.
Tom Yum Poh Tak – seafood combination in spicy soup with lemongrass, lime leaf, mushrooms, chilies paste and lime juice. Garnished with cilantro.
Tom Yum Kai – sliced chicken breast in spicy soup with lemongrass, lime leaf, mushrooms, chilies paste and lime juice. Garnished with cilantro.
Yum – grilled barbecue beef, pork or chicken steak, sliced and tossed with lime dressing, chilies, red onions, tomatoes, cucumbers and lemongrass. Garnished with lettuce, scallions and mint leaf or sweet basil.

Yum seafood – combination of seafood and tossed with lime dressing, chilies, red onions, tomatoes, cucumbers and lemongrass. Garnished with lettuce, scallions and mint leaf or sweet basil.

24.7.2 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 fat and oils. It is used to improve the flavour of some fish and can be used to flavour wines, sauces, etc. 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).

24.7.3 Oleoresin

Lemongrass oleoresin is mainly used in flavouring foods, drinks and bakery preparations.

24.8 Functional properties

24.8.1 Herb

Leaves of lemongrass can be used as a source of cellulose in the manufacture of paper and cardboard. Reduction in root-knot nematode disease was observed in soil amended with leaves of *C. flexuosus*. In the Carribbean, lemongrass is primarily regarded as a fever reducing herb (especially where there is significant catarrh). It is applied externally as a poultice to ease pain and arthritis. In India, a paste of leaves is smeared on patches of ringworm (Chevallier, 2001).

24.8.2 Essential oil

Lemongrass oil is one of the most important essential oils being widely used for the isolation of citral. Citral is the starting material for the preparation of ionones. α-ionone is used in flavours, cosmetics and perfumes. β-ionone is used for the synthesis of vitamin A. Citral b, the most common constituent of oil, could be a good inhibitor of β-glucuronidase. The oil has other uses as bactericide, as insect repellent and in medicine (Alam *et al*., 1994; Atal and Kapur, 1997; Rodriguez *et al*., 1997; Sasidharan *et al*., 1998; El-Kamali *et al*., 1998; Balz, 1999; Saikia *et al*., 1999). Wisprec antimicrobial cream, made from *Ocimum sanctum* and *C. citratus,* remains intact in its activity up to three years from the date of manufacturing (Tiwari *et al*., 1997; Prashanth *et al*., 2002). Its mosquito repellent activity lasts for 2–3 hours (Oyedele *et al*., 2002). It exhibits significant antifeedant and larvicidal activity against *H. armigera* (Rao *et al*., 2000). It is effective against storage pests (Rajapakse and Emden, 1997). The whole oil has fungicidal properties to plant and human pathogens (Yadav and Dubey, 1994; Mehmood *et al*., 1997; Handique and Singh, 1990; Dubey *et al*., 2000; Cimanga *et al*., 2002) and is potentially anticarcinogenic (Zheng *et al*., 1993; Vinitketkumnuen *et al*., 2003).

The essential oils from *C. citratus* have been tested for their cytotoxic activity against P_{388} leukemia cells (Dubey *et al*., 1997). It also exhibited antioxidant activities

comparable with α-tocopherol and butylated hydroxyl toluene (Baratta *et al*., 1998; Lean and Mohammad, 1999). It retards mould growth in butter cakes thereby increasing storage life. Oil of *C. citratus* caused egg hatch inhibition (Yadav and Bhargava, 2002). Oil of *C. pendulus* is used for the preparation of antibacterial drug trimethoxyprim. Z-asarone, a component of oil, is used as an antiallergic compound. It is used for the development of designer beverages and blends of oils with the desired odour characteristics. It strengthens the stomach, stimulates appetite, promotes digestion, and regulates the nervous system and vascular expansion. It is a stimulant, antiseptic, febrifuge, carminative, diuretic, anti-inflammatory, anti-diabetic and useful against rickets.

24.9 Quality issues

The results of routine physico-chemical analysis and chromatographic examination of the recovered oil are of greater value as criteria of authenticity and source (Humphrey, 1973; Rhyu, 1979). A method of fingerprinting essential oil has been described (AMC, 1980) and is widely accepted not only as a reliable method for determining the quality, source and authenticity of the raw material. From a sensory point of view, essential oils collected under laboratory conditions are of little value in indicating the quality of the bulk distilled under commercial conditions from the material under examination. The odour pattern and taste of small-scale distilled oils are not reliable and should not be used as a basis for quality judgement.

Various types of apparatus for the determination of essential oil proposed by Clevenger (1935) are available. The one recommended by the Council of Europe Pharmacopoeial Commission is in current laboratory use as it is convenient and facilitates the standardization of distillation conditions for obtaining consistent results. A method for the analysis of small amounts of essential oils by distillation in a microversion of a modified Marcusson apparatus, followed by capillary GC is described by Bicchi and Frattini (1980).

The degree of quality control applied to essential oils depends to a large extent on their source, whether they are unprocessed, have been concentrated or de-terpenated and on their intended use. Their sampling analysis and quality assessment demands considerable expertise, a close attention to test procedures and a good understanding of the relationship between physico-chemical characteristics and sensory attributes. Quality judgements should be based on the combined data obtained by physical, chemical and sensory analyses, particularly at the aromatic profile observed under defined conditions (Varghese, 1986).

The sensory qualities of essential oils should be paramount in any evaluation of quality and suitability for use. The evaporation pattern of oil exposed on a smelling strip over a period of time gives very valuable information about its source, age and often its authenticity. For most samples, the odour assessment should be carried out and a judgement made at the following intervals: immediately after dipping, after 1 hr, 2 hr and 6 hr and after standing overnight or for a period of not less than 18 hr. The flavour of the oil should be assessed at an appropriate dilution in diluted sugar syrup or some other appropriate medium (Heath, 1978). In each case, the material under examination should be compared directly with a reserve sample, regularly replaced from acceptable material and maintained under optimum storage conditions, usually refrigerated. Obviously, there will be natural variation between different lots

of oils, but these should be within acceptable limits judged by the experience of the assessor. Many of the commercially available essential oils originate in countries remote from those in which they are used so that control is of prime importance in both the selection and acceptance of these materials, particularly for use in food products.

Routine physical tests include

- moisture content (ISO 939-1980)
- specific gravity/relative density
- optical rotation
- refractive index
- freezing or congealing point
- solubility in diluted alcohol of a stated strength (a table for the preparation of diluted alcohol is given in British Standard BC 2073: 1976).

Chemical tests include

- acid value
- ester value before acetylation (for calculation of esters and combined alcohols)
- ester value after acetylation (for calculation of free alcohols)
- ester value after formulation (for calculation of free tertiary alcohols)
- carbonyl value
- phenol content.

There are specific tests which should only be used in commercial transactions after full agreement by both parties. In any event, the method employed must be clearly indicated in the test report. Test methods are defined in food chemicals codex III for determination of:

- acetals
- acid value
- total alcohols
- aldehydes
- aldehydes and ketones
  - hydroxylamine method
  - hydroxylamine-tert butyl alcohol method
  - neutral sulfite method
- chlorinated compounds
- esters
- linalool content
- phenols, free phenols,
- residue on evaporation
- solubility in alcohol
- volatile oil content.

Industrial methods use chromatographic techniques (TLC, paper chromatography, GLC (Humphrey, 1973), column chromatography, HPLC (Lego, 1984)); spectrophotometric techniques (visible range, UV range, IR range) and spectroscopic methods (NMR, mass spectroscopy (MS), usually coupled with GLC (Thomas, 1984)). Kumar and Madan (1979) have described a rapid method for the detection of adulteration in essential oils using an iodide monobromide/mercuric acetate to establish iodine values which can be directly compared with those for genuine oils.

The conventional method used for the determination of citral, the major constituent of lemongrass oil is the sodium bisulphite method (Guenther, 1948). This method involves treatment of a measured volume of lemongrass oil with excess of saturated metabisulphite solution. Aldehydes in the oil, the bulk of which is constituted by citral, reacts with the sulphite to form an adduct which is soluble in water. At the end of the reaction, the non-aldehydic components of the oil will form a layer floating on the aqueous portion. The volume of the non-aldehydic portion can be directly measured, from which the volume of aldehyde (citral) can be calculated by difference. The method suffers from a positive error depending on the volume of non-citral aldehydes present in the oil. Since this component is negligible in most lemongrass oils the method yields satisfactory results for quality evaluation of oil in trade.

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25

Long pepper

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25.1 Introduction

Long pepper commonly called 'Pippal' in Sanskrit, is an important medicinal spice. The long pepper of commerce is derived from more than one species. The Indian long pepper is derived from *Piper longum* L and *Piper peepuloides* Wall. while the much longer Indonesian or Java long pepper is derived from *P. retrofractum* Vahl. The products of these species are used for the same purposes, though they vary in their effectiveness. The products of *Piper longum* and *P. retrofractum* are often not clearly distinguished in the spice trade. The spikes of *Piper peepuloides* and sometimes another related species with globose spikes *P. mullesua* Ham. occur mixed with commercial *P. longum* (CSIR, 1969; Pruthi, 1976).

25.1.1 Vernacular/regional names

English: Long pepper; Bengal pepper, Java pepper, Balinese pepper; Unani: Pipal; Amharic: Timiz; Arabian: Dardildil; Persian: Filfilidaraz, filfildray, Maghzpipal, Pilpil, Pipal; Nepalese: Pipal, Piplamol, Popal; Malaya: Pit poot; Burmese: Peik-khyen, Peikchin; Malay: Lada, Mula-gu, Cuttaterpali, Chabai; Hungarian: Bali bors, Bengali bors; Sinhalese: Tippali; Greek: Pepper makron; Italian: Pepe lungo; Indonesian (Java): Cabe bali, Cabe jawa; Lada panjang; Mexican: Tlathancuaye; Potuguese: Pimenta longa; Dutch: Langwerpige peper; Duk.: Pipaliana; Swedish: Lang peppar; German: Langer Pfeffer, Stangenpfeffer, Balinesischer Pfeffer, Jaborandi Pfeffer, Bengalischer Pfeffer; French: Poivre long, Racindes de; Spanish: Pimentera larga; Khmer: Morech ansai; Laotian: Sa li pi, I lo; Japanese: Hihatsu; Chinese: Pipo, Bi ba; Estonian: Pikk pipar; Thai: Dee plee, Phrik hang, Dipli chuak; Tibetan: Dro-sman; Hindi: Pipli, Pipal, Pimpli, Piplamul, Pipulmul; Sanskrit: Chanchala, Chapala, Granthika, Kana, Kati, Kola, Korangi, Krishna, Krishnapippali, Magadhi, Pippali, Tiktatandula, Vaidehi; Urdu: Pipul; Bengali: Piplamor, Piplamul, Pipli, Pipul; Assami: Pipoli; Gujarathi: Piper, Pipli, Pipara; Marathi: Pimpli, Mothi, Piple; Punjabi: Darfilfil, Filfildaraz, Maghzpipal, Pipal; Sindhi: Filfildray, Tippili, Fil; Uriya: Baihehi, Krykola,

Mogodha, Pippoli; Santal: Ralli; Canarese (Kannada): Thippali, Hippali; Tamil: Argadi, Atti, Kalidi, Kaman, Kanna, Kindigam, Kolagam, Savundi, Sauyini; Telugu: Modi, Pippali; Malayalam: Chapal, Tippali (Singh *et al*. 2000). The roots have been described separately in Ayurvedic texts as granthika, Pippalimul, Ushna, Chatakashir, kolmul, Katugranthi, Chavikashir.

25.1.2 Origin and geographical distribution

Long pepper belongs to the family Piperaceae and is native to South and South East Asia. The three major species which constitute long pepper of commerce occur in three different geographical regions. *Piper longum* L. (Syn. *Chavica roxburghii* Miq.), commonly called Indian long pepper, occurs throughout India, Sri Lanka, Burma, Malaysia, Nepal, Singapore and other South Asian countries, but is most widely distributed in India and is a native of penisular India. It occurs from central Himalayas to Assam, Khasi and Mikir hills, lower hills of Bengal and evergreen forests of Western Ghats from Konkan to Kanyakumari as well as Nicobar Islands. Indian long pepper is mostly derived from the wild type mainly from Kerala, Assam, West Bengal, Nepal, Uttar Pradesh, North East region and Andhra Pradesh. It is also cultivated to a limited extent in parts of Bengal, Assam, Maharashtra, Tamil Nadu, Orissa, Andhra Pradesh, Arunachal Pradesh, Meghalaya and Manipur (Atal and Ojha, 1965). The chromosome number of *P. longum* varies from 2n = 24 to 2n = 96. The reported chromosome numbers of *P. chaba* are 2 n =24 and 104, *Piper peepuloides* 2n = 156 and *P. mullesua* 2n = 132. Many related species have been reported in India (Ravindran and Nirmal Babu 1994, Ravindran 2000).

Piper peepuloides Wall occurs mainly in North-Eastern India whereas *Piper retrofractum* Vahl (Syn. *P. chaba* Hunt), comes from South East Asia and is mostly cultivated in Indonesia and Thailand (Hooker, 1886).

25.1.3 Botanical notes and description

Family: Piperaceae.

Piper longum Linn.; syn.; *P. sarmentosum* Wall.; *P. latifolium* Hunter; *P. turbinarium* Noronha.; *Chavica roxburghii* Miq.; *C. sarmentosa* Miq.
Piper peepuloides Wall; Syn. *Chavica peepuloides* Miq.
Piper retrofractum Vahl: Syn. *P. chaba* Hunt,

Piper longum is a slender, aromatic, trailing, dioecious plant with perennial woody roots occurring in the hotter parts of India. It is a perennial creeping undershrub spreading on the ground. Stems creeping, jointed with erect fruiting branches, young shoots downy. Leaves are simple alternate, petiolate or sessile, distinctly dimorphic, 5–9 cm long, 3–5 cm wide, ovate, cordate with broad rounded lobes at base, sub-acute, entire, glabrous on creeping shoots; leaves on the fruiting branches oblong, lanceolate, base unequally cordate. Spikes cylindrical with peduncle, male longer and slender, female 1.3–2.5 cm long and 4–5 mm diameter, fruits ovoid, sunk in fleshy spike turns black from green when ripe. Flowering is throughout the year; flowers are dioecious. Inflorescence is a spike with unisexual, small or minute closely packed flowers and form small clusters of grey berries. The female spikes are with short thick stalk varying from 1.5 to 2.5 cm length and 0.5 to 0.7 cm thickness. The male spikes are slender and longer stalks (2.5 to 7.5 cm), slightly elongate. The fruits

are ovoid drupes, small and completely sunk in the fleshy spikes, fused laterally, pungent, aromatic, spicy, shining dark green when immature and blackish green when fully mature. Female spikes arising singly from leaf axil, is cylindrical, short and stout with multiple fruit. Male spikes also arise from the base of the leaf, is single, long cylindrical and of no economic value. The mature female spikes are collected and dried and this is the commercial form of pippali (Narayan Aiyer and Kolammal, 1966, Ravindran 2000).

The long peppers from Indonesia come from slender climbers rooting at nodes. The branches are swollen at the nodes and the leaves are alternate. Plants of *Piper retrofractum* and *P. peepuloides* are climbers with yellowish orange to red fruits. In addition *P. retrofractum* has reticulate leaves on its fruiting branches with much larger spikes. They have sparser-looking foliage than *P. longum*, the most noticeable difference between the two being that the fruits of Indian long pepper *(P. longum)* are smaller and more pungent than those of Javanese long pepper *(P. retrofractum).* The spikes of *P. retrofractum* are conical while those of *P. longum* (Viswanathan, 1995) are cylindrical.

25.1.4 Economic parts and importance

Long pepper is so called because the fruits are long, cylindrical spikes 5 mm in diameter and 2.5 to 4 cm long. The economic parts are roots and dry spikes of female plants, which are generally used for its several medicinal and spicy properties. Long pepper has a sweet and fragrant aroma but the flavour is bitingly hot, lingering and numbing, belying its innocent smell. Long pepper probably came to Europe much before the now dominant black pepper. During the Roman Empire it was priced about three times that of black pepper. With its taste pungent and sweet at the same time, it was perfect for Roman cookery especially as they were fond of these two taste sensations. Since terpene components are missing in its aroma, long pepper cannot be substituted by ordinary black pepper. Its hot-and-sweet taste goes well with spicy cheese specialities.

The 'Pippalmul' are the roots of *Piper longum* which are sometimes adulterated with those obtained from other wild species of *Piper*. These are mostly dried bits of roots 4–6 cm in length of a dark grey or grayish brown colour with the surface slightly shrunken, and having distinct internodes and swollen nodes with a number of small rootlets and root scars. There is a general resemblance in the anatomical structure between these bits and those of *Piper longum*. The number of primary xylem groups may vary from five to seven, so also the number of radiating bands of vascular tissue. Small thickened cells occur in the cortex of the roots of *Piper longum* but are not evident in the dried specimens. The phloem appears narrower and the cork much darker in colour. The powder is reddish brown to creamish grey and under the microscope shows scalariform vessels, aspetate fibres, simple and compound starch grains measuring 3–14 μm in diameter (*The Ayurvedic Pharmacopia of India*. Parts I and II. Ministry of Health and Family Welfare. Dept of ISM&H. 133–134.)

25.1.5 Histology of *Piper longum* root

The histology of *Piper longum* root was studied by Narayan Aiyer and Kolammal (1966). A transverse section of the root about 4 mm diameter is almost circular and the outline regular. The outermost cork is made up of three-five rows of thin-walled,

elongated cells and appears as a very narrow strip slightly brown in colour and is not evident in many specimens. The cortex has round to oblong, large thin walled parenchymatous cells with large intercellular spaces. The cell walls of the peripheral rows are slightly thickened but not lignified. Most of these cells contain starch grains. A few cortical cells contain minute prismatic crystals of calcium oxalate. Many thick-walled cells and secretary cells are found scattered in the cortex. A wavy endodermis composed of one row of rectangular cells with their side walls slightly thickened. The pith is surrounded by four-six wedge shaped radiating strips of vascular tissue having their wider ends towards the periphery. The cells of the pith are similar to those of cortex. Six groups of evenly spaced primary stem bundles are present outside the pith. In each vascular strip the xylem is composed of xylem vessels, xylem parenchyma and wood fibres and its wider end is crowned with a hemispherical strip of phloem.

One or two rows of thin-walled rectangular cambial cells are present between the xylem and phloem. The phloem is composed of many sieve tubes and companion cells and phloem parenchyma. One or two groups of two to three stone cells are present at the peripheral region of the phloem. There are four to six broad wedge-shaped medullary rays extending from the pith up to the endodermis, with their wider ends at the periphery and alternating with the radiating bands of vascular tissues. The ray cells are all thin walled and heavily loaded with starch grains. Narayan Aiyer and Kolammal (1966) also studied the histology of market samples of long pepper root and found that many samples showed histological similarity to long pepper root with minor differences.

25.2 Chemical composition of long pepper

The constituents responsible for the spicy properties of plants are always secondary metabolism products, that is, they are not involved in primary metabolism hence not vital for the plant. In some cases, it is supposed that the aroma molecules are essentially byproducts of metabolism, in most cases, though, they play an important rôle in attracting pollinators or drive away herbivorous animals. It is somehow a paradox that plants are grown and spread word-wide as food enhancers, although their tasty constituents' intention is to discourage the consumption of the plant.

Fruits contain volatile oil, resin, alkaloids and terpenoids. The dried spikes of long pepper on steam distillation yield an essential oil (0.7%–0.8%). The flavour is characteristic of pepper in pungency and taste, the important flavour compounds being piperine, piperlongumine (present in the major alkaloid in addition to piperine) and pipelartine. These components are responsible for the important medicinal functions, *viz*., laxative, carminative, thermogenic, anthelmintic, digestive, stomachic, emmenagogue.

Long pepper is similar to black pepper in composition but it is less expensive and used as an adulterant of ground black pepper. The approximate composition of the plant is:

Moisture	9.5%
Protein	12.2%
Starch	39.5%
Fibre	5.8%
Total ash	5.9%

Insoluble acid	4.2%
Volatile oil	1.5%
Fixed oil	6.6%
Piperine	4.5%

(All values except moisture are measured on dry basis.)

The active constituents of *P. longum* are the alkaloids. They exhibit characteristic mouthfeel, a pepper-like pungency and pronounced salivation and numbness. The highest content of piperine was found in the underground part of the stem and roots. Piperine content of fruits increases with maturity from 14–16 days (0.53%) to 40–45 days (0.9%).

Piperine is the active principle and principal alkaloid of long pepper (*Piper longum* L.) and constitutes 3–5% (on dry weight basis). The content of piperine (about 6%) is slightly higher than in black pepper and yields upon distillation with water, 1% of a bland, thickish, yellow-green oil of specific gravity 0.861, and resembling ginger in odour. The drug has a peculiar odour and a pungent bitter taste producing numbness on the tongue. It contains piperine (0.15–0.18%), piplartine (0.13–0.20%) and traces of yellow crystalline pungent alkaloid. Other constituents include triacontane, dihydrostigmasterol, a sterol, reducing sugars and glycosides (Pillai *et al*., 2000).

Piperine increases micelle formation, stimulation of active transport of amino acids (gamma-glutamyl transpeptidase), and epithelial cell wall modification due to the affinity of piperine towards fats and fatty substances. In view of these findings it is proposed that piperine ingested in relatively small amounts would act as a thermonutrient. Localized thermogenic action on the epithelial cells would in turn increase the rate of absorption of supplemented nutrient(s).

25.2.1 Chemical composition of *P. longum* oil

Long pepper on distillation yields 0.7–1.5% of light green, viscous oil with a spicy odour resembling that of pepper and ginger and has the following characteristics:

D_{20}	0.8484
N_{20}	1.4769
[α]D	40.1
m.p.	–6 °C
Acid value	7.2
Saponification value	8.9
Saponification value after acetylation	12.8
n-hexadecane	0.7%
n-heptadecane	6.0%
n-octadecane	5.8%
n-eisocane	4.7%
n-heneicosane	2.5%
α-thujene	1.7%
terpinolene	1.3%
zingiberene	7.0%
ρ-methoxy acetophenone	trace
dihdrocarveol	4.3%
phenethyl alcohol	2.1%

25.2.2 Chemical constituents of fruits

The long pepper fruits contain Sylvatin, Sesamin, Diaeuolemin, Piperine, Piplartine, Asarinin, Pluviatilol, Fragenin (E) and (Z), Pipercide, Guineenside, Longamide, piplasterol, Dihudropiperonaline (Shoji *et al*., 1986).

Fruits contain volatile oil, resin, alkaloids (4–5% piperine), a terpenoid substance, piplartine (m.p. 124–125°) and two liquid alkaloids. Sesamin ($C_{20}H_{18}O_6$, m.p. 122°), dihydrostigmasterol and piplasterol are also present. On the other hand, long pepper contains less essential oil than its relatives (about 1%), which consists of sesquiterpene hydrocarbons and ethers (bisabolene, *beta*-caryophyllene, *beta*-caryophyllene oxide, each 10–20%; *alpha*-zingiberene, 5%), and, surprizingly, saturated aliphatic hydrocarbons: 18% pentadecane, 7% tridecane, 6% heptadecane. Medicinal properties are attributed to the alkaloid piperine and piplartine (Atal and Ojha, 1965).

A sample of dried fruit of *P. longum* on steam distillation gave 0.7% of essential oil with spicy odour resembling that of pepper and ginger oils, and has the following characteristics: acid val. 7.2; sap. val. 8.9; sap. val. after acetylation 12.8; soluble in 20 vol. of 95% alcohol; specific gravity – 0.8484; refractive index: 1.4769; Optical rotation – 40.1°. The oil contained: *n*-hexadecane – 0.7; *n*-heptadecane – 6.0; *n*-octadecane – 5.3; *n*-nonadecane – 5.8; *n*-eicosane – 4.7; *n*-heneicosane – 2.5; α-thujene – 1.7; terpinolene – 1.3; zingiberene – 7.0; *p*-cymene – 1.3; *p*-methoxy acetophenone – trace; and a monocyclic sesquiterpene (Handa *et al*., 1963).

25.2.3 Chemical constituents of leaves

Hentriacontane, β-sitosterol, hentriacontane-16-one, triacontanol (Purnima *et al*., 1999).

25.2.4 Chemistry of Piplamool

Chemical constituents of roots

Piperine, Piperlongumine, Piperlonguminine, Piplasterol, Triacontane, Cepharanone B, Aristolactam AII, Piperolactam A, Piperlactam B, Cepharadione A, Cepharadione B, Norcepharadione B, Sesamin, 22,23 – dihrdostigmasterol, methyl-3,4,5-trimethoxy cinnamate, piperadione.

In *P. retrofractum*, piperine, piperlonguminine, sylvatine, guineensine, piperlongumine, filfiline, sitosterol, methyl piperate and a series of piperine-analog retrofractamides are reported. (Banerji *et al*., 1985).

Two alkaloids piperlongumine (probably identical with piplartine; $C_{17}H_{19}O_5N$, m.p. 124°; 0.2–0.25%) and piperlonguminine ($C_{16}H_{19}O_3N$, m.p. 166–168°; 0.2%) have also been identified in the roots (Parthasarathy and Narasimha Rao, 1954; Atal and Ojha, 1965) Long pepper is rarely used medicinally in the United States. *King's American Dispensatory.* 1898.

25.2.5 Structure of piperine

Chemical names: 1-piperoyl piperidine; (E,E) 1-[5-(1,3-Benzodioxol-5-yl)-1-oxo-2, 4-pentadienyl]piperidine; Molecular weight: 285.33; Percentage composition: C = 71.55%, H = 6.71% N = 4.91% O = 16.82%.

25.2.6 Method of extraction

Piperine can be isolated from the oleoresin of *P. longum*. The powdered fruits of the plant are extracted with dichloromethane at room temperature with stirring for 12 hours. The extract is filtered, concentrated in vacuum, and then the residue is purified on an alumina column. Pure piperine can also be obtained by crystallization from ethanol, which may be required for food and/or medicinal usage. Piperine is obtained directly from the crude residue in lesser amounts by extraction in alcohol, filtration and successive crystallization. Alkaline hydrolysis of piperine gives two compounds, one an acid, viz., piperic acid and the other an alkaloid, viz., piperidine. This confirms that piperine is the amide formed between piperidine and piperic acid.

$$\underset{\text{Piperine}}{C_{17}H_{19}O_3N} + H_2O \xrightarrow{NaOH} \underset{\text{Piperidine}}{C_5H_{11}N} + \underset{\text{Piperic acid}}{C_{12}H_{10}O_4}$$

25.2.7 Structure of piperidine

Piperidine is well-known as the hexahydro derivative of pyridine. It is a simple organic heterocyclic nitrogen compound. The structure is shown in Fig. 25.2.

25.2.8 Structure of piperic acid

Qualitative tests confirmed that piperic acid contains one carboxyl, two olefinic double bonds and no free hydroxyl groups. Piperic acid on permanganate oxidation gives first the aldehyde piperonal and then the acid piperonylic acid. Piperonylic acid does not contain any free phenolic hydroxyl groups. On heating with HCl under pressure, it gives the diphenolic acid protocatechuic acid and formaldehyde. This shows that only one carbon is eliminated to give a diphenolic compound. It has previously been established that protocatechuic acid is 3,4-dihydroxybenzoic acid (Fig. 25.3.)

The structure of some of the other important constituents of long pepper are shown in Fig. 25.4.

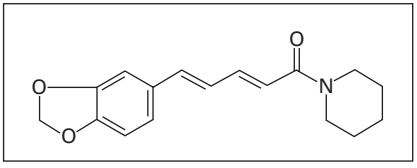
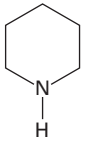
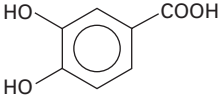


Fig. 25.1 Structure of piperine.



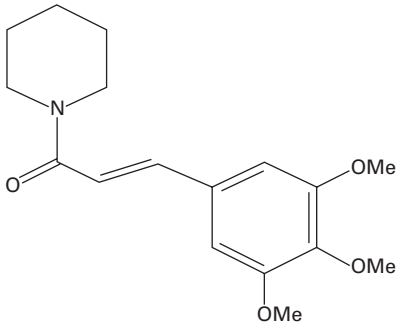
Piperidine

Fig. 25.2 Structure of piperidine.

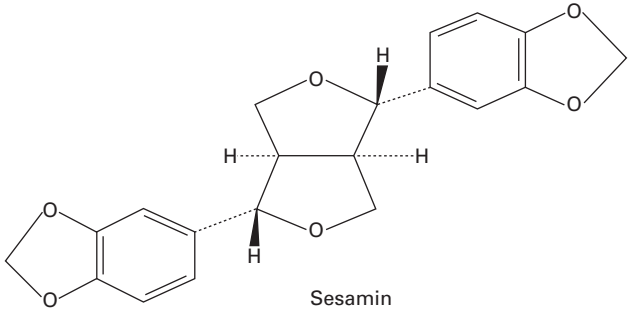


Protocatechuic acid

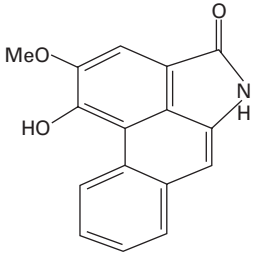
Fig. 25.3 Protocatechuic acid.



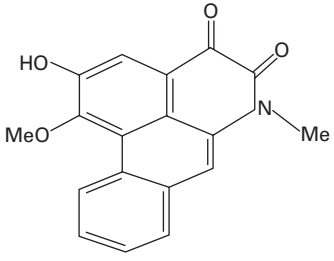
Piplartine (Piperlongumine)



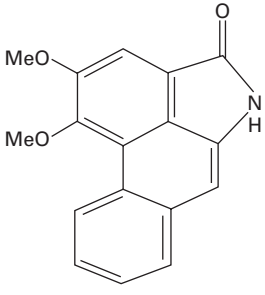
Sesamin



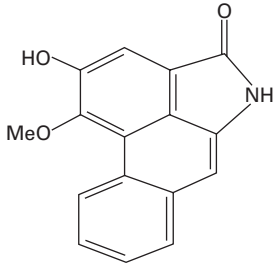
Piperolactam A



Piperadione



Cepharanone B



Aristolactam AH

Fig. 25.4 Structure of some of the other important constituents of long pepper.

25.3 Uses

The fruits are used as a spice and also in pickles, preservatives, foods, beverages, liquors and medicines. Rather remarkably, long pepper is also known and popular in parts of Africa, namely in the Islamic regions of North and East Africa, where it has been introduced by Arab traders therefore long pepper is sometimes found in the complex spice mixtures of Morocco. It is also of some importance for the cuisine of Ethiopia, where long pepper is usually found in the traditional meat stews (*wat*), mostly together with black pepper, nutmeg, clove and turmeric; the usage of turmeric exemplifies the Indian influence in Ethiopian cuisine.

The most important use of long pepper is as a medicinal ingredient in the Indian systems of medicine – Ayurveda, Sidha and Unani. Both fruit and dried roots are used. Besides the spikes thicker stem and roots are used in preparation of 'piplamool' in Ayurvedic Sidha and Unani medicines. The pungent root is considered as healing, stomachic, laxative, anthelmintic, carminative, improves the appetite, useful in bronchitis, abdominal pains, diseases of the spleen, tumour, ascites and relieves biliousness. The fruits as well as roots are attributed with numerous medicinal uses and may be used for diseases of respiratory tract, viz., cough, bronchitis, asthma, etc; as a counter irritant and analgesic when applied locally for muscular pains and inflammation; as snuff in coma and drowsiness and internally as carminative; as sedative in insomnia and epilepsy; as general tonic and haematinic; as cholagogue in obstruction of bile duct and gall bladder; as an emmenagogue and abortifacient; and for miscellaneous purposes as antihelmenthic and in dysentery and leprosy (Atal and Ojha, 1965; Atal *et al*., 1981).

Used in many Ayurvedic traditional remedies, *Piper longum* has been intensively studied. A large number of traditional medicinal preparations have long pepper as one of their constituents. *Piper longum* differs little in its medicinal values from *P. nigrum* as it is less aromatic and more acrid. It is widely used in Ayurvedic and Unani systems of medicine in the prevention and treatment of respiratory congestion and bronchial asthma. Whole spike and piplamool (dried roots and thick stem) are used. Unripe fruit is used as an alternative analgesic for muscular pains and inflammations, vermifuge, carminative, sedative, anti-diarrhoeic, anti-dysenteric against fevers, leprosy, jaundice and as an immunostimulant and tonic; used after childbirth to check post-partum haemorrhage, treat respiratory tract diseases. The dry spikes of female types are used in the Ayurvedic preparations like Pipalarishta, Pipplayasava, Panchakola, Pippalayadiluha and Lavanabhaskar churnam. It is the major constituent of an Ayurvedic preparation, 'Trikatu' which is prescribed routinely for a variety of diseases. The root is used for bronchitis, stomachache, diseases of spleen and tumours. It improves appetite also.

An infusion of the root is prescribed after parturition to induce the expulsion of placenta. The fruits are also used as carminative, sedative in insomnia and epilepsy, as general tonic and haematinic, as cholagogue in obstruction of bile duct and gall bladder; as an enumenagogue and abortifacient; as anthelmintic and in dysentery and leprosy. Ripe fruit is sweetish, pungent, heating, stomachic, aphrodisiac, alternative, laxative and anti-dysenteric.

Pungent root is considered as warming, stomachic, laxative, anthelmintic, carminative, improves the appetite, useful in bronchitis, abdominal pains, diseases of the spleen, tumour, ascites and causes of biliousness. The roots and stems are used for diseases of the respiratory tract like cough, bronchitis, asthma, etc., as counter-irritant and

analgesic when applied locally for muscular pains and inflammation; as snuff in coma and drowsiness.

The fruits contain volatile oil, resin, alkaloids (4–5% piperine) – a terpenoid substance, piplartine (m.p. 124–125°) and two liquid alkaloids. The first alkaloid is closely related to pellitorine producing marked salivation, numbness and a tingling sensation of mucous membranes of the mouth. It showed *in vitro* anti-tubercular activity against *Mycobacterium tuberculosis* H-37 Rv strain; inhibited the growth of the bacillus in 20 µg/ml concentrations (Pruthi, 1976). Piperine has diverse pharmacological activities including nerve depressant and antagonistic effect on electro-shock and chemo-shock seizures as well as muscular uncoordination. The alkaloids (Piperine, pipelartive and piper longument) present in long pepper proved to possess anti-tubercular activity. The fruits are also used as carminative, sedative in insomnia and epilepsy, as general tonic and haematinic, as cholagogue in obstruction of bile duct and gall bladder; as an enumenagogue and abortifacient; as anthelmintic in dysentery and leprosy. Alcoholic extracts of the dry fruits and aqueous extracts of the leaves showed activity against *Micrococcus phygenes* var. *aureus* and *Escherichia coli*. Ether extract of the fruits showed larvicidal properties (Pruthi, 1976). Alcoholic extracts of the dry fruits and aqueous extracts of leaves showed activity against *Micrococcus pyogenes* var. *aureus* and *Escherichia coli*. Ether extract of the fruits showed larvicidal activity (George *et al*., 1947).

A decoction of immature fruits and roots is given for chronic bronchitis, coughs and colds. Fruits and roots are used in palsy, gout, rheumatism, lumbago, an antidote for snake-bite and scorpion sting, as counter-irritant and analgesic when applied locally for muscular pains and inflammation; internally as carminative; as sedative in insomnia and epilepsy; as general tonic and haematinic; and for miscellaneous purposes as anthelmintic, in dysentery and leprosy (Atal and Ojha, 1965). It forms one of the ingredients in various compound preparations used for anorexia, piles, dyspepsia and also in snuffs used in coma and drowsiness (CSIR, 1969). A compound preparation of *P. longum* is also said to be a good remedy for leucoderma. The plant is considered by tribals (Santals) to be useful in splenetic disorders, cholera, dysentery, consumption, puerperal fever and diarrhoea (Jain and Tarafder, 1970).

Experiments were conducted to evaluate the scientific basis of the use of the trikatu group of acrids (long pepper, black pepper and ginger) in the large number of prescriptions in Ayurveda. [3H] vasicine and [3H] sparteine were taken as test drugs. *Piper longum* (long pepper) increased the blood levels of vasicine by nearly 233%. Under the influence of piperine, the active principle of *Piper* species, sparteine blood levels increased more than 100%. The results suggest that these acrids have the capacity to increase the bioavailability of certain drugs. It appears that the trikatu group of drugs increase bioavailability either by promoting rapid absorption from the gastrointestinal tract or by protecting the drug from being metabolized/oxidized in its first passage through the liver after being absorbed, or by a combination of these two mechanisms (Atal *et al*., 1981).

Components of the long pepper fruits have been shown to exert a significant level of protection against liver toxicity induced by tert-butyl-hydroperoxide and carbon tetrachloride by reducing *in vitro* and *in vivo* lipid peroxidation by decreasing the reduction of GSH (Koul and Kapil, 1993; Treadway, 1998).

Rasa (taste) is katu (pungent), Virya (energy) is ushna (hot) and Vipak (post digestive action) is madhura (sweet). The berries are a cardiac stimulant, carminative, tonic, laxative, digestive, stomachic and antiseptic. It is a mild diuretic, alterative,

hepatic and expectorant. The fruit contains volatile oil, starch, protein, alkaloids-piperine and piperlongumine, saponins and lignans. Pippali, like its relative Black pepper, is a powerful stimulant for the digestive and respiratory systems. It is strongly healing, removes colds, congestion and toxins and revives weak organ functions.

In an Indian study published in 1999, *Piper longum* was tested for its efficacy against experimental infection of *Giardia lamblia* in mice. *Piper longum* possessed a demonstrable immunostimulatory activity, both specific and non-specific. In another study, piperine, an active alkaloidal constituent of *Piper longum* was evaluated for its anti-hepatotoxic potential in order to validate its use in traditional therapeutic formulations. The alkaloid exerted a significant protection against tert-butyl hydroperoxide and carbon tetrachloride hepatotoxicity by reducing both *in vitro* and *in vivo* lipid peroxidation and by reducing the depletion of glutathione and total thiols. (Tripathi *et al*., 1999). In an analogous way to the digestive tract delivering nutrients, air passages deliver the most important nutrient of all – oxygen. In fact, the main Ayurvedic formula for better delivery of nutrients at the gastrointestinal level is used in bronchopulmonary conditions as well.

Piper longum, traditionally known in Sanskrit as Pippali, has been used in Ayurveda and related Unani medicine in the prevention and treatment of bronchial asthma. In a study involving 20 children, five to twelve years old, suffering from bronchial asthma with confirmed sensitivity to house dust mite (HDM), long pepper fruits were administered in form of 150 mg (children five years old or younger) or 250 mg (children five to twelve years old) capsules for five weeks (week 1, one capsule a day, week 2, two, week 3, three, week 4, two, week five, one). At the end of five weeks all patients showed significant clinical improvement as assessed by the pulmonary functions tests and decrease in frequency and severity of asthma attacks and decreased sensitivity to HDM skin test. The FVC, FEV1 and MMEFR values were significantly ($p < 0.05$) increased: 1.2253 (before treatment)/1.5123(after); 852.17/1061; 48.88/73.38 respectively. The follow-up of the patients' status after one year found 11 patients with no recurrence of asthma attacks. *Piper longum* contains a minimum of 1% of alkaloid piperine, however, other yet to be identified components may be responsible for the therapeutic action in patients with asthma. (Muhammed and Vladimir 1997).

The dried spikes are thermogenic, carminative (cures flatulence), expectorant, drives off fever, laxative, digestive, antiseptic and tonic. Pippali finds usage in anorexia, indigestion, flatulence, cold, cough, bronchitis, and hiccups, fevers and stomach disorders. The root of long pepper is also attributed with several medicinal properties. The extract is used in cough syrups and as a counter-irritant in analgesics and for all other ailments where fruits are used.

Antiallergic activity of the fruit has been studied. It effectively reduced passive cutaneous anaphylaxis in rats and protected guinea pigs against antigen-induced bronchospasm; a 30% protection of mast cells was observed in an *in-vitro* study (Dahanukar *et al*., 1984). Both alcoholic extract and piplartine extracted from the stems showed significant inhibition of ciliary movements of oesophagus of frog (Banga *et al*., 1964). Piperine decreased the rate and amplitude of respiration and showed nonspecific blockade of acetylcholine, histamine and 5-hydroxytryptamine induced spasm on isolated guinea pig and rabbit intestine (Neogi *et al*., 1971). The oil of fruit has been found to possess significant paralytic action on the nerve-muscle pre-paration of A. lumbricoides (D'Cruz *et al*., 1980). The hepatoprotective effect has been shown in carbon tetrachloride-induced liver damage in rats (Rege *et al*.,

1984). A common use of the fruit is in the prevention of recurrent attacks of bronchial asthma (Pandeya, 1983).

Another important indication is in chronic malaria (Gogate, 1983). In a study of 240 children treated long-term with fruit 58.3% had decreased severity of attacks (Athavale, 1980). In another study 20 children were studied for one year with the same treatment. Eleven had no recurrence. All patients had a strongly positive skin test which became negative in six and decreased significantly in 12 after five weeks of treatment. Along with *Piper nigrum* and *C. officinale* it has been useful in viral hepatitis (Dahanukar and Karandikar, 1984).

25.3.1 Contraindication

Piper longum has been in widespread use for many centuries. The standard doses are well tolerated. No mortality was observed with the powder of the fruit boiled in milk and water administered orally to albino rats in a dose of 1 gm/kg. Acute toxicity studies with piperine, piperlongumine and piperlonguminine were carried out in mice, rats and dogs using oral and intraperitoneal routes. In mice, oral LD (50) was 56.2 + 8.0, 110.1 + 7.8 and 115.3 + 9.5 mg/kg with piperine, piper-lonigumine and piperlonguminine respectively (Singh *et al*., 1973).

25.4 Cultivation

Long pepper is successfully cultivated in well-drained forest soils rich in organic matter. Laterite soils with high organic matter content and moisture holding capacity are also suitable for cultivation. Areas with high rainfall and high humidity with an elevation of 100–1000 m are ideal. It grows well under semi-shady conditions (25–50% shade) in irrigated coconut gardens.

In some hilly parts of Vishakapatnam district of Andhra Pradesh, long pepper is grown for its roots. It is grown as a perennial in small plots of 25–50% and the roots are collected for 10–30 years, the first harvest commencing from 18 months after planting. The stems close to the ground are cut and the roots dug up, cleaned and heaped in shade for a day, after which they are cut into pieces of 2.5–5 cm long. On an average 500 kg of roots are obtained per hectare (Parthasarathy and Narasimha Rao, 1954)

25.4.1 Varieties and cultivars

Viswam is the only released variety in the country so far. The variety was developed by Kerala Agricultural University, Thrissur, India, through clonal selection. It was recommended to grow as an intercrop in irrigated coconut and arecanut gardens. It has a prolonged flowering phase and bears stout, short and thick fruits. Unripened mature fruits are blackish green. The variety gives economic yield for about 240–270 days in a year. Fruits contain about 20% dry matter and 2.83% alkaloid.

25.4.2 Soil and climate

It is grown in the natural habitat and indigenous to wet and warmer parts of India and requires partial shade for ideal growth. It is cultivated as a rainfed crop in Assam and

Meghalaya and as an irrigated crop in other parts. The crop thrives in a variety of soils – fertile forest soil rich in organic matter, laterite soils with high organic matter content and water-holding capacity, limestone soil and well drained fertile black cotton soil. However, light, porous and well drained soil rich in organic content is most suitable for its cultivation. It requires high humidity, high rainfall or frequent irrigation and partial shade for good growth and can be cultivated up to 1000 m elevation.

25.4.3 Planting material

Propagation is through vine cuttings mainly by layering of mature branches or by suckers. Three to five noded cuttings, 15–20 cm long with three 5 cm nodes, taken from any part of the stem, serve as planting material. However, terminal shoots are usually used for planting. The cuttings can be easily rooted in pot mixture and planted in polythene bags or in nursery beds and irrigated on alternate days. Rooting takes about 15–20 days after planting. The rooted cuttings will be ready for transplanting in two months; 100% establishment of cuttings can be observed. March–April is the best time for raising the nursery. Cuttings can be directly planted in the field at the beginning of the rainy season or rooting can be initiated in the nursery before they are transplanted in the field. Mealy bugs attack the roots in the nursery. Spraying or drenching Aldrin 10% reduces Mealy bug attack (Philip *et al*., 1991; Satyabrata Maiti and Presanna Kumari).

25.4.4 Land preparation and planting

With the onset of the monsoon in June, the field is ploughed well, levelled considering the slope of land to facilitate drainage of excess of water, and raised beds of convenient length and breadth are taken. On these beds, pits are dug at 60×60 cm spacing and well-decomposed organic manure at the rate of 100 g/pit is applied and mixed with soil. Rooted vine cuttings or suckers (two/pit) are then transplanted to these pits. The plant will trail on the ground or it can be staked for better yields. The crop cannot survive in waterlogged conditions. Hard wood cuttings of *Sesbania grandiflora* or *Erythrina varigata* or both are planted near the sprouted cuttings of long pepper for providing support and shade. In south India, it is also successfully cultivated as an intercrop in irrigated coconut and arecanut gardens.

25.4.5 Manuring and intercultural operations

Piper longum requires heavy organic manuring (20–25 tonnes of farmyard manure/ha/year) as split application will give a good yield during the economic period of three years. During the first year, organic manure can be applied in pits at the time of planting. In subsequent years, manuring is done by spreading in beds and covering with soil. Crop growth and spike production increases by the application of wood ash. There is no report so far about the use of inorganic fertilizers. No chemical fertilizer has been recommended so far for this crop. A study conducted at Kerala Agricultural University to find out the optimum spacing and manorial recommendation revealed that plant height, number of branches, number of leaves and total dry matter increased with a high dose of organic manure and 30:30:60 kg NPK/ha with an optimum spacing of 50×50 cm. In soils with low fertility the growth of the plant is very poor.

Regular interculture operations can be done as and when weeds grow in beds during the first year. Generally two or three weedings are sufficient. When the crop covers the broad interspaces at the time of manuring the weeds can be removed and manure can be spread in beds and earthed up. The crop should be irrigated during summer months once a week.

25.4.6 Irrigation

It is reported that an unirrigated crop after the onset of monsoon grows vigorously and shows more hardiness than the irrigated crop. But irrigation is most essential during summer months. One or two irrigations a week, depending upon the water-holding capacity of the soil, is needed. Even in the monsoon period, if there is a failure of rain for quite some time, irrigation needs to be given. In irrigated crops, fruit production continues even in summer months.

25.4.7 Diseases and pests

Bordeaux mixture can be applied in pits at time of field planting. Diseases reported are rotting of vine and leaves due to *Colletotrichum* during monsoon season and Necrotic spot and blight of leaves by *Colletotrichum* and *Cercospora* in summer months which sometime cause total or partial crop loss. This can be controlled by Bordeaux mixture (1%) spray during May and subsequently during rainy season. The crop is also affected by mealy bugs especially during summer. The mealy bug infected root of the crop shows stunted growth and yellowing. The insect attacks the healthy roots and sucks its sap. Application of systemic insecticides like Rogar, Nuvacron or Dimecron is recommended. Severe attack of *Helopeltis theivora* is also reported by feeding on tender foliage. Application of neem kernel suspension at 0.25% is recommended for controlling it. *Phytophthora* leaf and stem rot and anthracnose are important diseases of long pepper. Spraying of 0.5% Bordeaux mixture at 15 day intervals and soil drenching of 1.0% Bordeaux mixture at monthly intervals reduce the loss caused by these diseases effectively.

25.4.8 Harvesting

The vines start flowering six months after planting and flowers are produced almost throughout the year. The spikes are harvested, two months after flowering, when they are full-grown but yet unripe, as it is the most pungent stage, and are sun dried. If left without picking they ripen and their pungency is lost to a great extent. Harvesting over-matured or ripened fruits also reduces the quality of the produce and it does not break easily after full drying. Indian long pepper is usually cultivated as a four- to five-year crop as yield starts declining and gradually becomes uneconomic after the fifth year and should be replaced. In such cases fruits, roots and thicker basal stem portions are also collected before crop is abandoned. Stems and roots are cleaned, cut into cylindrical pieces of 2.5–5 cm length and 0.5–2.5 mm thickness, dried in shade and marketed as piplamool. This is not the case with other species (*Piper retrofractum* and *P. peepuloides*) of climbing long peppers which continue to give increased yields even after 15 years. The yield of pipalmul is much higher in these species depending on the year of harvesting.

25.4.9 Post harvesting operation

Harvested spikes are repeatedly exposed in the sun for four to five days until they are perfectly dry. The green spike to dry spike ratio is around 10:1.5. The dried spikes have to be stored in moisture-proof containers. Produce should not be stored for more than a year. Thicker parts of stems and roots are cut and dried for making piplamool and graded depending on the size of roots and stems (Parthasarathy and Narasimha Rao, 1954).

25.4.10 Yield

In Kerala, three to four pickings are made depending upon the maturity of the fruits. The yield of dry spike is 400 kg/ha during the first year when irrigated, increases to 1.0 to 1.25 t/ha in subsequent years and decreases thereafter. Rain-fed crop has a shorter flush of fruiting, resulting in reduced yield. Average yield is 500 kg dry roots/ha. Stems and roots are cleaned, cut into cylindrical pieces of 2.5–5 cm length and 0.5–2.5 mm thickness, dried in shade and marketed as piplamool. The market for medicinal plants is volatile and the economics may vary from year to year.

25.5 Quality specifications

There are three grades of *Piplamul*, Grade I with thick roots and underground stems fetching a higher price than Grade II or III, which comprise either thin roots, stems or broken fragments. Commercial drugs consist almost entirely of transversely cut pieces (length 5–25 mm, diam. 2–7 mm), which are cylindrical, straight or slightly curved, and some with distinct, swollen internodes showing a number of leaf and rootlet scars. Surface of the pieces is dirty light brown in colour.

25.6 Biotechnology

According to the World Health Organization 80% of the world population is dependent upon medicinal plants for primary health care, 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). The popularity of traditional health care in most parts of the world has created a tremendous demand for medicinal plants, which are still collected from their natural habitats leading to their depletion and finally extinction. Medicinal and aromatic plants need to be multiplied faster to meet the demand, with minimum loss to their natural habitats. Micro-propagation technology for fast multiplication of required planting material could be very useful. The advent of molecular biology, gene technology and cell biology has helped understand diseases on the molecular/gene level. Novel target-directed screening assay, automation and miniaturization have resulted in high throughput screening (HTS) approaches thereby improving the industrial drug discovery process drastically (Grabley and Thiericke 1999). Moreoever, *in vitro* gene banks can play a very crucial role in providing the feedstock for this revolution.

Protocols were standardized for rapid clonal multiplication of *Piper longum* and *P. chaba* from shoot tip explants (Sarasan *et al*., 1993; Nirmal Babu *et al*., 1994).

Conversion of root meristem into shoot meristem and its subsequent development to plantlets was reported in *P. longum* (Nirmal Babu *et al*., 1993b). Plants were regenerated from leaf and stem explants of *Piper longum*, *P. chaba,* through direct and indirect organogenesis (Bhat *et al*., 1992, 1995; Sarasan *et al*., 1993). In *P. longum*, root explants were directly regenerated into plantlets (Nirmal Babu *et al*., 1993a).

Piper longum and *P. chaba* could be successfully micropropagated on McCown's Woody Plant Medium (WPM) supplemented with BAP and kinetin. WPM with 3 mgl^{-1} BAP and 1 mgl^{-1} kinetin was found to be ideal for shoot regeneration and their subsequent growth from both leaf and stem explants either with or without intervening callus phase in both the species. Within another 20–30 days, organogenesis in the form of numerous (10–100) shoot primordials could be obtained and over 40% of these primordials showed good elongation and continued normal development. These shoots developed good root systems when growth regulators were removed from the culture medium. When these rooted plantlets were grown in culture medium with 3 mgl^{-1} BAP and 1 mgl^{-1} kinetin there was conversion of root meristem to shoot meristem, which subsequently developed into shoots and then plantlets. Over 90% of the regenerated plantlets could be easily established in soil (Nirmal Babu *et al*., 1993a,b; 1994; 1997; 1999; 2000).

Shoot tips could be conserved under minimal growth conditions with yearly subculture in WPM without any growth regulators. The plantlets could be multiplied normally after one year of storage and the rooted plantlets were successfully planted out. This helps in conservation of long pepper genetic resources in *in vitro* gene banks (Nirmal Babu *et al*., 1999; Peter *et al*., 2002).

Ajith (1997) used RAPD profiling to study the micropropagated plants of *Piper longum* and reported that they are genetically stable. Nirmal Babu *et al*., 2000 and Parani *et al.,* (1997) have standardized RAPD fingerprinting for selecting micropropagated plants of *Piper longum* for conservation. Philip *et al*., (2000) have studied RAPD polymorphism in three different collections of *P. longum*. Banerjee *et al*., (1999) have developed RAPD markers to identify male and female lines of *P. longum*.

25.7 Future

Long pepper is an important medicinal plant used in many drugs and medicinal formulations. There is tremendous demand for commercial long pepper and pipalmul. In India, most of the long pepper is still collected from the wild leading to destruction of these populations in their natural habitats. It is important to encourage commercial cultivation on a larger scale to ensure a continous supply of genuine raw material. Adequate availability of planting material is also a limiting factor for commercial cultivation. Micropropagation supplemented with vegetative propagation can meet these lacunae. The pricing of medicinal plants is highly volatile due to unorganized marketing, discouraging farmers to take up cultivation of medicinal plants on a larger scale. A properly regulated market with guaranteed pricing would help in popularization and cultivation of these important plants.

Identifying genotypes which contain high amounts of the required drug/alkaloid is another area which needs intensified research. It is known that the environment adversely affects the quality parameters of many medicinal plants. Information on suitable soil nutrient and water requirements need to be generated for producing

high-quality products suitable for the pharmaceutical industry. Though some information is available, clinical validation of drugs from long pepper and identification of the new drugs are important areas, which need intensified research.

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26

Lovage

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26.1 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 otu* in Turkish; *Robejji* in Japan, and *Anjedan e roomi* in Iran.

In a 12th-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).

26.1.1 Origin and habitat

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).

26.1.2 Botanical characteristics

Lovage (*Levisticum officinale* W.D.J. Koch) is a dicotyledon 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 (Rechinger, 1987; Simon *et al*., 1984). 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 reaching 1 m spread. The stems are stout, furrowed, striate and tubular, which branches and develops over 2–2.5 m tall every year. 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 tap root, which is ringed crosswise. 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, 1989; Hornok, 1992; Evans, 2002).

26.1.3 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. In 2005, 15 ml, 100 ml and 1 kg of lovage oil are 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.

26.2 Chemical composition

All parts of the plant contain essential oil. The herb oil (*Levistici herba*) is a colorless or very pale yellow and extremely diffusive. Lovage root oil (*Levistici radix*) is an amber to olive-brown colored liquid with root-like odor, suggestive of celery, angelica, liquorice extract, oleoresin and oak moss. The yield and its chemical composition differ significantly depending on the individual genetic, geographical variability, plant age, different plant parts and developmental stages, as well as any post-harvest treatments. 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; Dauksas *et al*., 1999; Bylait *et al*., 2000; Dauksas *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; Dauksas *et al*., 1998). The chemical composition of essential oils distilled from separate botanical parts of this plant is 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, isovaleric acid, maleic acid, angelic acid) (Gijbels *et al*., 1981, 1982; Toulemonde *et al*., 1987; Cu *et al*., 1990; Hogg, 2001; Hogg *et al*., 2001; Ibrahim, 1999).

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 (Dauksas *et al*., 1998). The chemical structures of major phthalides are shown in Fig. 26.1. It was found that 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

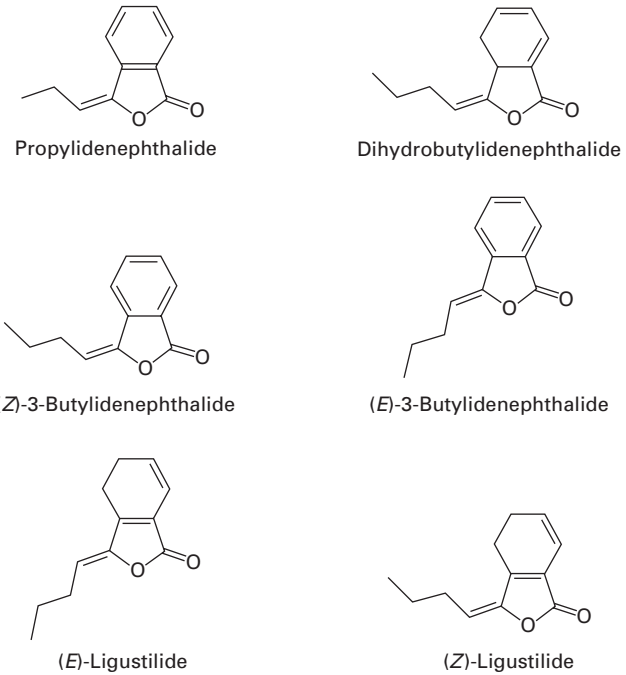


Fig. 26.1 Chemical structures of major phthalides in the essential oil of lovage.

reported to contain β-phellandrene (69.3%), terpinenyl acetate (4.2%) and α-terpineol (2.1%) as the major components (Dyduch *et al*., 2003). The major volatile oil components of lovage parts are shown in Table 26.1.

In the study by Stahl-Biskup and Wichtmann (1991), the essential oil composition of lovage root between seedlings and adult plants was compared. In adult plants the essential oil, *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 are begun 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 flavor substances of the commercial lovage extract were studied by Blank and Schieberle (1993). Aroma extract dilution analysis resulted in six odorants having high sensory relevance. They were identified as 3-hydroxy-4,5-dimethyl-3(*2H*)-furanon (sotolon) with seasoning-like odor, (*E*)-β-damascenone with honey-like odor, 2-ethyl-4-hydroxy-5-methyl-3-(*2H*)-furanone (homofuraneol) and 4-hydroxy-2,5-dimethyl-3(*2H*)-furanon with caramel-like odor, 3-methylbutanoic acid with rancid odor and acetic acid with pungent odor (Fig. 26.2). 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.

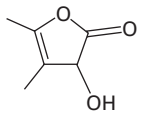
Lovage, as the other plants of the Apiaceae family, contains furocoumarins (Fig. 26.3) (Nielsen, 1970; Murray *et al.*, 1982). Some furocoumarins such as psoralen, 5-methoxypsoralen (5-MOP) and 8-methoxypsoralen (8-MOP) are potent photosensitizers when activated by near-UV light (300–380 nm). They intercalate 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 (1968) as being present in the lovage root. Other cumarines such as umbelliferone and apterin were also isolated and characterized from the lovage (Karlsen, 1968; Fischer and Svendsen, 1976) (Fig. 26.3).

Najda *et al.* (2003) determined the phenolic acids and tannins content of different anatomical parts of the plant (Table 26.2). Total phenolic acid content of different parts of the plant has been reported as: roots (0.12–0.16%), herb (0.88–1.03%), stems

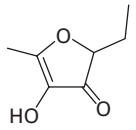
Table 26.1 The major volatile oil constituents of lovage parts

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

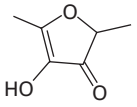
Source: Hogg (2001).



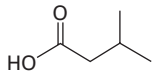
3-Hydroxy-4,5-dimethyl-3(2*H*)-furanone (sotolon)



2-Ethyl-4-hydroxy-5-methyl-3-(2*H*)-furanone

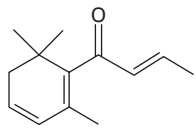


4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone



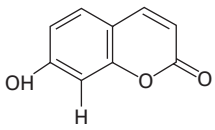
3-Methylbutanoic acid

Acetic acid

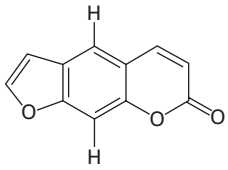


(*E*)-beta-Damascenone

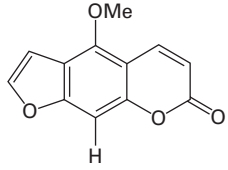
Fig. 26.2 Chemical structures of seasoning-like substances of lovage.



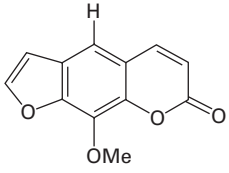
Umbelliferone



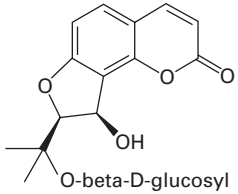
Psoralen



5-Methoxypsoralen



8-Methoxypsoralen



Apterin

Fig. 26.3 Chemical structures of some coumarins isolated from lovage.

Table 26.2 Water content, tannins and total phenolic acids in different anatomical parts of lovage

Anatomical part	Water content %	Tannins %	Total free phenolic acids (mg/100g 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).

(0.30–0.39%), 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%). Lovage also contains β-sitosterol (Nielsen and Kofod, 1963).

26.3 Cultivation and production

26.3.1 Ecological requirements

Lovage can be cultivated in any temperate climate and 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 6–18 °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).

26.3.2 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 to 7.8. Soils originating from swamp are especially suitable for cultivation and harvesting, rooting is easy in these types of soils. For cultivation, the field is prepared for fall sowing with 30–50 cm deep plowing in August. For sowing it is necessary to prepare the soil so as obtain a fine structure and a well-compacted seed-bed. Organic manure application is not recommended directly and is preferable for previous plants. 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, and 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 were 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.

26.3.3 Propagation

Lovage can be propagated by direct seeding, dividing roots or transplanting the transplants. Seeds retain their viability for two 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 42–55 thousand 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.

26.3.4 Pests and diseases

The leaf miner (*Liriomyza* sp.) is the first threat to the well-being of a lovage plant. These pests are tiny black flies, 0.1 inch 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 defense against this pest. Remove and destroy infested leaves. Shallow cultivation of the earth in fall 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.

26.3.5 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, Merkazin (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).

26.3.6 Harvesting and handling

Lovage can survive for 6–8 years, however, in practice 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 one-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 which 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 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 concentration 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 odor (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 May 15. 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 (July 19) (Bylaite *et al.*, 1998).

Harvesting of lovage seeds depends on the market demand and kind of 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 (Dauksas *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 one-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 of roots, handling manners (e.g. cleaning and drying) are very necessary. The soil is shaken off the roots and then before processing the roots are washed, and then they are split into pieces 0.1–0.15 m in length and dried under shade conditions or by artificial driers at 40–50 °C.

26.4 Use 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 soaps, 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 flavoring 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 flavor components in major food products, such as beverages, frozen dairy dessert, candy, gelatins and pudding, 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 dessert, respectively. Lovage (crude) is also mentioned in alcoholic beverages, baked foods, savory and sweet sauces. In this case, largest level used is 0.015% in beverages (Leung and Foster, 1996). According to Opdyke (1978), the acute oral toxicity of root oil has an LD_{50} of 3.4 g/kg and an acute dermal toxicity of LD_{50} 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:

Lobster and potato salad with lovage

Ingredients	*Amount*
Cooked lobster meat	$2^1/_2$ pounds
Red bliss potatoes (cooked and cut into $^1/_2$ inch dice)	1 pound
Mayonnaise	$^1/_2$ cup
Sour cream	$^1/_2$ cup
Freshly squeezed lemon juice	1 tablespoon

Chopped shallots	3 tablespoons
Chopped fresh flat leaf parsley	1/2 cup
Chopped lovage leaves	1/2 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 the small bowl.
2. Add the mayonnaise mixture to the lobster and potatoes.
3. Toss gently until the mixture combined.
4. 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 and cut into 1/2 -inch dice)	1 1/2 pounds
Fresh corn kernels	3 cups
Milk or light cream	2 cups
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 seven 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. Taste to salt and 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	6–10 inches

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)	$^1/_2$ 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 in a soup for five minutes.
2. Add the lovage and cook for one 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. Puree 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 boiling. It can be served hot or cold with chopped lovage as garnish.

26.5 Functional/health benefits

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 angelica but lovage is less known as a herb. The leaves and seeds are often used in seasoning, and the rhizome and roots are used medicinally. Today lovage is still the principal ingredient in many diuretic tea mixtures and 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 disordered stomach, 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, hemorrhoids 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, tablets, and tea ingredients, however, the use of lovage as a herb has cavests (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 Horhammer, 1976; Leung and Foster, 1996). Lovage has been indicated for pedal edema 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 has a value for potential use in antitumor 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 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, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27852, and *Klebsiella pneumoniae* ATCC 3583 (Table 26.3). The oils indicated high activity against tested Gram-positive bacteria especially, *Bacillus subtilis* that was more sensitive than others and a Gram-negative bacterium, *Escherichia coli*. The antibacterial activitiy of the oils has also been determined by measuring the minimal inhibitory concentrations (MICs) against tested bacteria (Table 26.3). The essential oils of mature and ripened seeds exhibited the highest activity against *Bacillus subtilis* with MIC value of 0.93 mg/ml. Also high sensitivity of *Staphylococcus epidermidis* to the mature seed oil was observed with a MIC value of 0.93 mg/ml. The oils showed lowest activity against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, with MIC values more than 15 mg/ml.

Table 26.3 Antibacterial activity of the essential oil of lovage seeds

Microorganisms	IS[a]		MS[b]		RS[c]		Ampicillin[d]
	DD[e]	MIC[f]	DD	MIC	DD	MIC	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

[a] Immature seed; [b]mature seed; [c]ripened seed; [d]tested at a concentration of 10 µg/disk; [e]diameter of inhibition zone (mm) including disk diameter of 6 mm; [f]minimum inhibitory concentration, values as mg/ml oil, inactive (–), moderately active (7–14), highly active (>14).

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27

Pandan wangi

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27.1 Description

Pandan wangi is a common name of a shrub, *Pandanus amaryllifolius* Roxb., in the family *Pandanaceae*. This plant family comprises about 250 species of evergreen trees, shrubs, and scramblers (Bown, 2002). Many of these plants are grown for their architectural appearance, either as landscape plants or as ornamentals under cover, whereas most species can be found in the forest and some are coastal plants. The plants in this family are often known as 'screw pine' because they resemble *Ananas* plants (pineapple) with the spiral arrangement of long, narrow, and strap-shaped leaves. Leaves of some species have a toothed edge along their margin. Among these various species, there are two types of fragrant screw pine, Pandanus leaves (*Pandanus amaryllifolius*) and Pandanus flowers (*Pandanus odoratissimus*), which are divided according to the part of plant that bears scent. *P. amaryllifolius* or pandan wangi is the only Pandanus species with fragrant leaves. It is a short shrub of 1.2–1.5 m (4–5 ft) in height and 60–90 cm (24–36 inch) in width with a stout stem and usually branched low down. Their aromatic, linear, pointed leaves, with no toothed edge, are about 80 cm (32 inch) long and 5 cm (2 inch) wide (Bown, 2002). The plant never flowers, thus the fruits are unknown. Natural distribution is found over Southern India, the Southeast Asia peninsular, Indonesia and Western New Guinea. Nowadays, it is well-known as a characteristic herb of Southeast Asia cuisines, in which its leaves are mainly used as food flavorings.

The genus name, *Pandanus*, is derived from the Indonesian name of the tree, pandan. Common names of *P. amaryllifolius* in many European countries are similar to its origin which include pandanus (French), pandanusz levél (Hungarian) and pandano (Italian, Portuguese and Spanish). It is noted that in European languages, there is no distinction between the single species yielding pandanus leaves and the group of species yielding pandanus flowers. Unlike in Asian countries, the different vernacular names of *Pandanus* plants clearly indicate their identities. For *P. amaryllifolius*, names given include pandan wangi (Malaysian), daun pandan (Indonesian), bai toey or toey hom (Thai), taey (Khmer), tey ban, tey hom (Laotian),

dua thom (Vietnamese), and ban yan le (Chinese). In India and Sri Lanka, the plant is named 'rampe' (Singhalese and Hindi) (Katzer, 2001).

27.2 Cultivation, production and processing

Pandan wangi is mainly grown by farmers of Southeast Asia. Its large distribution as well as the lack of a wild population, especially in Southeast Asia, implies a long tradition of cultivation. As the male flowers are extremely rare, and there is no scientific description of a female flower for this species, its main propagation is by cutting. The easiest and most effective way to propagate *P. amaryllifolius* for landscape and household uses is to place the cuttings of stem or stem tip having at least three or more nodes with root into damp soil located in a hot and dry area with good and indirect sunlight. The young plantlets will grow up to about 2 ft high within 12–18 months depending on the conditions of soil and sunlight. If a mature plant is left to grow longer, a number of young plantlets will develop along the main stem of the mother plant. At the same time, the new prop roots will reach down to the ground like stilts supporting the whole plant. Its population then expands to the surrounding area regardless of soil type and condition. This systematic natural propagation of pandan wangi also reflects the high adaptability of the plant.

Nevertheless, attempts have been made by groups of scientists for the expeditious propagation of this interesting aromatic herb by applying biotechnology. A complete method for the micropropagation of *P. amaryllifolius* through tissue culture has been established (Neelwarne *et al*., 2004). By this method, shoot buds of the mature plant, terminal and lateral, can be cultured in a suitable tissue culture medium until the plantlets are obtained and ready to transfer to soil. Detailed information on the most suitable nutrient and growth regulator compositions of the culture medium as well as the best combination of light and temperature conditions for maximizing the multiplication of the shoot cultures is also provided. Another study emphasizing a protocol for clonal propagation of *P. amaryllifolius* has been reported at the same time (Gangopadhyay *et al*., 2004). In this study, the genetic fidelity of the tissue-culture-raised plantlets was ascertained through identical isozymic and RAPD profiles. Additionally, concentrations of the impact aroma compound, 2-acetyl-1-pyrroline, were comparatively determined in both the mother population and tissue-cultured clones. Micropropagation has been revealed by this study as one of the most viable biotechnological tools for conservation of *P. amaryllifolius* germ plasms.

A sweet and delightful flavor of pandan wangi, which is well-known throughout the world as an important component in Asian cookery, has made the industrial production of both natural extracts and artificial flavorings containing green food colors for use as food additives in Southeast Asian countries enlarge during the past two decades. Because of their strong flavor, cheap prices and ready availability, many types of artificial pandanus essence with deep green color are widely sold in the markets of Southeast Asian countries and replacing the fresh pandanus leaves. In Western countries, Pandanus leaves are purchasable in many forms: powder, paste, fresh frozen or whole dried leaves sealed in plastic bags, most of which are imported from Southeast Asia. During industrial processing, there is not only a decomposition of the impact aroma compounds but also a formation of some off-flavors, which can diversify aroma quality of the Pandanus products. Thus, processing conditions such

as temperature, pH, and heating time have considerable effects on the overall aroma of the processed Pandanus leaves.

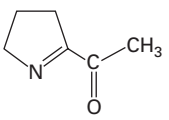
27.3 Chemical structure

Chemical constituents of *P. maryllifolius* Roxb. have been studied in both volatile and higher molecular weight fractions. Early studies reported a number of volatile compounds in groups of alcohols, aromatics, carboxylic acids, ketones, aldehydes, esters, hydrocarbons, furans, furanones and terpenoids. Some of these volatiles were suggested to play a role in aroma of *P. amaryllifolius* leaves, obtained in both fresh and dried forms. Their chemical structures were identified utilizing mainly a combined gas chromatographic and mass spectrometric technique (Teng *et al*., 1979, Jiang, 1999, Wijaya and Hanny, 2003). It was not until the report of Buttery in 1982 that the compound mostly contributed to the flavor of pandanus leaves has been well known, namely, 2-acetyl-1-pyrroline (**1**) (Fig. 27.1). This five-membered N-heterocyclic ring compound was identified for the first time as the important aroma component of cooked rice (Buttery *et al*., 1982) and freeze-dried leaves of *P. amaryllifolius* Roxb. (Buttery *et al*., 1983).

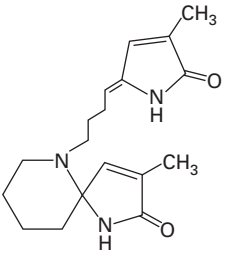
The very low odor threshold value, 0.1 nL/L of water (Buttery *et al*., 1988), has made this volatile the key impact aroma compound frequently found in processed and cooked foods. Its formation in foods has been suggested by many researchers to occur during food processing at elevated temperature through a reaction called 'Maillard' (Weenen, 1998). So far, 2-acetyl-1-pyrroline has been found to occur naturally only in *P. amaryllifolius* Roxb., *Vallaris glabra* Ktze. (bread flower) (Wongpornchai *et al*., 2003), and some aromatic rice varieties such as Basmati rice of India, Khao Dawk Mali 105 of Thailand and Kaorimai of Japan (Buttery *et al*., 1986, Laksanalamai and Ilangantileke, 1993, Tava and Bocchi, 1999). In *P. amaryllifolius*, the compound was found not only in plant leaves but also in stem and root (Gangopadhyay *et al*., 2004). However, its concentrations in fresh plant parts are varied depending on age and growth stage of the plant as well as climate and location of planting. Fresh pandanus leaves are found to contain higher amounts of 2-acetyl-1-pyrroline than those at dried stage, though they hardly smell. The reason lies in the partial distribution of the aroma compound inside and outside the leaves.

A polar moiety of 2-acetyl-1-pyrroline has made the compound more readily dissolve in the fresh leaf tissue where the percentage of water is relatively high. When the leaves are withering and the water content is reduced, the compound is then forced to partition into the gas phase, resulting in the pleasant smell continually released from the withering leaves. Therefore, the extraction of the aroma fraction from *P. amaryllifolius* is more efficient when fresh leaves are used. Solvent extraction at room or lower temperature employing a non-toxic solvent or carbon dioxide, so called supercritical fluid extraction, has been the method of choice and gained higher popularity among food research institutions and industries (Laohakunjit and Noomhorm, 2004; Bhattacharjee *et al*., 2005). Apart from 2-acetyl-1-pyrroline, some other odorants reported include ethyl formate, 3-hexanol, 4-methylpentanol, 3-hexanone and 2-hexanone, *trans*-2-heptenal, β-damascenone, 4-ethylguaiacol and 3-methyl-2-(5H)-furanone. This furanone has often been found as a major component.

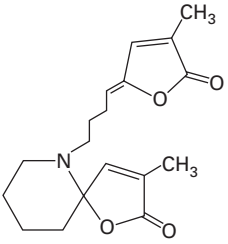
In the polar fractions of *P. amaryllifolius* leaf extracts, a number of alkaloids were identified starting with a piperidine-type alkaloid, (±)-pandamarine (**2**). This



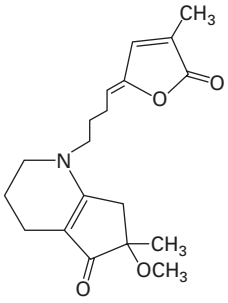
2-Acetyl-1-pyrroline **(1)**



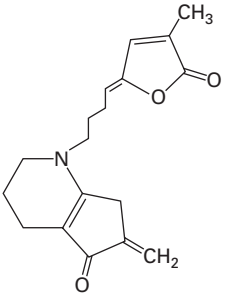
(±) Pandamarine **(2)**



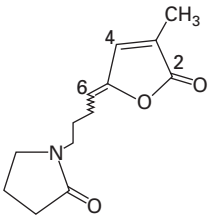
Pandamarilactone-1 **(3)**



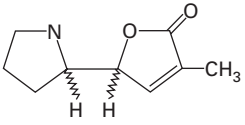
Pandamarilactone-31 **(4)**



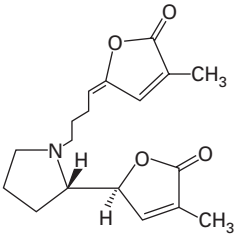
Pandamarilactone-32 **(5)**



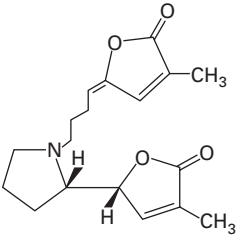
Pandamarilactam-x, –y **(6, 7)**



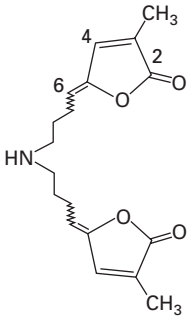
Norpandamarilactonine-A, B **(10, 11)**



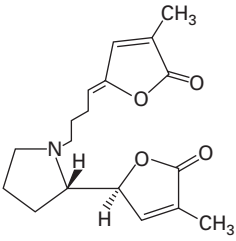
Pandamarilactonine-A **(8)**



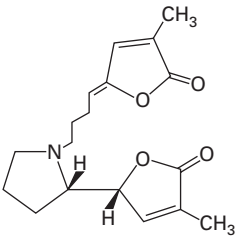
Pandamarilactonine-B **(9)**



6*E*-Pandanamine **(14)**
6*Z*-Pandanamine **(15)**



Pandamarilactonine-C **(12)**



Pandamarilactonine-D **(13)**

Fig. 27.1 Structures of alkaloids found in leaves of *P. amaryllifolius.*

compound was isolated as a crystal and its structure was determined by X-ray diffraction (Byrne *et al.*, 1992). Three more piperidine alkaloids, pandamarilactone-1(**3**), pandamarilactone-31(**4**) and pandamarilactone-32 (**5**), were isolated later on (Nonato

et al., 1993). Their structures were elucidated using two-dimensional nuclear magnetic resonance spectroscopy (2D NMR) techniques. These alkaloids have a C9-N-C9 skeleton and were suggested to be derived biologically from 4-hydroxy-4-methylglutamic acid.

Two pyrrolidinone alkaloid isomers, namely pandamarilactam-3x (**6**) and pandamarilactam-3y (**7**) were isolated from the leaves colleted in Jambi, Indonesia (Sjaifullah and Garson, 1996). Six pyrrolidine alkaloids, pandamarilactonine-A (**8**), pandamarilactonine-B (**9**), (Takayama *et al*., 2000), norpandamarilactonine-A (**10**), norpandamarilactonine-B (**11**), (Takayama *et al*., 2001a), pandamarilactonine-C (**12**), and pandamarilactonine-D (**13**) (Takayama *et al*., 2002), were isolated from fresh young leaves planted in Thailand. The alkaloids (**8**), (**9**), (**12**) and (**13**) are stereoisomers and all of them comprise γ-butylidene-α-methyl α,β-unsaturated γ-lactone and pyrrolidinyl α,β-unsaturated γ-lactone moieties in the molecules as shown in Fig. 27.1. Only the later moiety is found in a pair of diastereomeric alkaloids (**10**) and (**11**). These two alkaloids were isolated as amorphous powder and present as minor constituents. Two pandanamine isomers, *6E*-(**14**) and *6Z*-(**15**), were isolated in unequal amounts from dried leaves of *P. amaryllifolius*. These pandanamine isomers have a symmetrical structure with two α-methyl α,β-unsaturated γ-lactone moieties and therefore, have been postulated to be a biogenetic precursor of pandamarilactonines and pandamarilactone-1 (Takayama *et al.*, 2001b, Salim *et al*., 2004).

The leaves of *P. amaryllifolius* have also been reported as a rich source of a number of lipophilic antioxidants (Lee *et al*., 2004). These antioxidants include compounds in the group of carotenoids: neoxanthin, violaxanthin, α-carotene, β-carotene, lutein, zeaxanthin, and vitamin E analogues: δ-tocotrienol, γ-tocopherol, α-tocopherol, as well as all-*trans*-retinol. Among all carotenoids of *P. amaryllifolius*, lutein accounts for the majority having the highest concentration of slightly more than half the amount of total carotenoids. The concentration of α-tocopherol is more than 90% of the amount of total vitamin E analogues while all-*trans*-retinol is present in a small amount. The total carotenoid content of *P. amaryllifolius* is as much as those of curry leaves (*Murraya koenigii*), anise basil leaves (*Ocimum basilicum*) and laksa leaves (*Polygonum odoratum*) but eightfold more than those of alfalfa and bell capsicum. The only biomolecule isolated from fresh leaves of *P. amaryllifolius* is an unglycosylate protein, lectin, called pandanin. This single polypeptide chain has the molecular weight of 8.0 kDa and exhibits hemagglutinating activity toward rabbit erythrocytes. Pandanin also possesses antiviral activities against two types of human viruses, herpes simplex virus type-1 (HSV-1) and influenza virus (H1N1) with 3 day's EC50 of 2.94 and 15.63 μM, respectively (Ooi *et al*., 2004).

27.4 Uses in food

The leaf is the main used part of most Pandanus plants that can be utilized in various ways. Fragrant leaves of *P. amaryllifolius* have their center of usage in Southeast Asia: Thailand, Malaysia and Indonesia. Their main function is food flavoring, especially in desserts and sweets. The leaves are usually applied into food as fresh whole leaf or juice. In this way, Pandanus leaves impart not only flavor, but also green color to the food. Since the flavor of *P. amaryllifolius* is similar to that possessed by some famous aromatic rice varieties, for example, Basmati rice of India and Thai jasmine rice known as *khao hom mali*, the leaves often find their way into the rice pot to

enhance the aroma of lesser rice varieties. They are also used to wrap food for cooking, such as chicken wrapped in pandanus leaves, a Thai recipe (*gai haw bai toey*), and are neatly folded into small baskets for filling with puddings and cakes. Also in Thailand, country folk use the leaves to boil with water for drinking purposes, adding refreshment. Dried leaves are available only in the form of herbal tea.

27.5 Functional properties

Besides its culinary value, *P. amaryllifolius* is known in folk medicine for its healing properties. The water extract of fresh leaves has a cooling effect and is excellent for the treatment of internal inflammations, colds, coughs, and measles. A drink made by boiling finely chopped fresh stem or root in water is also used to cure urinary infections. The juice extracted from fresh leaves in combination with that of *Aloe vera* is used to cure some skin diseases. The aromatic herbal tea of well-processed leaves has a cardiotonic function. Additionally, similar to some tropical aromatic herbs, fresh leaves of *P. amaryllifolius* possess repellent activity toward some household insects.

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28

Peppermint

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28.1 Introduction

Mentha is a genus of perennial aromatic herbs belonging to family Lamiaceae, distributed mostly in temperate and sub-temperate regions. The herb (foliage) on distillation, yields essential oil containing a large variety of aroma chemicals in varying composition. These oils and their aroma chemicals command a huge and world-wide demand in international trade. The genus contains about 25 species, of which Japanese mint (*M. arvensis*), Pepper mint (*M. piperita*) Spear mint (*M. spicata*) and Bergamot mint (*M. citrata*) are the better known species of commerce for their oil and aroma isolates like menthol, carvone, linalyl acetate and linalool, etc., for use in pharmaceutical, food, flavour, cosmetics, beverages and allied industries. Peppermint, also known as black mint, candy mint or Mitcham commercially, is a source of fragrant volatile essential oil, known for its sweet flavour. The constituents of peppermint oil are very similar to Japanese mint oil. However, the menthol content is lower in peppermint (35–50%) with other constituents like menthyl acetate, menthone, menthofuran and terpenes like pinene and limonene. Peppermint oil is one of the most popular and widely used essential oils in flavouring of pharmaceuticals and preparations for oral care, cough syrups, chewing gums, confectionery and beverages. The leaves and also its oil have medicinal properties as carminative and gastric stimulant. The oil has been reported to have antibacterial, antiviral, antiparasitic and antifungal activities.

28.2 Description

28.2.1 Botanical description

Peppermint, botanically known as *Mentha piperita* L. is an aromatic perennial herb, producing creeping stolons and belongs to family Lamiaceae. It is the natural hybrid (2n = 6x = 72) from *M. aquatica* L. (water mint, 2n = 96) and *M. spicata* L. (spearmint, 2n = 48). The genus name *Mentha* is derived from the Greek *Mintha*, the name of a mythical nymph who metamorphosed into this plant; and its species name *piperita* is

from the Latin *piper*, meaning pepper, alluding to its aromatic and pungent taste (Tyler *et al.*, 1988). There are three varieties of *M. piperita* L.: variety v*ulgaris* Sole or Mitcham mint, the most widespread throughout the world; variety *sylvestris* Sole or Hungarian mint, and variety *officinalis* Sole. Two varieties of the species, black mint (which has violet-coloured leaves and stems) and white mint (which has pure green leaves) are under cultivation (Briggs, 1993; Bruneton, 1995; Leung and Foster, 1996; Wichtl and Bisset, 1994). The most extensively cultivated is the so-called English or black mint, *M. piperita officinalis rubescens* Camus. This variety yields more volatile oil than white mint (Masada, 1976).

The plant grows from 45 to 80 cm tall, resembling *M. spicata* closely and differing in relatively long petiolated opposite lanceolate leaves and broader inflorescence. The stem is quadrangular, channelled, purplish, somewhat hairy and branching towards the top. The leaves are opposite, petiolate, ovate, sharply seriate, pointed, smoother on the upper than on the under surface, and of a dark green colour, which is paler beneath. The leaf lamina (4–14 cm) possesses hair and glandular trichomes on both the surfaces. Usually the lower surface of leaves contain more glandular trichomes than the upper surface. The inflorescence is verticillate. The flowers are small, purple, and in terminal obtuse spikes, interrupted below, and cymosely arranged. Late in the season, the growth of the lateral lower branches often gives to the inflorescence the appearance of a corymb. The calyx is tubular, often purplish, furrowed, glabrous below, and five-toothed, the teeth being hirsute. The corolla is purplish, tubular, with its border divided into four segments, of which the uppermost is broadest, and notched at its summit. The four short stamens are concealed within the tube of the corolla; the style projects beyond it, and terminates in a bifid stigma. The presences of volatile essential oils in the leaves and other parts of the plant gives the plant a very appealing scent and fills the surrounding air with a pleasant aroma of mint.

28.2.2 Distribution and history of cultivation

Peppermint is found growing wild throughout Europe, North America and Australia along stream banks and in moist wastelands, and is also cultivated under a number of varieties, strains, or chemotypes (Trease and Evans, 1989). It has a long history of cultivation in northern and southern temperate regions along stream banks and in other moist areas. It was believed to be cultivated in ancient Egypt, although the record of cultivation was known to be near London in 1750. In the late 1600s and early 1700s it was first recognized as a distinct species by John Ray, a botanist. The peppermint of commerce today is obtained mostly from cultivation in Bulgaria, Greece, Spain, northern Europe, and the United States (BHP, 1996). The United States is the leading producer of peppermint oil, especially in Washington, Oregon, Idaho, Wisconsin, and Indiana (Tyler *et. al.*, 1988).

Mint leaves have been used in medicine for several thousand years, according to records from the Greek, Roman, and ancient Egyptian eras (Briggs, 1993; Evans, 1991). The origin of peppermint cultivation is disputed, though there is some evidence that it was cultivated in ancient Egypt. Roman naturalist Pliny the Elder (ca. 23–79 C.E.) wrote of its uses by the Greeks and Romans. Peppermint was first recognized as a distinct species by botanist John Ray in his *Synopsis Stirpium Britannicorum* (second edition, 1696), and his *Historia Plantarum* (1704). It became official in the *London Pharmacopoeia* in 1721 (Briggs, 1993; Tyler *et al.,* 1988). Today, peppermint leaf and/or its oil are official in the national pharmacopoeias of Austria, France,

Germany, Great Britain, Hungary, Russia, and Switzerland, and the European Pharmacopoeia.

28.2.3 Economic aspects

The peppermint plant and its many parts are used throughout the world in many different ways and for diverse purposes. The production of peppermint oil by distillation of the cultivated herb is an extensive industry in the United States and around the world. Cultivation of the plant is required because the plants found in the wild are not suitable for the distillation process and the cultivated plants contain much more and better quality oil. The United States is the leading producer of peppermint oil in the world, with Michigan, California, Washington, Oregon, Idaho, Indiana, and Wisconsin leading the way. Peppermint oil is used as a flavouring agent in many different products including decongestants, mouthwashes, chewing gum, toothpastes, and other mint flavoured candies and breath-freshening products. Peppermint oil can cause burning and gastrointestinal upset in some people. Peppermint tea, made from the dry leaves of the peppermint plant, is considered safer than peppermint oil for regular consumption. Peppermint tea has antiseptic properties and is considered a stimulant. It is effective in treating digestive pains caused by gas, colic, gallstones, gingivitis, irritable bowel syndrome, morning sickness, headaches, sore throats, common colds, fevers, insomnia, nervous tension, and it may also increase flow of bile from the gall-bladder.

In Germany, peppermint leaf is one of the most economically important individual herbs. It is licensed as a standard medicinal tea, is official in the German pharmacopoeia, and approved in the Commission E monographs (leaf and oil). It is used as a mono-preparation and also as a component of many cholagogue, bile-duct, gastrointestinal, and liver remedies, and some hypnotic/sedative drugs (Wichtl and Bisset, 1994). In the United States, peppermint leaf is used singly and as a main component of a wide range of digestive, common cold, and decongestant dietary supplement and OTC drug products, in fluid and solid dosage forms. Peppermint leaf and peppermint oil are official in the *U.S. National Formulary* (Briggs, 1993; Tyler *et al.*, 1988).

28.3 Cultivation and production

Peppermint essential oil is of great economic value; however, the cultivation and the production of essential oil are limited by agricultural and environmental factors, the presence of specific pathogens, and by differences in comparative costs (Maffei, 1999). Based on a literature survey, some of the factors affecting essential oil production of *M. piperita* in India have been discussed by Baslas (1970). These factors include type of soil, climate, altitude, fertilizers and drying conditions.

28.3.1 Soil and climate

Peppermint adopts itself well to a wide range of soil and climatic conditions but it thrives best in a fairly cool, preferably moist climate, and in deep soils rich in humus and retentive of moisture but fairly open in texture and well drained, either naturally or artificially. It can be profitably cultivated in plains as well as foothill areas having a sub-tropical climate. For peppermint cultivation, a rich and friable soil, retentive of

moisture should be selected, and the ground should be well tilled 20–25 cm deep. The crop cannot tolerate highly acidic or alkaline soils and requires near neutral soil pH. The crop initially requires lower temperatures and later on, a mean temperature of 20–40 °C is suitable for its vegetative growth. The crop grows well in humid areas which receive 100–110 cm well distributed rainfall. The plants cannot tolerate frost, particularly during the sprouting period. They require ample sunshine during most part of the growing period, and shade is undesirable as it induces higher ester and menthone content in the oil. Day length is a determining factor contributing towards higher oil yield and its quality and 15 h day length is essential. Ellis (1960) has established that for economic production of the oil, the day length must approach 16 h.

Its cultivation has been tried in heavy metal polluted soils in Bulgaria by Zheljazkov and Nielsen (1996). It was established that heavy metal pollution of soil and air at a distance of 400 m from the source of pollution decreased the yields of fresh herbage by 9–16% and the yield of essential oil by up to 14% compared to the control, but did not negatively affect the essential oil content and its quality. Oils obtained from a distance of 400 m from the source of pollution have not been contaminated with heavy metals. Cultivar response to heavy metal pollution was also established. A positive correlation between Pb concentration in leaves and in essential oil was found. Heavy metal concentration in the plant parts was found to be, in order, Cd: roots > leaves > rhizomes > stems; Pb: roots = leaves > rhizomes = stems; Cu: roots > rhizomes = stems = leaves; Mn: roots > leaves > stems = rhizomes; and Zn: leaves > roots > stems = rhizomes. Despite the yield reduction (up to 14%), due to heavy metal contamination, mint still remained a very profitable crop and it could be used as a substitute for the other highly contaminated crops.

28.3.2 Planting and varieties

The field for peppermint planting should be prepared by repeated ploughing and harrowing. The FYM or compost at 10–15 t/ha should be mixed properly at the time of field preparation. All stubble and weeds should be removed before the land is levelled and laid out into beds with appropriate irrigation and drainage arrangements. The new crop is raised through planting stolons although suckers, runners and transplanting of sprouted plants can also be successful. The stolon is an underground stem, formed at the end of the creeping rootstock during winter to overcome the dormancy period. These are white to light cream coloured, smooth, fragile and juicy, which are dug out fresh for planting. The plants are propagated in the spring, when the young shoots from the crop of the previous year attain a height of about 10 cm. January end to early February is the best season for planting stolons. The suckers are transplanted into new soil, in shallow furrows about 50–60 cm apart, lightly covered with about 5 cm of soil. The planted field is irrigated immediately. The new sprouts are produced within 1–2 weeks. They grow vigorously during the first year and throw out numerous stolons and runners on the surface of the ground. After the crop has been removed, these are allowed to harden or become woody, and then farmyard manure is scattered over the field and ploughed in. In this way the stolons are divided into numerous pieces and covered with soil before the frost sets in, otherwise if the autumn is wet, they are liable to become sodden and rot, and the next crop fails.

The Central Institute of Medicinal and Aromatic Plants, Lucknow, has developed improved peppermint cultivars. These are Kukrail, Tushar, Pranjal, CIM Madhuras and CIM Indus, having herb yield potential between 200–225 q/ha and oil content

between 0.40–0.45%. Out of these, CIM Indus provides menthofuran rich oil, while other varieties yield sweet smelling oil usually rich in menthol with a balanced quantity of menthone, menthyl acetate and menthofuran.

The chromosomes in *Mentha* are of relatively small size, responsible for its wide adaptability and world-wide distribution. Shehudka and Korneva (1980) used the seed produced through free pollination of *M. piperita*, characterized by different degrees of seed productivity and made selection of seedlings and clones with high essential oil content (up to 4%) and menthol (up to 65%). Induction of variability through physical and chemical mutagens has also been attempted by several workers. Only a few mutants have reached the stage of advanced field testing. A broad-leafed natural mutant in peppermint crop was identified in a population of Mitcham mint by Singh *et al*. (1982), who found it to grow better under sub-tropical conditions in India.

Interspecific somatic hybridization by protoplast fusion was carried out between *M. piperita* L. cv. Black mint and ginger mint (*M. gentilis* L. cv. Variegata). These protoplasts divided to form calli under the conditions developed for peppermint protoplast culture. Callus-derived shoots were grown to whole plants, and some with intermediate character between the parental plants were tested for their volatile constituents by gas chromatography. Among the four plants investigated, one had three major volatile constituents, menthone, menthol and linalool (the major component of ginger mint oil). Chromosome counts and random amplified polymorphic DNA analysis indicated that it was an inter-specific somatic hybrid between two species (Satoa *et al*., 1996).

Jullien *et al*. (1998) developed an efficient protocol for plant regeneration from protoplasts of peppermint by stepwise optimization of first cell division, microcalli formation and shoot differentiation. Transgenic peppermint plants were obtained by using *Agrobacterium tumefaciens*-mediated gene transfer. Transgenic plants were successfully acclimatized in the greenhouse (Diemera *et al*., 1998). Genetic transformation of *Mentha arvensis* and *M. piperita* with the neomycine phosphotransferase marker gene and a 4S-limonene synthase cDNA from *M. spicata* led to the regeneration of 47 transformed plants. Quantitative and qualitative modifications in monoterpene levels were observed in transgenic plants. Four *M. piperita* transgenic plants contained higher levels of total monoterpenes, whereas monoterpene levels were lower in two *M. arvensis* transgenic lines. In both *M. piperita* and *M. arvensis*, transgenic lines, altered levels of compounds formed directly from geranyl diphosphate such as cineole or ocimene and monoterpene end-products such as pulegone or piperitone were observed (Diemera *et al*., 2001). These data demonstrate the feasibility of modifying essential oil content by the introduction of a cDNA encoding an enzyme involved in monoterpene metabolism.

In temperate conditions, a plantation lasts about four years, the best output being the second year. The fourth-year crop is rarely good. A crop that yields a high percentage of essential oil exhausts the ground as a rule, and after cropping with peppermint for four years, the land must be put to some other purpose for at least seven years. The monocultures of peppermint pose the potential hazards of continuous cropping including eventual suppression of soil fertility, productivity, soil structure, and microbial activity. Intercropping peppermint with soybean in Italy resulted in yield and quality increases in the essential oil, compared to sole peppermint cultivation. The yield was higher by about 50% on an equal land area basis and higher percentages of menthol and lower percentages of menthofuran and menthyl acetate improved the quality of the oil (Maffei and Mucciarelli, 2003). Peppermint is grown as an annual

crop in India. Depending upon nature of land and sub-soil water, a combination of crop rotations and inter-cropping models are practised. The Maize-Lahi-Peppermint, Maize-Potato-Peppermint, Late paddy-Peppermint, and Early paddy-Lahi-Peppermint crop rotations have been found feasible and profitable for sub-tropical conditions.

28.3.3 Nutrient and water management

Peppermint, being a leafy crop, responds favourably to both organic and inorganic fertilizers. Liberal manuring is essential, and the quantity and nature of the manure has a great effect on the characteristics of the oil. Mineral salts are found to be of much value. In the U.S.A., peppermint is given 150 kg N per ha at 60 days age together with 50–80 kg of P_2O_5 and potash (Guenther, 1961). Depending upon the status of the soil fertility, a basal dose of 50–90 kg P_2O_5 and 60–90 kg K_2O should be applied per ha at planting. Of the 120 kg N fertilizer, two-thirds is recommended to be applied in early spring and one-third after the first harvest. Singh *et al*. (1978) in Jammu and Bharadwaj *et al*. (1980) in H.P. found that peppermint crop responds to high N levels such as 120–160 kg/ha. It was applied in three equal splits – at planting, at 60 days age and after the first harvest.

Gupta and Gulati (1971) found that 80, 40 and 20 kg/ha of N, P and K produced maximum herb yield in Tarai tract. Potash is particularly useful against a form of chlorosis or 'rust' (*Puccinia menthoe*) due, apparently, to too much water in the soil, as it often appears after moist, heavy weather in August, which causes the foliage to drop off and leave the stems almost bare, in which circumstances the rust is liable to attack the plants. In the south of France, sewage is extensively used, together with Sesame seeds from which the oil has been expressed. The residues from the distillation of the crop are invariably used as manure. Chemical fertilizers alone are equally unsatisfactory in soils poor in organic matter, but they give excellent results in conjunction with organic manures. Growth and terpene production of *in vitro* generated *M. piperita* plants in response to inoculation with a leaf fungal endophyte were characterized by Mucciarelli *et al*., 2003. The endophyte induced profound effects on the growth of peppermint, which responded with taller plants bearing more expanded leaves. The observed increase of leaf dry matter over leaf area suggested a real improvement of peppermint metabolic and photosynthetic apparatus. A sustained lowering of (+)-menthofuran and an increase of (+)-menthol percentage concentrations were found in plants from both *in vitro* and pot cultures.

Peppermint requires frequent irrigation. It is important to keep the soil constantly moist, although well drained. Absorption of water makes the shoots more tender, thus facilitating cutting, and causes a large quantity of green matter to be produced. Adequate and timely irrigation is necessary for obtaining high herb yield in drier sub-tropical climates. The frequency of irrigation depends upon the soil texture and weather conditions. Gupta and Gulati (1971) estimated through trials in *Tarai* region that peppermint requires 8–10 irrigations of 2 acre inch of water per irrigation during dry summer months till rain sets in. Randhawa *et al*. (1984) reported 5 cm depth of irrigation at 60 mm of cumulative evaporation in Punjab as optimum for maximum yield. Water logging should be avoided by ensuring adequate drainage of rain and irrigation water.

In a study by Clark and Menary (1980), peppermint was harvested twice during the growing season. The first harvest was conducted on 16 February 1979, and the subsequent regrowth was harvested on 25 April 1979. High peppermint oil yields

were associated with high rates of nitrogen fertilizer (100, 200 and 300 kg nitrogen/ha), and high levels of irrigation (50 mm per week) throughout the last half of the growing season. The composition of oil extracted from herb at the commercial harvest date was not significantly affected by either nitrogen or irrigation treatments. The oil yield from regrowth within the same growing season was significantly affected by irrigation and nitrogen treatments applied prior to the first harvest. When 300 kg nitrogen/ha and 50 mm of irrigation weekly (during the last half of the growing season) were applied, the oil yields from regrowth approached the commercial yield obtained from one harvest. Oil from regrowth contained high levels of menthol, menthyl acetate, menthofuran and limonene, and low levels of menthone and cineole.

28.3.4 Pest management

The crop requires frequent tillage as successful mint-growing implies clean culture at all stages of progress. The presence of weeds among the peppermint, especially other species of *Mentha*, is an important cause of deterioration on quality of the oil. Use of herbicides significantly reduces the cost of weed management. Due to slow sprouting and growth rate of the crop in the initial stages, weeds may dominate the crop if proper weed management is not followed. The critical periods for weed interference in peppermint has been found to be between 30–50 days after planting and 15–30 days after the first harvest. Usually 2–3 manual hand weedings are required to keep the weed growth under check. Pre-emergence spray of herbicides like Oxyflourofen, Pendimethalin and Diuron has been found quite effective. However, considering the use of herb and oil for edible purposes, the use of herbicides should be avoided.

Experiments at different locations in Poland were carried out in 1971–1973 on the usefulness of a herbicide Sinbar, which can be successfully applied for the control of dicotyledonous weeds. Successful results were obtained when the preparation was applied at 1–1.5 kg/ha dose before or after the shooting growth of mint (Golcz *et al*., 1975). Sinbar does not control monocotyledonous weeds but it inhibits their development. Sprayings with Sinbar in an appropriate dose and at fixed dates do not have any negative effect on the development of mint and the concentration of essential oil in the crude drug. The residual terbacil (an active ingredient of Sinbar) in Herba Menthae piperitae is 0.008 ppm during harvesting, when Sinbar is applied before and after drying of mint. Later sprayings increased the concentration of terbacil in the crude drug (0.21–0.27 ppm).

Briggs (1973) applied three polybutene preparations to healthy peppermint plants and assessed the effects of these products on the yield and quality of oil derived from the plants. The treatments caused premature ageing of the leaves and the quantitative composition of the oils had altered relative to controls, resulting in low quality. Preparations containing polybutenes are unsuitable for application to peppermint and should not be applied to crops that are to be extracted by steam distillation. The effect of ten different herbicides on the yield of herb, oil yield and oil composition of peppermint was studied by Skrubis (1971). The herbicides increased the yield of fresh herb, but the yield of peppermint oil was not affected. A gas chromatographic examination of the oil showed that the composition of the oils varied with different herbicides.

In another study, 40 different chemical substances with weed killing properties were studied by Pank *et al.* (1986) in cultures of peppermint over thirteen years duration. It was summarized that Chlorbromuron, Simazin and Terbacil can be

recommended for application just before shooting begins, while a mixture of Prometryn and Simazin can be used in established cultures. Alloxydim-Na has proved effective against undesired grasses and Bentazon against dicotyledonous weed. Using Simazin after the first harvest of peppermint plants prevents the growth of weed in the autumn and winter to come. A combination of Diuron and Propyzamid killed *Agropyron repens* in established peppermint cultures in late autumn. In cultures laid out to obtain new planting material Chlorbromuron proved successful, while in the period of beginning growth Desmetryn was applied. The use of chemicals to fight the weed did not result in any yield reduction or morphological variation and did not influence the proportion of essential oil. Similarly, Vaverkova *et al*. (1987) did not find any significant effect of Simazine application on essential oil constituents of peppermint.

The essential oil content and constituent concentrations in various parts of *M. piperita* during growth were studied before and after administration of Sinbar (terbacil). The highest oil content was found in the leaves of the youngest upper part of the stem. Menthol increased during growth with maximum concentrations observed in the flowering stage. Sinbar application caused no changes in essential oil content or in proportional amount of components (Vaverkova and Felklova, 1988). The results of another study have shown that the beginning of bloom may be regarded as a vegetation period giving the highest content of the essential oil in herb and leaves of peppermint, and its greatest amount was found in the youngest leaves (Vaverkova *et al*., 1997). Content of menthol gradually increased to its top in the blooming phase while that of menthone was decreasing. The treatment of peppermint with Terbacil did not influence the essential oil content and its changes during vegetation when compared with that of untreated plants. Similarly, the application of a herbicide formulation did not cause detectable changes in relative representation of main and secondary components of the essential oil. To study Lindane residue dynamics, peppermint was sprayed with a 0.05% formulation in May, and samples taken two months later were found to contain 0.4 ppm lindane; which was reduced to below 0.1 ppm, similar to those in the untreated controls after four months (Beitz *et al*., 1971).

Diseases are generally not the major constraint in the cultivation of peppermint in temperate conditions. Under Indian conditions, rust, powdery mildew, wilt, leaf blight and stolon rot are the five major fungal diseases in regions with high humidity. Of these, the recurrence of leaf blight and rust is more frequent. Leaf blight is caused by *Alternaria tenuis* and *Rhizoctonia* species. These can be checked by application of Mancozeb. The hilly regions are more prone to occurrence of rust, which can also be prevented by Mancozeb. The broad-spectrum, systemic fungicides, Propiconazole and Tebuconazole are used to control rust in peppermint. Garland *et al*. (1999) determined their rate of dissipation in peppermint. At harvest, 64 days after the final application, Propiconazole was detected at levels of 0.06 mg/kg and 0.09 mg/kg of dry weight, and Tebuconazole was detected at 0.26 and 0.80 mg/kg dry weight, in identical trials. Rates of dissipation of Propiconazole and Tebuconazole were lower at a second trial site, where three applications of 125 g/ha a.i. for each fungicide resulted in residue levels of 0.21.

A large number of insect pests attack the aerial and underground part of the crop. Among these, the important ones are leaf roller, white fly and hairy caterpillar, which damage the aerial part. The underground parts are damaged by white grub and termites. Crickets, grasshoppers and caterpillars may also do some damage. Two spotted mite (TSM) is one of the most difficult horticultural pests to control and constitutes a very

significant and real risk to stable commercial peppermint oil production. The incidence of TSM has increased annually in commercial peppermint crops. Under dry conditions high levels of TSM infestation result in excessive leaf loss, particularly lower leaf and can affect the oil quality.

Bienvenu conducted a field survey of commercial peppermint crops to establish which pests are present in peppermint fields and the range of controls currently used or available. Evaluation of the potential to develop an effective integrated pest management program for peppermint production in south eastern Australia based on effective control of TSM was successfully completed. It was noted that the predator mite *Phytoseiulus persimilis* can survive and reduce TSM populations during the critical months of peppermint production (http://www.rirdc.gov.au/comp02/eoi1.html#DAV-178A, Project Title: Potential for IPM in Peppermint growing in South East Australia, RIRDC Project No.: DAV-178A).

Shukla and Haseeb (1996) evaluated some nematicides (aldicarb, carbofuran, ethoprop) and oil cakes (linseed, mustard, neem) against *Pratylenchus thornei* infesting *Mentha citrata, M. piperita* and *M. spicata* in glasshouse experiments. All the treatments were effective in increasing herb weight and oil yield, and minimizing nematode reproduction of all the test species of mint as compared to untreated-inoculated plants. Neem cake was most effective in reducing the reproduction rate of *P. thornei.* The humid-adapted species *Neoseiulus fallacis* (Garman) was the most common phytoseiid mite collected in either humid (>100 cm annual rainfall) or arid (20–45 cm annual rainfall) mint growing regions of Washington, Oregon, Montana, Idaho, and California during 1991–1995. In experimental field plots, this predator gave excellent biological control of *Tetranychus urticae* Koch on peppermint grown under arid conditions in central Oregon when evaluated by an insecticide check method or by the caging of mites (Morris *et al*., 1999).

28.3.5 Harvest and post-harvest management

The herb is cut just before flowering according to local conditions. Sometimes when well irrigated and matured, a second crop can be obtained in next 60–75 days. Harvesting should be carried out on a dry, sunny day, in the late morning, when all traces of dew have disappeared. The first crop is always cut with the sickle to prevent injury to the stolons. In India, the crop planted in January–February, becomes ready for the first harvest in April–June, depending upon crop management. The second harvest is taken after 60–70 days of the first harvest. After harvesting, the herb is spread in shade to reduce the bulk and increase the recovery of oil. The average yield varies around 15–20 t/ha of herb and 60–70 kg oil/ha. The oil yield depends upon the period of wilting, period of stalking (between wilted hay and distillation) and efficiency of distillation.

Changes in essential oil content, CO_2 exchange rate and distribution of photosynthetically fixed $^{14}CO_2$ into essential oil, amino acids, organic acids and sugars were determined in developing peppermint leaves by Srivastava and Luthra (1991). The incorporation of $^{14}CO_2$ into sugars was maximal followed by organic acids, amino acids and essential oil at all stages of leaf development. The incorporation into sugars and amino acids declined as the leaf matured whereas that in essential oil and organic acids increased with leaf expansion and then decreased. The seasonal variations in fatty acid composition were studied by Maffei and Scannerini (1992) in developing peppermint leaves. Chalchat *et al.* (1997) studied the effects of harvest

time on the composition of the essential oil of peppermint. Harvesting at the end of flowering afforded an inversion of the menthone/menthol ratio, yielding an oil that was richer in menthol and therefore more valuable commercially. It was possible to harvest twice a year, thereby increasing the annual yield per hectare. Batches of differing qualities were obtained with a range of menthol/menthone ratios, according to harvest time. Pre-drying of *M. piperita* herbage before distillation did not affect chemical composition, but allowed steam distillation of greater amounts of plant material. Composite oil samples from a field trial having six harvest dates were analyzed to determine the effect of age on their physicochemical properties (Duhan *et al*., 1976). Oil and free menthol content increased with time to a maximum in those plants harvested between 163–178 days. Thereafter, oil content and stem to leaf ratio decreased.

The results of another field trial on the effect of crop age on the yield of herb oil and quality of essential oil of *M. piperita* are reported by Gulati *et al*. (1978). It is indicated that in India, proper harvesting occurs between 145 to 160 days for first harvest and 97 to 111 days for second harvest of the crop. The content of oil and its chemical constituents vary with the growth and developmental stage of the plant. Vaverkova *et al*. (1997) found that the beginning of bloom may be regarded as a vegetation period giving the highest content of the essential oil in herb and leaves of peppermint, and its greatest amount was found in the youngest leaves. Content of menthol gradually increased to its maximum in the blooming phase while that of menthone was decreasing.

On suitable soil and with proper cultivation, yields of 15 to 17 tons of peppermint herb per hectare may be expected. In many places, the custom is to let the herb lie on the ground for a time in small bundles or cocks. In other countries the herb is distilled as soon as it is cut. Again, certain distillers prefer the plants to be previously dried or steamed. The subject is much debated, but the general opinion is that it is best to distil as soon as cut, and the *British Pharmacopoeia* directs that the oil be distilled from the fresh flowering plant. Even under the best conditions of drying, there is a certain loss of essential oil. If the herbs lie in heaps for any time, fermentation is bound to occur, reducing the quality and quantity of the oil, as laboratory experiments have proved. Exposure to frost must be avoided, as frozen mint yields scarcely half the quantity of oil, which could otherwise be secured. A part of the exhausted herb is dried and used for cattle food, for which it possesses considerable value. The rest is cut and composted and eventually ploughed into the ground as fertilizer. There is also a market, chiefly for herbalists, for the dried herb, which is gathered at the same time of year. It should be cut shortly above the base, leaving some leafbuds, not including the lowest shrivelled or discoloured leaves, and dried.

Professor Robert Menary of the University of Tasmania examined the current cultivation practices for peppermint in Australia where growers were struggling to obtain yields comparable to the north-eastern states of the U.S.A., where most of the world's mint oil is produced. He summarized that planting material was often of poor quality, no practical benchmarks were being used to regulate irrigation and nitrogen fertilizer, the two most important inputs during the growing season, in many fields and for many growers, a lack of uniformity of inputs, and resulting variation in yield of herb and oil were the main contributors to the poor overall yields and that many plantings lapsed after relatively few years of production, usually due to poor weed control, general loss of vigour or change of enterprise (http://www.rirdc.gov.au/99comp/eoi1.htm#_Ref460804616, Project Title: Best Practice in Peppermint, RIRDC Project No.: UT-16A).

28.4 Chemical composition

Peppermint oil is a colourless, yellowish or greenish liquid, with a peculiar, highly penetrating odour and a burning, camphorescent taste. It thickens and becomes reddish with age, but improves in mellowness, even if kept as long as ten or fourteen years. The essential oil contains alpha- and beta-pinene, Cineole, Jasmone, Isomenthol, Isomenthone, Ledol, Limonene, Menthofuran, Menthol, Menthone, Menthyl acetate, Neomenthol, Piperitone, Pulegone and Viridiflorol. The chief constituent of peppermint oil is Menthol, but it also contains menthyl acetate and isovalerate, together with menthone, cineol, inactive pinene, limonene and other less important bodies. On cooling to a low temperature, separation of menthol occurs, especially if a few crystals of that substance be added to start crystallization. The value of the oil depends much upon the composition. The principal ester constituent, menthyl acetate, possesses a very fragrant minty odour, to which the agreeable aroma of the oil is largely due. The alcoholic constituent, menthol, possesses the well known penetrating minty odour and characteristic cooling taste.

Peppermint leaf contains luteolin, hesperidin, and rutin; caffeic, chlorogenic, and rosmarinic acids, and related tannins; choline; α- and β-carotenes; gum; minerals; resin; α and γ tocopherols; α-amyrin and squalene triterpenes; volatile oil (1.2–3%) composed mostly of monoterpenes – 29–55% menthol, 10–40% menthone, 2–13% cineole, 1–11% pulegone, 1–10% menthyl acetate, 0–10% menthofuran, and 0.2–6% limonene (Bradley, 1992; Bruneton, 1995; Leung and Foster, 1996; Wichtl and Bisset, 1994). Gherman *et al.* (2000) analyzed seven different peppermint samples by gas chromatography and gas chromatography–mass spectrometry (GC:MS). The main volatile compounds identified by the gas chromatography–mass spectrometric analysis of *M. piperita* were menthol, menthone, isomenthone, 1,8-cineole, menthyl acetate, limonene, β-myrcene and carvone. The active principles of the oil are menthol, menthone, isomenthone, menthyl acetate, α-pinene, β-pinene, champhor, limonene, linalool and piperitone. The qualitative fatty acid composition is dominated by palmitate (16:0), linoleate (18:2) and linolenate (18:3) (Maffei and Scannerini, 1992).

From *M. piperita* leaves, 16 free lipophilic flavonoid aglycones were isolated and identified by Voirin and Bayet (1992). The variation of this flavonoid composition studied by the means of HPLC techniques from the youngest to the oldest leaves showed that the A- and B-ring O-methylation patterns of leaf pairs differ according to leaf age and would indicate the sequential activity of 4′-O- and 6-O-methyl-transferases. Different steps of monoterpene metabolism – disappearance of limonene, accumulation of 1,8-cineole, reduction of menthone to menthol and acetylation of menthol – have been studied in different parts of *M. piperita* leaves of different ages. The analyses of different samples (leaf strips, disks and individual peltate trichomes, translucent or containing crystals from either epidermis) show that all these dynamic changes start at the distal extremity of the leaf and shift progressively towards the base. Except for the peltate trichomes localized within the leaf area, in which a metabolic step is being realized, the trichomes of other parts present a homogeneous monoterpene composition. The measurements of amounts of chlorophyll in two parts, distal and basal, of youngest leaves of terminal buds show that chlorophyll biosynthesis starts also at the distal extremity of the leaf (Voirin and Bayet, 1996).

In *M. piperita* leaves, the peltate glands may be divided into two types according to the presence or the absence of crystals in the head of the trichomes. In both types, the free lipophilic flavonoid aglycones, characterized by UV and chromatographic data, are located in the head of the peltate glands, together with monoterpenes (Voirin

et al., 1993). A qualitative study of free aglycones from one clone of *M. piperita* was carried out weekly for two months by Voirin *et al*. (1994). They found that the flavonoid pattern of the whole plant remained invariable. Principal component analysis recognized three flavonoid groups, corresponding to three terpenoid groups. The study of the effects of photoperiodic treatments showed that the flavonoid pattern is affected by day length.

The variability of the enantiomeric distribution of biologically active chiral terpenes in *M. piperita* plants from different geographical origins was evaluated by solid phase microextraction–gas chromatography–mass spectrometry (Ruiz del Castillo *et al*., 2004) For all chiral terpenes, the enantiomeric composition varied within a very narrow range all over the samples. The enantiomeric composition of chiral terpenes appeared to be independent of the geographical origin of the plant and, thus, any alteration in the characteristic value may be related to an adulteration or inadequate sample handling.

The stereoselective synthesis and organoleptic properties of π-menthane lactones 7α-η are described by Jean-Marc Gaudin (2000). Apart from correcting the published data concerning these compounds, this work has also allowed an unambiguous identification of 7α, 7β and 7γ in Italo Mitcham black peppermint oil. In addition, these lactones are of considerable interest to the perfume industry, due to their exceptional odour intensity and typical coumarin-like note. Areias *et al*. (2001) proposed a reversed-phase high-performance liquid chromatography procedure for the determination of ten phenolic compounds (eriodictyol 7-*O*-rutinoside, eriodictyol 7-*O*-glucoside, luteolin 7-*O*-rutinoside, luteolin 7-*O*-glucoside, hesperetin 7-*O*-rutinoside, apigenin 7-*O*-rutinoside, rosmarinic acid, 5,6-dihydroxy-7,8,3′,4′-tetramethoxyflavone, pebrellin and gardenin B) in peppermint.

28.5 Commercial uses

Peppermint oil, peppermint extract and peppermint leaves are obtained from *M. piperita* plant. In 1998, the peppermint oil was used in 102 cosmetic formulations as a fragrance component. Peppermint extract was used in 35 formulations as a flavouring agent and fragrance component. Peppermint leaves were used in two formulations (Anonymous, 2001).

28.5.1 Uses in food industry

Chemical constituents with antioxidant activity found in high concentrations in plants determine their considerable role in the prevention of various degenerative diseases (Hu and Willett, 2002). Besides the fruits and vegetables that are recommended at present as optimal sources of such components, the supplementation of human diet with herbs, containing especially high amounts of compounds capable of deactivating free radicals (Madsen and Bertelsen, 1995), may have beneficial effects. The incorporation of seasoning based on herbs into everyday meals may be of crucial importance. The benefits resulting from the use of natural products rich in bioactive substances has promoted the growing interest of pharmaceutical, food and cosmetic industries as well as of individual consumers in the quality of herbal produce. Among the important constituents participating in the cell defence system against free radicals are phenolic compounds and also ascorbic acid and carotenoids (Diplock *et al*., 1998, Szeto *et al*., 2002).

Herbs, regardless of the purpose they serve, are used fresh or dried. Enzymatic processes during drying fresh plant tissues may lead to significant changes in the composition of phytochemicals. The evaluation of antioxidant properties of the raw material allows the determination of its suitability as high-quality food beneficial for human health and therefore is of considerable importance. Capecka *et al*. (2004) has presented the results of two assays of antioxidant capability and the content of total phenolics, ascorbic acid, and carotenoids in the fresh and air-dried herbs of peppermint. The highest antioxidant ability, expressed as inhibition of LA peroxidation (TAA), was found for extracts from both fresh and dried oregano. The TAA value for peppermint and lemon balm was significantly lower. In the case of these two species, the process of drying decreased their total antioxidant activity. The ability of scavenging DPPH – free radical measured after five minutes was very high in the extracts from all the tested herbs, exceeding 90%. Comparison of RSA measurements after one and five minutes allowed estimation of the rate of DPPH neutralization. In the case of peppermint and lemon balm extracts, obtained both from fresh and dried plant material, this parameter reached its maximum level after one minute. The content of total soluble phenolics was very high (2600 mg GA 100 g^{-1} f.m.) in dried peppermint. Drying resulted in a considerable increase of total phenolics in the case of oregano and peppermint. A very important compound in herbs of Lamiaceae family is rosmarinic acid, showing a high scavenging DPPH potential. The rosmarinic acid content in peppermint was about 30,000 ppm.

To find the most suitable antioxidant for the stabilization of sunflower oil, the kinetics of peroxide accumulation during oxidation of sunflower oil at 100 °C in the presence of different concentrations of hexane, ethyl acetate and ethanol extracts of six herbs including *M. piperita* was studied by Marinova and Yanishlieva (1997). The strongest action in retarding the autoxidation process was exhibited by the ethanol extracts from *Saturejae hortensis*, followed by the ethanol extracts from *M. piperita* and *Melissa officinalis*. The stabilization factor F for the ethanol extracts (0.1–0.5%) from *Saturejae hortensis* was 1.8–2.3, which is higher than F for 0.02% butylated hydroxytoluene (BHT, F = 1.2).

The effect of different concentrations (0–1.2% v/v) of peppermint oil on the growth and survival of *Salmonella enteritidis* and *Staphylococcus aureus* was studied in nutrient broth by Tassou *et al*. (2000). The addition of mint essential oil reduced the total viable counts of *S. aureus* about 6–7 logs while that of *S. enteritidis* only about 3 logs. The percentage of glucose utilization in the growth medium of both pathogens, was reduced drastically with the addition of essential oil and as a consequence, the assimilation or formation of different compounds, such as lactate, formate and acetate in the growth medium was also affected.

Despite the beneficial effects of *M. piperita* in digestion, we should also be aware of the toxic effects when the herb is not used in the recommended fashion or at the recommended dose. To justify the effects of *M. piperita* herbal teas on plasma total testosterone, luteinizing hormone, and follicle-stimulating hormone levels and testicular histologic features, Akdogan *et al*. (2004) performed a study an its adverse effects on the male reproductive function and found that the follicle-stimulating hormone and luteinizing hormone levels increased and total testosterone levels decreased in the experimental groups compared with the control group; and the differences were statistically significant.

Whole plants of peppermint after etheric oil distillation were tested for *in situ* degradability and *in vitro* gas production (Djouvinov *et al*., 1997). Digestibility of

these by-products was also determined in *in vivo* trials. Peppermint, after etheric oil distillation, contained more crude protein (130 g kg^{-1} DM), and less neutral detergent fibre (583 g kg^{-1} DM) and acid detergent fibre (425 g kg^{-1} DM).

28.5.2 Uses in the pharmaceutical industry

Peppermint oil is the most extensively used of all the volatile oils, both medicinally and commercially. The characteristic anti-spasmodic action of the volatile oil is more marked in this than in any other oil, and greatly adds to its power of relieving pains arising in the alimentary canal. From its stimulating, stomachic and carminative properties, it is valuable in certain forms of dyspepsia, being mostly used for flatulence and colic. It may also be employed for other sudden pains and for cramp in the abdomen. It is also widely used in cholera and diarrhoea.

It is generally combined with other medicines when its stomachic effects are required, being also employed with purgatives to prevent griping. Oil of peppermint allays sickness and nausea, and is much used to disguise the taste of unpalatable drugs, as it imparts its aromatic characteristics to whatever prescription it enters into. It is used as an infants' cordial. The oil itself is often given on sugar and added to pills, also a spirit made from the oil, but the preparation in most general use is Peppermint Water, which is the oil and water distilled together. Peppermint Water and Spirit of Peppermint are official preparations of the *British Pharmacopoeia*. In flatulent colic, Spirit of Peppermint in hot water is a good household remedy, also the oil given in doses of one or two drops on sugar.

Peppermint is good for assisting the raising of internal heat and inducing perspiration, although its strength is soon exhausted. In slight colds or early indications of disease, a free use of peppermint tea will, in most cases, effect a cure, an infusion of one ounce of the dried herb to a pint of boiling water being employed, taken in wineglassful doses; sugar and milk may be added if desired. An infusion of equal quantities of peppermint herb and elder flowers (to which either Yarrow or Boneset may be added) will banish a cold or mild attack of influenza within thirty-six hours, and there is no danger of an overdose or any harmful action on the heart. Peppermint tea is used also for palpitations of the heart. In cases of hysteria and nervous disorders, the usefulness of an infusion of peppermint has been found to be well augmented by the addition of equal quantities of Wood Betony, its operation being hastened by the addition to the infusion of a few drops of tincture of caraway.

The *British Herbal Compendium* indicates peppermint leaf for dyspepsia, flatulence, intestinal colic, and biliary disorders (Bradley, 1992). The European Scientific Cooperative on Phytotherapy indicates its use for symptomatic treatment of digestive disorders such as dyspepsia, flatulence, gastritis, and enteritis (ESCOP, 1997). The German Standard Licence for peppermint leaf tea indicates its use for gastrointestinal and gall bladder ailments. In German pediatric medicine, peppermint leaf (67%) is combined with chamomile flower (33%) as a herbal tea to treat gastric upset in children. It is also used as a component of various 'kidney and bladder' teas for children. Peppermint oil is used as a component of Inhalatio composita (45% eucalyptus oil, 45% pumilio pine oil, 10% peppermint oil) specifically indicated for coryza and nasal catarrh in children (Schilcher, 1997). Peppermint oil is used in the United States as a carminative in antacids, a counter-irritant in topical analgesics, an antipruritic in sunburn creams, a decongestant in inhalants and lozenges, and as an antiseptic or flavouring agent in mouthwashes, gums, and toothpastes (Briggs, 1993; Tyler *et al.*, 1988).

Most modern human studies have investigated peppermint oil rather than peppermint leaf as a treatment for stomach ache (May *et al.*, 1996), spastic colon syndrome (Somerville *et al.*, 1984), postoperative nausea (Tate, 1997), relief of colonic muscle spasm during barium enema (Sparks *et al.*, 1995), irritable bowel syndrome (Carling *et al.*, 1989; Dew *et al.*, 1984; Koch, 1998; Lawson *et al.,* 1988; Pittler and Ernst, 1998; Rees *et al.*, 1979), and headaches (Gobel *et al.*, 1994). The use of peppermint oil for irritable bowel syndrome is based on preparations in enteric-coated capsules, causing a spasmolytic activity on smooth muscles of the gut. In animal tests, the probable mechanism of action has been shown to be the inhibition of smooth muscle contractions by blocking calcium influx into muscle cells (Forster *et al.*, 1980; Giachetti *et al.*, 1988).

Peppermint oil is the major constituent of several over-the-counter remedies for symptoms of irritable bowel syndrome (IBS). Pittler and Ernst (1998) conducted a study to review the clinical trials of extracts of peppermint as a symptomatic treatment for IBS by computerized literature searches to identify all randomized controlled trials. The study indicates that peppermint oil could be efficacious for symptom relief in IBS. In view of the methodological flaws associated with most studies, no definitive judgement about efficacy could be given.

In one double-blind, placebo-controlled multi-centre trial, Enteroplant®, consisting of peppermint oil (90 mg) and caraway oil (50 mg) in an enteric-coated capsule, was studied in 45 patients with non-ulcerous dyspepsia. After four weeks of treatment both the intensity of pain and the global clinical impression were significantly improved for the group treated with the peppermint/caraway combination compared with the placebo group ($p = 0.015$ and 0.008, respectively) (May *et al.*, 1996).

The *British Herbal Compendium* reported carminative, spasmolytic, and choleretic activity (Bradley, 1992). The approved modern therapeutic applications for peppermint are supportable based on its history of use in well established systems of traditional and conventional medicines, extensive phytochemical investigations, *in vitro* studies, *in vivo* pharmacological studies in animals, and human clinical studies.

To examine the antibacterial effects of a wide variety of essential oils (including peppermint oil) on major respiratory tract pathogens, the antibacterial activity of 14 essential oils and their major components was evaluated by agar-plate dilution assay under sealed conditions. Of the selected strains of four major bacteria causing respiratory tract infection, *Haemophilus influenzae* was most susceptible to the essential oils, followed by *Streptococcus pneumoniae* and *Streptococcus pyogenes* (Inoye *et al.*, 2001).

28.5.3 Other economic uses

Peppermint oil was evaluated for larvicidal activity against different mosquito species: *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus.* Application of oil at 3 ml/m^2 of water surface area resulted in 100% mortality within 24 hours for *C. quinquefasciatus*, 90% for *A. aegypti* and 85% for *A. stephensi* (Ansari *et al.*, 2000). The oil showed strong repellent action against adult mosquitoes when applied on human skin. The virucidal effect of peppermint oil against herpes simplex virus was examined by Schuhmachera *et al.* (2003). The inhibitory activity against herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2) was tested *in vitro* on RC-37 cells using a plaque reduction assay. The 50% inhibitory concentration (IC50) of peppermint oil for herpes simplex virus plaque formation was determined

at 0.002% and 0.0008% for HSV-1 and HSV-2, respectively. Peppermint oil exhibited high levels of virucidal activity against HSV-1 and HSV-2 in viral suspension tests. At noncytotoxic concentrations of the oil, plaque formation was significantly reduced by 82% and 92% for HSV-1 and HSV-2, respectively.

The deterrent and toxicity effects of mint, *M. virdis*. and *M. piperita* on mite (*Tetranychus urticae* Koch) were studied under laboratory conditions. Leaf discs treated with increasing concentrations of both materials showed a reduction in the total numbers of eggs laid (Momen *et al*., 2001). The fumigant toxicity of 28 essential oils extracted from various spice and herb plants and some of their major constituents were assessed for adult coleopterans, major stored products insects. The compound 1,8-cineole and the essential oils anise and peppermint were active against *T. castaneum* (Shaaya *et al*., 1991).

28.6 Quality issues

The flavouring properties of the oil are due largely to both the ester and alcoholic constituents, while the medicinal value is attributed to the latter only. The most important determination to be made in the examination of peppermint oil, is that of the total amount of menthol, but the menthone value is also frequently required.

European pharmacopeial grade peppermint oil is the volatile oil distilled with steam from the fresh aerial parts of the flowering plant. Its relative density must be between 0.900 and 0.916, refractive index between 1.457 and 1.467, optical rotation between –10° and –30°, among other quantitative standards. Identity must be confirmed by thin-layer chromatography (TLC), organoleptic evaluation, and quantitative analysis of internal composition by gas chromatography. It must contain 1.0–5.0% limonene, 3.5–14.0% cineole, 14.0–32.0% menthone, 1.0–9.0% menthofuran, 1.5–10.0% isomenthone, 2.8–10.0% menthylacetate, 30.0–55.0% menthol, maximum 4.0% pulegone, and maximum 1.0% carvone (Ph.Eur.3, 1997). French pharmacopeial grade peppermint oil must contain not less than 44% menthol, from 4.5–10% esters calculated as menthyl acetate, and from 15–32% carbonyl compounds calculated as menthone. TLC is used for identification, quantification of compounds, and verification of the absence of visible bands corresponding to carvone, pulegone, and isomenthone (Bruneton, 1995).

The English oil contains 60–70% of menthol, the Japanese oil containing 85%, and the American only about 50%. The odour and taste afford a good indication of the quality of the oil, and by this means it is quite possible to distinguish between English, American and Japanese oils. Menthol is obtained from various species of *Mentha* and is imported into England, chiefly from Japan. The oils from which it is chiefly obtained are those from *M. arvensis* var. piperascens in Japan, *M. arvensis* var. glabrata in China, and *M. piperita* in America. Japan and China produce large quantities of *Mentha* oil, which is greatly inferior to those distilled from *M. piperita*, but have the advantage of containing a large proportion of menthol, of which they are the commercial source. The cheapest variety of peppermint oil available in commerce is partially dementholized oil imported from Japan, containing only 50% of menthol. Adulteration of American peppermint oil with dementholized Japanese oil, known as Menthene, which is usually cheaper than American oil, is frequently practised. The Japanese oil, termed by the Americans corn-mint oil and not recognized by the *United States Pharmacopoeia*, is at best only a substitute in confectionery and other

products, such as tooth-pastes, etc. There are other varieties of so-called peppermint oil on the market that are residues from Menthol manufacture and are inferior even to the oil imported from Japan. These are not suitable for use in pharmacy.

Felklova *et al*. (1982) examined the qualitative and quantitative aspects of different cultural varieties of *M. piperita* volatile oils and found that the Ukrainian variety had the highest content of oil. Ruiz del Castillo *et al*. (2004) found that the enantiomeric composition of chiral terpenes in *M. piperita* is independent of the geographical origin of the plant and thus any alteration in the characteristic value may be related to an adulteration or inadequate sample handling. The enantiomeric composition of bioactive chiral terpenes in *M. piperita* can be used in authenticity studies.

The effects of mint type, planting density, and planting time on the composition and yield of mint oils in *Mentha piperita* and other *Mentha* species in northern Finland were studied by Galambosi *et al*. (1998). The content of menthol in peppermint grown in Finland was low in comparison to international standards. The highest menthol percentage was obtained with the highest plant density (spacing of 10 cm) while the highest yield was achieved by planting in the early spring.

28.6.1 Pesticide residues

Pharmacopoeial grade peppermint leaf must be composed of the dried whole or cut leaf with not more than 5% stem fragments greater than 1 mm in diameter and not more than 10% leaves with brown spots caused by *Puccinia menthae*. The whole leaf must contain not less than 1.2% (ml/g) and the cut leaf must contain not less than 0.9% volatile oil. Botanical identity must be confirmed by macroscopic and microscopic examinations and organoleptic evaluation (Wichtl and Bisset, 1994). The ESCOP peppermint leaf monograph requires that the material comply with the European pharmacopoeia (ESCOP, 1997).

The residue levels of broad-spectrum, systemic fungicides propiconazole and tebuconazole, used to control rust in peppermint were studied by Garland *et al*. (1999). An analytical method, using gas chromatography combined with detection by high-resolution mass spectrometry was developed to allow for the simultaneous monitoring of both pesticides in peppermint leaves and oil. At harvest, 64 days after the final application, propiconazole was detected at levels of 0.06 mg/kg and 0.09 mg/kg of dry weight, and tebuconazole was detected at 0.26 and 0.80 mg/kg dry weight, in identical trials. The Lindane residue dynamics in peppermint was studied by Beitz *et al*. (1971). In the crop sprayed with a 0.05% formulation in May, the residue was reduced to below 0.1 ppm, similar to those in the untreated controls after four months.

Golcz *et al*., (1975) observed that sprayings with Sinbar herbicide in an appropriate dose and at fixed dates do not have any negative effect on the development of mint and the concentration of essential oil in the crude drug. The residual terbacil (an active ingredient of Sinbar) in *Herba Menthae piperitae* was 0.008 ppm during harvesting, when Sinbar was applied before and after drying of mint. Later sprayings increased the concentration of Terbacil in the crude drug (0.21–0.27 ppm). The treatment of peppermint with Terbacil did not influence the essential oil content. Similarly, the application of a herbicide formulation did not cause detectable changes in relative representation of main and secondary components of the essential oil (Vaverkova *et al*., 1997).

28.6.2 Adulterants and safety assessment

Camphor oil, and also cedar wood oil and oil of African Copaiba are occasionally used as an adulterant of peppermint oil, The oil is also often adulterated with one-third part of rectified spirit, which may be detected by the milkiness produced when the oil is agitated by water. Oil of rosemary and oil of turpentine are sometimes used for the same purpose. If the oil contains turpentine it will explode with iodine. If quite pure, it dissolves in its own weight of rectified spirits of wine.

In a report on safety assessment of *M. piperita*, the following has been summarized: the 24-hour oral LD50 for peppermint oil in fasted mice and rats was 2410 and 4441 mg/kg, respectively. Several (but not all) short-term and subchronic oral studies noted cyst-like lesions in the cerebellum in rats that were given doses of peppermint oil containing pulegone, pulegone alone, or large amounts (>200 mg/kg/day) of menthone. Results of a host-resistance assay suggested immunosuppression and/or increased susceptibility to bacterial-induced mortality. Studies on human basophil suspensions suggested that peppermint oil induced histamine release by non-immunological mechanisms. It was negative in a plaque-forming assay. Repeated intradermal dosing with peppermint oil produced moderate and severe reactions in rabbits. Peppermint oil did not appear to be phototoxic. Peppermint oil was negative in the Ames test and a mouse lymphoma mutagenesis assay but gave equivocal results in a Chinese hamster fibroblast cell chromosome aberration assay.

In a carcinogenicity study of toothpaste, mice treated with peppermint oil developed neoplasms at the same rate as those treated with the toothpaste base. In some instances, the rates were comparable to those in mice of the untreated control group. Isolated clinical cases of irritation and/or sensitization to peppermint oil and/or its constituents have been reported, but peppermint oil (8%) was not a sensitizer when tested using the Kligman maximization protocol. In assessing the safety of peppermint oil, extract and leaves, we must be concerned about oral-dosing studies that reported cyst-like spaces in the cerebellum of rats. The results of these studies were difficult to interpret. The findings were not consistent among studies (lesions were noted in some studies but not others), and though the lesions appeared to depend on the pulegone content, no definitive conclusion could be made (a greater NOAEL was reported in a 90-day study using a peppermint oil containing 1.1% pulegone versus a 28-day study that tested a Peppermint Oil containing 1.7% pulegone). The Panel also noted that the large differences between doses within each study made it impossible to pinpoint exactly the dose at which changes first appeared. Noting the lack of dermal exposure studies on peppermint oil, the Panel expected its absorption would be rapid, following that of menthol, a major component. Dermal absorption, however, was not expected to be greater than absorption through the gastrointestinal tract. Metabolism from either route of exposure would be similar-phase 1 metabolism followed by transport to the liver. The Panel was of the opinion that the oral-dose data contained in this report were sufficient to address concerns resulting from the expected rapid absorption. However, the Panel noted the evidence that menthol can enhance penetration. Formulators are cautioned that this enhanced penetration can affect the use of other ingredients whose safety assessment was based on their lack of absorption.

Clinical dermal testing demonstrated that 8% peppermint oil was not a sensitizer, and that 2% peppermint oil produced a small number of positive reactions in dermatitic patients. Because pulegone is toxic, the panel limited it to < = 1% in cosmetic grade peppermint oil, extract, leaves, and water. The panel was confident that this concentration was achievable both by controlling the time of harvest, and through the patented

techniques. Recent data reported that peppermint oil is used at a concentration of < = 3% in rinse-off formulations and < = 0.2% in leave-on formulations. This concentration of use data coupled with the < = 1% restriction on pulegone suggested to the panel that pulegone toxicity would not be seen with cosmetic use. On the basis of the available data, the CIR Expert Panel concluded that peppermint oil, extract, leaves, and water are safe as used in cosmetic formulations. The concentration of pulegone in these ingredients should not exceed 1%.

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29

Perilla

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29.1 Introduction

Perilla (also known as wild coleus, Chinese basil, Perilla mint or purple mint) is a widely used flavouring herb that belongs to the family Lamiaceae. The natural habitat extends from Northeast India to China, occurring up to an altitude of 1200 m. The major producing countries are China, Japan, Korea and India. (Axtell and Fairman, 1992). In South Asia, *Perilla* extends from Kashmir to Bhutan, and from Champaran in India to Myanmar (Misra and Hussain, 1987). *Perilla* was introduced to Japan in the 8–9th century where it is grown extensively now. *Perilla* is widely grown in Korea, where there is also a strong research base for the improvement of the crop. Currently *Perilla* occupies the largest area in Korea, about 50,000 ha. The Asian immigrants introduced *Perilla* into the USA in the 1800s. The Japanese brought *Perilla* seeds with them to cultivate, and the cultivation began mainly on the West Coast. The plants spread to the Ozarks and the Appalachian mountains, where the species become naturalized and spread widely. It is now widely grown in the USA as an ornamental bedding plant.

Perilla is an annual short day plant. Two types occur: green leaved and purple leaved varieties, and each of them has several cultivated forms. The green variety has been described (Yu, 1997) as: *P. frutescens* (L.) Britt., *P. frutescens* var. *acuta* (Kudo) forma *viridis* (Makino); *P. frutescens* var. *crispa* (Decne) forma *viridis* (Makino); *P. frutescens* var. *arguta* (Benth.) Hand-Mazz; *P. frutescens* var. *acuta f. albiflora; P. frutescens* var. *stricta f. viridifolia.*

The purple leaved variety has the following forms:

P. frutescens var. *acuta* (Kudo)
P. frutescens var. *typica* (Makino)
P. frutescens var. *stricta*
P. frutescens var. *crispa*
P. frutescens var. *atropurpurea*
P. frutescens var. *crispa* f. *purpurea* (Makino)

In Japan the following varieties have been recognized (Yu, 1997) for cultivation:

P. frutescens var. *japonica* (Hara)
P. frutescens var. *citriodora* (Ohwi)
P. frutescens var. *crispa* f. *discolor*
P. frutescens var. *crispa* f. *hirtella*
P. frutescens var. *crispa* f. *atropurpurea*
P. frutescens var. *acuta* f. *crispidiscolor*
P. frutescens var. *oleifera.*

Possibly much natural crossing and introgression might have taken place that led to many varieties and forms. The taxonomy of *Perilla* becomes confusing due to the presence of intergrading populations of interspecific and intraspecific hybrids. Recently genetic diversity analysis has been attempted using molecular taxonomic tools (Lee *et al.,* 2002). Yu *et al.* (1997) have compiled the various aspects of *Perilla* and its uses in medicine.

29.1.1 Botanical notes

Perilla is an annual, short-day plant growing about 1.5 m, resembling *Coleus* and basil in its general appearance. The stem is purple, square, leaves opposite, oval, 4–12 cm long and 2.5–10 cm wide; margin serrated, petiole about 2–7 cm long. Leaves are pubescent, gland-dotted and aromatic. Inflorescence is a terminal raceme, 6–20 cm long; flower purple or white; flowering in June–August. Fruit is a greyish brown nutlet containing 1–4 seeds, seeds small, ovoid, and greyish brown to blackish brown, having a mild pungent taste. Seeds contain 38–45% fixed oil.

Cultivated varieties and types

Perilla plants are broadly classified as green and purple varieties, the latter is grown more commonly. From the usage point of view there are bud *Perilla* (*Mejiso*), leaf *Perilla* (*Hajiso*), head *Perilla* (*Hojiso*) and seed *Perilla* (*Shiso-no-mi*). Bud *Perilla* is used as spice together with raw fish. Head *Perilla*, with seeds partially set, is used to spice dried fish and shrimp. Mature seed is used as spice in processed foods, while leaf is used with a variety of dishes (Tanaka *et al.,* 1997). The commonly cultivated types are the following:

P. frutescens var. *acuta* ('Aka Shiso' in Japanese).
Leaves purple, flowers pale purple.
Var. *acuta f. crispidiscolor* ('Katamen Shiso' in Japanese).
Leaves are green above, purple striped beneath, strongly fragrant, mainly used as *Mejiso* and *Hajiso.*
Var. *crispa f. atropurpurea* ('Aka Chirimen Shiso' in Japanese).
Stem reddish purple, leaves light purple below and dark purple above, flower pale purple. Used mainly as *Mejiso* and *Hajiso.*
'Wase Chirimen Shiso': Dwarf type of the above variety, fast growing, used as *Mejiso.*
Var. *crispa* ('Ao Chirimen Shiso' in Japanese).
Leaves green on both sides, flower white. Used for all purpose (as *Mejiso, Hajiso, Hojiso* and *Shisonomi*)

Chemovars and their genetics

Several chemically distinct varieties (chemovars) exist in *Perilla*. Ito (1970), Koezuko *et al.* (1984) and Nishizawa *et al.* (1990) classified *Perilla* based on the predominant

chemical components into:

PA type – perillaldehyde (ca. 71%) and L-limonine (ca. 9%) are the major compounds.
EK type – elsholziaketone is the major component.
PK type – perillaketone (or iso egomaketone) is the major constituent.
C type – major constituent in *trans*-citral.
PP type – contains phenyl propanoids such as myristicin, dill-apiol, elemicin or caryophyllene.
PL type – perillone is the major constituent.

The above chemotypes are genetically stable showing no segregation in self-pollinated progenies. Genetic experiments had elucidated the mechanism behind the inheritance (Tabata, 1997). Many improved cultivars have also been released (Oh *et al.,* 1998; Park *et al*., 1998).

The chemical composition of the various chemotypes is controlled by single genes and follows a simple Mendelian inheritance pattern. A series of multiple alleles (G1, G2 and g) and an independent pair of allele (H, h) have been reported; G1 and G2 are essential for the initiation of the monoterpenoid biosynthesis in *Perilla*. G1 is responsible for the development of EK type, and is dominant over G2 that produces the PK type. Homozygous (gg) plant is the PP type. G1 or G2 in presence of the homozygous H(G-HH) produces the PA type, while the heterozygous ones (G-Hh) produce large quantities of L-limonene, an intermediate in the synthesis of perillaldehyde. The PL type is produced in the presence of the polymeric genes Fr1 and Fr2, which are involved in the conversion of citral into perillene. The C-type, which controls the production of large quantities of citral, is homozygous recessive for the two genes (Fr1 Fr1 Fr2 Fr2). (Koezuko *et al.,* 1986; Yu *et al.,* 1997; De Guzman and Siemonsma, 1999). The biosynthetic pathways and the genes controlling them are given in Fig. 29.1.

29.2 Crop production and management

29.2.1 Cultivation in Japan

Perilla is propagated through seeds. Seeds are often refrigerated (at 0–3 °C). Such

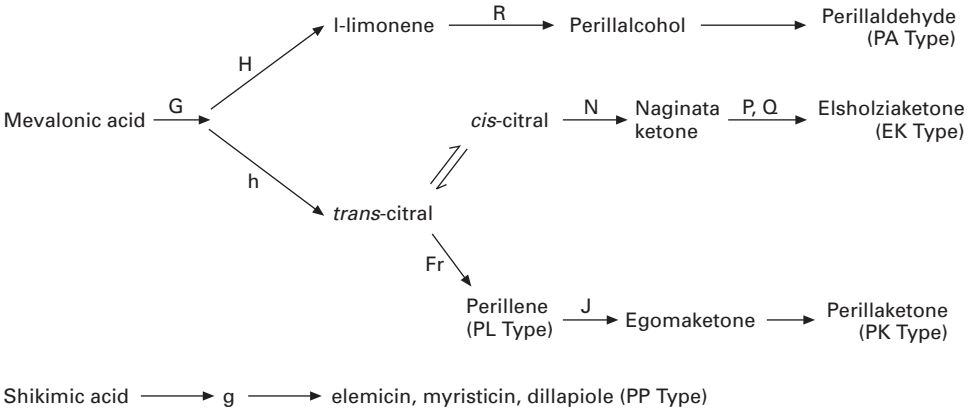

Fig. 29.1 Biosynthetic pathways and genes controlling them (adapted from Tabata, 1997).

seeds are kept at room temperature for about a week before sowing. Seeds are often soaked in gibberellic acid solution (50 ppm) for breaking dormancy. Seeds germinate in 6–10 days, and the optimum temperature is 22 °C (Tanaka *et al*., 1997). Seedbeds 1.2–1.5 m wide and of convenient length are used for seed sowing. Pre-soaking is essential for good germination. Seeds are then suspended in water and spread evenly on the bed at the rate of 9–10 ml per m^2. Bed is then covered with sand and pressed down using a board. The beds are covered with polythene sheeting to retain high humidity.

When *Perilla* is grown for green bud, harvesting is done when the cotyledons are expanded fully and the first pair of true leaves has grown out; which takes about ten days in summer and 15–20 days in other seasons. The purple variety (purple bud) is harvested when two pairs of leaves have grown out. When *Perilla* is grown for heads (head perilla) seedlings are transplanted in field and a basal dressing of 1.5 kg nitrogen, 2 kg phosphorous and 2 kg potash are given per 100 m^2 area. The inflorescences (heads) are harvested when five or six flowers open. The protocol for *Perilla* cultivation is given in Table 29.1.

29.2.2 Cultivation in Korea

In Korea, *Perilla* is also an important oil seed crop, and the annual production is around 36,800 tons. *Perilla* leaves are a by-product and consumed in a salted form or wrapped form with meat and fish (Tanaka *et al.,* 1997). The important cultivars for seed production are Sciwon No. 8, Sciwon No. 10 and Guppo; many local genotypes are also in use. Seeds are sown in open fields in April–June. Leaves are harvested from mid-June to September. Winter cultivation is from October (sowing) to March (harvesting). In winter, additional illumination is required for proper growth. Seed rate is 3 kg/ha, and the plant density is around 250,000/ha. Fertilizer dose recommended is: compost 1000 kg, N 4, P_2O_5 3, K_2O 2 kg/10 ares (1 are = 100 m^2). The hydroponic system of cultivation is also prevalent in Korea and Japan. Both nutrient film and deep flow techniques are used (Park and Kim, 1991).

Table 29.1 Procedure for *Perilla* cultivation in Hokkaido

Sowing time	End of April–early May
Planting field	Any kind of soil except for the field of natural growth and also the field in which *Perilla* was cultivated the previous year.
Planting density	800 plants/are, row width 60 cm, spacing 20–25 cm.
Sowing	30 ml of seed per are. 68,000 seeds weigh 55 g (100 ml). Sow when the soil contains enough moisture, lightly cover the seed with the soil and press it down carefully.
Fertilizer	Fertilizer standard (per are): N 1 kg, P 0.65 kg and K 0.66 kg.
Weeding	Middle of June–middle of July. Remove the weeds before they grow too thick when the weather is favourable. Tall weeds must be removed.
Thinning	Thinning should be started after the 4th or 5th leaf has appeared and should be completed before the plants reach a height of 15 cm.
Supplementary sowing	If the germination is poor, supplementary sowing is carried out on vacant hills.
Pest control	Chemicals used to control insects such as striated chafer, aphid, spider mite and cabbage army worm, must not be applied one month before harvesting.
Harvesting	Hand or machine cutting applied so as to obtain as much as possible.
Drying	Dry the leaves in the sun to a moisture content of about 13%.

(1 are = 100 m^2).
Source: (Tanaka *et al*., 1997).

29.2.3 Cultivation in China

Both direct seed sowing and transplanting are practised in China. The sowing season is March–April. Seeds are sown in rows. Machine sowing is also practised. Frequent application of fertilizer is practised for boosting growth. Chemical fertilizer at the rate of 4–6 kg/ha is applied every week, i.e., about 15–20 kg during the whole growth period (appr. 1.5 kg N, 1.5 kg P and 1 kg K). Barnyard manure is applied in June–August at the rate of about 15–20 tons. Plants require regular watering. For herb oil production, harvesting is done when the flower heads have just grown out, during August–September. Usually 225 kg sun dried leaf yield 0.2–0.25 kg oil. When the whole plant is harvested for oil extraction it is usually in October.

29.3 Chemical composition

Perilla leaves and stems contain an essential oil, commercially known as Perilla oil (*ao-jiso* in Japanese). The oil is produced through steam distillation. The oil is a mixture of mono and sesquiterpenes. The important monoterpenes are: (–) perillaldehyde and (–)-limonene. The important sesquiterpenes are β-caryophyllene and α-farnesene (Masada, 1976). About 50–60% of the essential oil consists of perillaldehyde, which has a powerful fatty-spicy, oily-herbaceons odour and sweet herbaceous taste (Arctander, 1969). Its anti-oxime is about 2000 time sweeter than sucrose (Fujita and Nakayama, 1997). Kang *et al*., (1992) reported the following composition: perillaldehyde (74%), limonene (12.8%), β-caryophellene (3.8%), β-begamontene (3.5%), linalool (2.6%) and benzaldehyde (1.6%). Other characteristic minor compounds having 'perilla-like' odour are (–)-perillyl alcohol, *trans*-shisool, *cis*-shisool, linalool. α-pinene, β-pinene, camphene, 3-octanol, 1-octen-3-ol, allofarnasene, β-farnasene, etc. *Perilla* having this type of oil is classified as perillaldehyde type (Fujita and Nakayama, 1997). Table 29.2 summarizes the important chemical constituents reported from *Perilla*.

29.3.1 Non-Volatile compounds

A number of non-volatile compounds having various biological activities have been reported. They include the following more important ones. Triterpenoids and sterols: perillic acid, steroids such as β-sitosterol, stigmasterol, campesterol, ursolic acid, oleanolic acid, tormentic acid, higher terpenoids and carotenoids, (β-carotene, lutein, neoxanthin, antheraxnathin, violaxanthin), etc. Flavonoids and anthocyanins: apigenin, luteolin, scutellarin and their glycosides, cyanidin glycoside, malonyl shisonin and shisonin. Koezuko *et al.* (1985) have reported genetic studies on anthocyanin production in perilla. Glycosides: perilloside A to D [(4S)-(–)-perillyl β-D-glucopyranoside and its isomers]; eugenyl β-D-glucopyranoside, benzyl β-D-glucopyranoside, phynylpropanoid glucoside perilloside E (6-methoxy-2,3-methylenedioxy-5-allylphenyl β-D-glucopyranoside); two cyanogenic glycosides prunasin and amygdaline; two jasmonoid glucosides (a phenyl valeric acid glucoside, and decenoic acid glucoside), etc. have been reported.

29.3.2 *Perilla* seed lipids

Perilla seed contains about 38–45% fixed oil; the Indian type has 51.7% oil. *Perilla* oil is a highly unsaturated oil having the characteristics (Shin, 1997): refractive

Table 29.2 Important components of essential oil occurring in the various types of *Perilla**

Source/Variety/Type*	Compounds
Shiso, ao-jiso	(–)-perillaldehyde, (–)-limonene, α-pinene
Egoma (1)	Elsholtziaketone, Naginaketone Perillaketone
Lemon-egoma	Citral, Perillone
Ao-jiso (1)	Perillaldehyde (Ca 50%)
(Commercial oil)	Perillyl alcohol, Pinene, Camphene, etc.
Egoma (2)	Elsholtziaketone, Naginaketone Linalool, 1-octen-3-ol, etc.
Several species of Perilla	β-caryophyllene, Elemicin Myristicin, Dillapiole, Isoegomaketone, etc.
Ao-jiso (2)	α-farnesene, allo-farnesene
Ao-jiso (3)	(–) Perillaldehyde, (–) Limonene Perillylalcohol, Linalool Trans-shisool, Cis-shisool
Shiso	Perillaketone, Isoegomaketone, etc.
Shiso, Katamen-jiso	Perillaldehyde, Perillyl alcohol β-caryophyllene, Elemicin Carvone, Phenethyl alcohol, etc.
Tennessee	Perillaketone
Bangladesh	Rosefuran, β-caryophyllene, Perillaketone, etc.

*Japanese names for the varieties Source: Fugita and Nakayama, 1997.

index, 1.4760–1.4784 (25 °C); iodine value, 192.0–196.3; saponification value, 192.7–197.7; unsaponifiable matter, 1.3–1.8%.

The major classes of seed lipids include neutral lipids (91.2–93.9%), glycolipids (3.9–5.8%) and phospholipids (2.0–3.2%). Tsuyuki *et al.* (1978) reported that the total lipids of seed were composed of triglycerides (79.79–82.46%), sterol esters (1.74–1.81%), free fatty acids (2.46–2.65%), diglycerides (1.58%), sterol (0.72–0.89%), pigments (3.06–4.18%), monoglycerides (0.59–2.19%), complex lipids (2.37–2.91%) and others (3.19–5.83%). The important fatty acids present in *Perilla* seed oil are linolenic (54–64%), linoleic, and oleic acids; palmitic and stearic acids are present as minor components.

29.4 Biotechnological approaches

Perilla plants have been subjected to many biotechnological studies, ever since the first report of tissue culture was published by Sugisawa and Ohmishi (1976). In the years that followed many reports came out on callus culture, cell suspension culture, etc. The topic has been recently reviewed by Zhong and Yoshida (1997). Zhong *et al.* (1991) as well as Zhong and Yoshida (1997) summarized the efforts made in anthocyanin production through cell suspension culture, and the process has been scaled up (Table 29.3). Yamazaki *et al.* (1997) summarized the efforts made for the isolation of specifically expressed genes in chemotypes using the technique of differential display of mRNA. cDNAs coding for Shisonin synthesis have been isolated and cloned. The cDNA coding for limonene cyclase was cloned from the PA type of *Perilla* (Yamasaki *et al.,* 1996, Yuba *et al.,* 1995). Hwang *et al.* (2000) have isolated and cloned the cDNAs coding for 3-ketoacyl-ACP synthase in the immature seeds.

Table 29.3 Cell and tissue culture studies of *Perilla*

	Culture conditions	Authors (year)
Metabolites:		
Perilla pigments	MS medium, 100 ppm NAA, 2 ppm KT, 25 °C, with light	Ota (1986)
	LS medium, 10 mM NAA, 1 mM BA, 25 °C, light 3000 lux for 12 h	Koda *et al.* (1992)
	LS medium, 1 mM 2,4-D and 1 mM BA, 25 °C, light at 17–20.4 W/m^2	Zhong *et al.* (1991, 1993, 1994)
Phenylpropanoids	B_5 medium, 5 ppm NAA, 1 ppm KT, 25 °C, light at 2000 lux	Tamura *et al.* (1989)
Caffeic acid	MS medium, 1 ppm 2,4-D, 0.1 ppm KT	Ishikura *et al.* (1983)
Monoterpenes	MS medium, 1 ppm 2,4-D, 5 ppm KT, 25 °C, slightly dark	Sugisawa and Ohnishi (1976)
Sesquiterpene	MS medium, 1 ppm NAA, 1 ppm, KT, 25 °C, light 3000 lux	Nabeta *et al.* (1985)
	Modified MS, 1 ppm 2,4-D, 5 ppm KT	Shin (1986)
Ursolic acid	LS medium, 1 mM NAA, 10 mM KT	Tomita & Ikeshiro (1994)
Cuparene	MS medium, 1 ppm NAA, 1 ppm KT, 25 °C, light at 3000 lux	Nabeta *et al.* (1984)
Essential oil	Modified MS, 1 ppm NAA, 5 ppm KT, 27 ± 2 °C	Shin (1985)
Glucosylation	LS medium, 1 mM 2,4-D, 25 °C, dark	Tabata *et al.* (1988)
	MS medium, 1 mM 2,4-D, 26 °C, dark	Furukubo *et al* (1989)
Resolution	LS medium, 2,4-D, 26 °C, dark	Terada *et al.* (1989)
Morphogenesis	MS medium, NAA, or 2,4-D, BA, NOA	Tanimoto and Harada (1980)

Abbreviations: BA, benzylamino-purine; 2-4,D,2,4-dichlorophenoxyacetic acid; KT, kinetin; NAA, 1-naphthaleneacetic acid; NOA, naphthoxyacetic acid; MS, Murashige and Skoog's; LS, Linsmaier and Skoog's.
Source: Zhong and Yoshida, 1997.

29.5 Functional properties and pharmacological studies

29.5.1 Anti-microbial activity

Perilla leaves are extensively used for food preservation and also for detoxifying fish and crab poisons. *Perilla* leaf extract is reported to be toxic to *Staphylococcus aureus*. However Honda *et al.* (1984) reported that the aqueous extract is inactive against Gram-negative bacteria, and weakly inhibits Gram-positive microbes. Either extract is reported to inhibit dermatophytic fungi such as *Trichophyton, Microsporum, Sabourandites* and *Epidermophyton.* Perillaldehyde is the active ingredient. The steam distillate of leaves inhibited *Salmonella choleraesuis* (Kang *et al.,* 1992). Perillaldehyde has shown a wide spectrum of microbicidal activity.

Effects on CNS

Dried leaf of *Perilla* is prescribed for neurosis in Kampo medicine. Sugaya *et al.* (1981) studied the effect of aqueous extract on CNS and obtained the following positive results: (i) a decrease in motility in rats following oral administration of aqueous extract; (ii) inhibition of nervous reflex following intravenous injection in cats; (iii) significant prolongation of hexabarbitol induced sleep by oral administration of aqueous extract. The sleeping time was prolonged by 80–90% for the PA, PK and TK genotypes; 170% for the PP-M (phenyl propanoid-myristicin) type and 380% for the PP-DM (phenylpropanoid-dillapiole-myristicin) and 52% for the L-PA (limonene-

perilladehyde) type. The sleep prolongation principle in the PP genotype was later identified as dillapiole and myristicin, the former was four times more active than the latter. In the PA type the potentiality factor was identified as perillaldelyde-stigmasterol in combination (Tabata, 1997).

Promotion of intestinal propulsion
Koezuko *et al.* (1985) investigated the effects of *Perilla* leaves on excretory activity. Only the leaves of the PK (perillaketone) type were found to promote intestinal propulsion. The active principle was identified as perillaketone. This compound stimulates the motility of the circular muscles of the intestine.

Toxicity
Perillaketone causes serious lung edema in cattle grazing wild perilla plants. This toxicity was reported to be due to perillaketone. This validates the non-use of the PK type in traditional medicines. Further studies have confirmed that perillaketone is a pulmonary-edema inducing agent (Wilson *et al.,* 1977). In traditional medicine only the PA type is used.

Allergic contact dermatitis
Contact dermatitis due to prolonged contact with *Perilla* is well known in the growing countries. The symptoms include vesicular eruption, diffuse erythrema, mild edema and marked hyperkeratosis with eruptions on fingers. The chemical constituent responsible for the malady is perillaldehyde. However the symptoms can be treated easily with corticosteroid creams.

Antitumour activity
Samaru *et al.* (1993) reported that administration of *Perilla* leaf significantly prolonged the lifespan of mice inoculated with the MM_2 ascites tumour. *Perilla* seed oil, which is rich in n-3-polyenoic fatty acids, inhibits carcinogenecity in the large intestine of rats (Narisawa *et al.,* 1990). Hori *et al.* (1987) reported the effect of *Perilla* seed oil on the pulmonary metastasis of ascites tumour cells in rats.

Yonekura and Sato (1989) showed that *Perilla* seed oil protects rats from induced breast cancer. Narisawa *et al.* (1990) reported the inhibition of MNU (methyl nitrosourea) induced cancer in the large intestine, and inhibition of induced colon cancer was reported by Park *et al.* (1993).

Inhibition of TNF-α over production
Tumour necrosis factor-α (TNF-α), a protein secreted by macrophages shows a strong necrotic activity on tumour cells. However, the over production of TNF-α causes damage to tissues, aggravating inflammation. Ueda and Yamakasi (1993) found that *Perilla* leaf extract from var. *acuta,* reduces the TNF in mice treated with TNF-triggering agents. The reduction is about 86%. This reduction in TNF and subsequent reduction of inflammation could be behind the use of *Perilla* in asthma, allergy, bronchitis, etc. The extract is now used in various products such as foods, cosmetics and other items. Perillaldehyde face cream applied to the skin has been shown to be beneficial in the treatment of contact dermatitis.

Influence of Perilla seed oil in lipid metabolism
The seed oil of *Perilla* has attracted considerable attention in recent years as an ideal health food, because it is rich in unsaturated fatty acids, in particular linolenic acid,

which is essential in maintaining health. Feeding trials have shown that *Perilla* oil reduced levels of cholesterol, phopsholipids and triglycerols in the blood (Nanjo *et al.,* 1993). *Perilla* oil diet also reduces the level of arachidonic acid, a precursor of prostaglandin biosynthesis by 67%. The production of prostaglandin E_2 in kidney is reduced by 75% in the case of *Perilla* oil-fed rats.

Perilla in the treatment of allergy

Allergy is the most widespread immunological disorder in humans, and is regarded by health experts as the most rapidly increasing chronic health problem. Investigations have shown that cytokines such as the tumour necrosis factor (TNF) are constantly associated with allergic reactions. Plasma TNF level becomes elevated in the serum of patients with atopic dermatitis and the level is tightly correlated with plasma histamine (Cooper 1994; Sumimoto *et al*., 1992). The treatment of allergy depends, in addition to allergy avoidance, on antihistamines, corticosteroids, sodium chromoglycate, etc., and is only symptomatic in approach. In Chinese traditional medicine, *Perilla* and its products are used very successfully for allergy treatment. Many reports (Oyanagi, 1997; Yamagata, 1992; Mitsuki, 1992; Kabaya, 1994) indicated that administration of *Perilla* extract – orally, nasally and topically – can relieve the allergy symptoms. The treatment period ranges from one week to three months, and the effect remains for substantially long periods. Chemical studies carried out by Japanese workers (Okabe, 1990; Okuhira, 1993; Oyanagi, 1997) were quite promising, and 73.5% and 80.6% of the patients in two test groups showed significant improvement. The use of *Perilla* in the treatment of allergy has been reviewed by Yu *et al*. (1997).

29.5.2 *Perilla* as a spice

Perilla leaves are strongly aromatic with a strong mint flavour, and having a pleasant, sweet taste. *Perilla* leaves are used as a spice, cooked as potherbs or fried and combined with fish, rice, vegetables and soups. It is also chopped and mixed with ginger rhizome and then added to stir-fries, tempuras and salads in many Asian countries. It is most widely used in Japanese, Korean, Vietnamese, Thai and Chinese cuisines. In India it is used in the north-eastern regions. The purple variety is used to impart colour along with flavour to many pickled dishes, the most famous of such dishes being the Japanese pickled plum. *Perilla* leaf extract was once the most important ingredient in sarsaparilla. It is also used to flavour dental products. The entire plant is very nutritious and is a rich source of vitamins. In Vietnam and Korea *Perilla* leaves are used as a fragrant garnish to noodle soup and spring rolls. In these countries it is the essential flavouring ingredient in dog meat soup (known as Bosintang), in which the *Perilla* leaves not only suppresses the meat smell, but also add flavour and colour (Anon. 2005).

In Japan, *Perilla* is one of the most widely used flavouring herbs. *Perilla* is believed to detoxify the toxic principles of shellfish and other crustaceans and hence is an essential ingredient in all such dishes. *Perilla* leaves are used to garnish '*Sashimi*', the famous Japanese raw fish dish. It is also used in tempuras, a dish of seafood deep fried in sesame oil. *Perilla* leaves are very widely used in pickling Japanese plum, the product is known as '*Umeboshi*'. For this, unripe fruits are harvested, packed with red *Perilla* leaves and pickled. The anthocyanin in the leaves imparts an attractive red colour and flavour to the plum. *Umeboshi* is traditionally served with Tofu, the sea

fish dish that is invariably garnished with *Perilla* leaves, and some tempuras. *Perilla* leaves are also used for pickling and canning a variety of Japanese vegetables. In the USA *Perilla* plants are used widely by Asian immigrants, who introduced the herb to that country.

In spite of the fact that it is a wonderful culinary herb – contributing flavour and colour to a variety of dishes, and its seeds have perhaps the highest concentration of unsaturated fatty acids of class omega 3, and it has varied uses as a herbal medicine for the treatment of cancer, it has not received due attention in other parts of the world and its use is still mainly restricted to South East Asian countries. In view of its great medicinal value at least, it needs to be promoted and its use popularized.

29.6 References and further reading

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30

Potato onion (Multiplier onion)

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30.1 Introduction

Onion is an important crop worldwide and is cultivated commercially in more than 100 countries. Onions and garlic are the most important bulb vegetable crops grown in India, and onion is the only vegetable where India figures prominently in world production and export (Singh and Joshi, 1978), with India being second only to China in terms of area under onion and production.

Alliums are among the oldest cultivated plant species. References to edible alliums can be found in the Bible, the Koran, and in the inscriptions of the ancient civilizations of Egypt, Rome, Greece and China. They are mentioned as a source of food for the builders of the great pyramid of King Cheops, and the Israelites wandering in the desert after the exodus from Egypt bemoaned the lack of appetizing onions. The botanical classification of alliums has recently been reviewed and summarized by Hanelt (1990) and the comments here are largely based on this account, together with the well-known earlier summary by Jones and Mann (1963). The Alliaceae have been included in both the Liliaceae and the Amaryllidaceae by different authorities, but they are now regarded as a separate family. There are more than 500 species within the genus alliums. The best-known feature of the alliums is their characteristic smell and taste.

Cultivated types of *Allium cepa* fall into two broad horticultural groups, the common onion group and the aggregatum group (Hanelt, 1990). Members of the common onion group are grown mostly from seed. They form large single bulbs, and constitute the vast bulk of the economically important varieties. The bulbs of the Aggregatum group are smaller than the common onion because they rapidly divide and form laterals, hence forming clusters of bulbs. Jones and Mann (1963) distinguished two bulb-forming sub-groups: multiplier or potato onions, and shallots. The multiplier or potato onions divide into between three and 20 bulbs which are wider than they are long. These are usually propagated vegetatively. The commercial importance of the Aggregatum group varies between countries. Multiplier onions are cultivated in domestic gardens in Europe, North America, The Caucasus, Kazakhstan and the south-east of

European Russia (Kazakova, 1978). They are grown commercially in Brazil, Southern India and Thailand, where the taste is preferred to that of the common onion group. The bulblets of multiplier or potato onions are widely used in cooking.

30.2 Chemical composition and uses

Potato onions are rich in minerals, inlcuding potassium, phosphorus, nitrogen and calcium. They also contain protein and ascorbic acid. The chemical composition of the onion bulb is presented in Table 30.1. The pungency of onion makes it an important food item, particularly in India. The bulbs and leaves are used raw or cooked (Organ, 1960). The pungency in onion odour is formed by an enzymatic reaction when tissues are damaged, and is due to a volatile oil known as allyle propyl disulphide. The chemical composition of onion varies from variety to variety. The soil, climate, cultural factors, agricultural practices and nutrient application are reported to affect the chemical composition. The onion ranks medium in caloric value, low in protein and very low in vitamins. Small onions contain more nutrients than larger onions.

Table 30.1 Chemical composition of multiplier onion bulbs

Content	Quantity
Moisture (%)	78.32
T.S.S. (%)	19.50
Dry matter (%)	21.68
Drying ratio	5.75:1
Reducing sugar (%)	1.13
Non-reducing sugar (%)	11.00
Total sugar (%)	12.13
Pyruvic acid umd/g	10.13 micro-mol/g
Reconstitution ratio	1.5.76
NEB on fresh bulbs	0.130
NEB on dehydrated bulbs	0.320
Protein (%)	2.220
Sulphur (mg)	154.65 per 100 g
Potassium	229.93 mg/100 g
Nitrogen (mg)	356.47 per 100 g
Calcium (mg)	71.69 per 100 g
Magnesium (mg)	12.65 per 100 g
Ascorbic acid (mg)	56.73 per 100 g
Total ash (%)	0.69
Crude fat (%)	0.17
Sodium (mg)	7.26 per 100 g
Phosphorus (mg)	115.66 per 100 g
Copper (mg)	1.82 per 100 g
Iron (mg)	1.80 per 100 g
Chlorine (mg)	29.35 per 100 g

Source: Singh *et al*., 2004.

30.3 Production

The potato or multiplier onion is also known as underground onion. It forms closely packed clusters of bulbs underground, rather than on the surface like the shallot. It is difficult to obtain reliable data regarding total area and production of potato onion because in many countries it is grown only domestically. However, in Thailand, Indonesia, the Philippines, Sri Lanka and India, potato onions are grown on a commercial scale for export and for internal consumption. *The Food and Agriculture Organization* (FAO) *Production Yearbook* contains production figures for all onions combined. According to the FAO website (www.fao.org), the total area under onion during 2004 was about 3.07 million hectares (ha), production was about 53.59 million tonnes and productivity was 17.46 tonnes/ha. Compared to 1994 figures, there has been an increase of 43.34% in area, 56% in production and 9% in productivity. In India, it is estimated that out of a total onion production of approximately 6 million tonnes, about 1.2 million tonnes is potato onion.

Several varieties of potato onion have been developed in India. The varieties CO1, CO2, CO3, CO4 (Fig. 30.1) and CO On5 were developed at the Tamil Nadu Agricultural University. The variety Agrifound Red (Fig. 30.2) was developed at the National Horticultural Research and Development Foundation. Bulblets of traditional potato onion varieties are much smaller than those of Agrifound Red (Fig. 30.3). The growing period is normally 60–65 days, and almost all varieties of potato onions are propagated vegetatively. The variety CO On5 has a longer growing period of 90 days, and also has the ability to flower and set seeds, so it can be propagated vegetatively or by seed. CO On5 is planted in March and harvested in July.

For other varieties, grown in the South, the bulblets are planted in April–May and October–November. The bulblets are planted on both sides of ridges and spacing is 45 cm × 10 cm. Fertilizers and manures are applied as required. Thrips, insect and leaf spot are the most common insects and diseases for potato onion crops. Thrips is controlled by administering Methyl demeton 25EC (1 ml / litre of water), and leaf

Fig. 30.1 Variety CO4 developed by T.N.A.U., Coimbatore, India.

Fig. 30.2 Agrifound Red variety developed by NHRDF.

Fig. 30.3 Comparison of Agrifound Red variety with traditional variety of potato onion with leaves and bulbs at maturity stage.

spot diseases are sprayed with Mancozeb (2g/litre of water). Yields for CO1, CO2, CO3, CO4 and Agrifound Red are 12–16 t/ha for a growing period of 65–90 days, and 18 t / ha for a growing period of 90 days for CO On5. Bulbs are harvested and cured in the field for four days (Fig. 30.4) and then under shade (Anonymous, 2005).

Fig. 30.4 Harvesting and curing in the field.

30.4 Uses in food processing

Potato onions are mostly used fresh, but the bulblets can also be used as a pickle in vinegar and brine. Dehydrated products potato onion are not common (Shinde and Sontakke, 1986).

30.5 Medicinal properties

Onion is a good cleanser and healer. It is believed that onions help prevent colds, catarrh, anaemia, fever, gastric ills and insomnia. Onion has been considered an excellent diuretic since antiquity. Onion juice is applied to burns, chilblains, bites and stings. It is believed to be very effective in the cure of sores and ulcers, and certain kinds of dropsy. It is also claimed that onion has benefits as a digestive stimulant, an anti-fermentative and as an anti-diabetic. In case of nose bleeds, an onion is cut in halves and placed on the nose. Roasted onions are applied as a poultice to boils, bruises and wounds to relieve heat and, in the case of boils, bring them to maturity. Fresh onion juice promotes perspiration, relieves constipation and bronchitis, induces sleep, and is good for cases of scurvy and lead colic. It is given as an antidote in tobacco poisoning. Cooked with vinegar, onions are given in cases of jaundice, splenic enlargement and dyspepsia. Onion promotes bile production and reduces blood sugar. It has germicidal properties and is recommended for tuberculosis. When used regularly in the diet, it affects tendencies towards angina, arteriosclerosis and heart attack. After warming, onion juice can be dropped into the ear to treat earache. It is also useful in preventing oral infections and toothache (Chevallier, 1996).

30.6 Toxicity

There have been cases of poisoning among some mammals caused by excessive consumption. Dogs seem to be particularly susceptible (Cooper and Johnson, 1984).

30.7 Quality

The quality of the raw material affects demand, both locally and for export. In order to improve quality, it is essential to understand the nature of the product and its possible defects. The bulbs of potato onions should be harvested at proper maturity and kept in windrows to cure. After about a week, when the bulbs and leaves have dried thoroughly, the bulbs are topped by cutting off the leaves, leaving about 2 cm of the top, and the roots. Diseased and damaged bulbs should be sorted out in the field. The bulbs should be thoroughly sorted and graded.

There are various quality guidelines (Anonymous, 2003). These include colour (should be red to bright pink), and size of bulblets (20–30 mm). The dried outer skin should be fully intact. This is achieved through proper curing as described above, and careful handling to avoid removing the skin. Storage diseases are prevented through appropriate storage conditions and, where necessary, the use of Carbendazim (0.1%) against basal rot and Steptocycline (0.02%) against bacterial soft rot.

The pungency of the onion is due to the presence of very small quantities (about 0.065%) of sulphur compounds in the volatile oil of the plant juice. Pungency can be tested in the laboratory, or more simply by cutting the bulbs and noting the effect on the eyes – if the eyes fill with tears, it is clear that the onions are pungent. Potato onions have strong pungency. TSS should be about 18 to 19%, and dry matter should be about 21 to 22%. The TSS and dry matter differs according to variety, growing season and agricultural practices. Immature bulbs have low TSS/dry matter. Rain-damaged bulbs also have low TSS/dry matter. It is important to avoid pesticide residues in the crop. Sprays used against diseases and insects should be diluted and administered according to the manufacturer's instructions and at the safest dosage level. Harmful insecticides, fungicides and other chemicals should not be sprayed on the onions during the last month before harvesting.
Additional important hygienic practices include:

- The onions should not be irrigated with water containing harmful industrial chemicals.
- After harvesting, onions should not be left out in fields where they may be contaminated by industrial waste.
- Chemicals used on the crop should always be administered at levels that are safe to human health.
- Jute bags, bullock carts, trucks, tractor trolleys or any other material used for handling the crop should not be contaminated with chemicals which are hazardous to health or which may create pesticide residue problems.
- Materials and waste products that are unfit for human consumption should be disposed off in such a manner as to avoid contaminating the good produce.
- The onions should be stored and packed in well-ventilated areas free of rotten or other onion wastes.

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31

Spearmint

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31.1 Introduction

Mentha spicata L., one of the total of about 25 species of the genus *Mentha* (*Lamiaceae*) is indigenous to northern England and is known by several names such as nature spearmint, brown mint, garden mint, lady's mint, sage of Bethlehem, etc. The plant is now grown practically all over the world as an important spice plant and a natural source of carvone rich essential oil which is widely traded in the world. The major spearmint growing countries are the USA, Russia, Germany, Australlia and China. The world market for spearmint oil is approximately 1500 t/year (Peterson and Bienvenu, 1998).

Following Husain *et al.* (1988) and Patra *et al.* (2001) spearmint can be botanically described as follows. Like other mints, *M. spicata* is perennial, propagating mostly by underground stolons from which a 50–56 cm aerial stem arises. Erect ascending branches, each measuring 30–60 cm develop from each stem. Leaves are sessile or nearly so, smooth, lanceolate or ovate-lanceolate, sharply serrate, smooth above and glandular below, acute apex and up to 7.0 cm × 2.0 cm in size. The leaves possess a characteristic smell and pungent taste, lacking a cooling after-effect in contrast to that of peppermint and Japanese mint. Flowers are sharply pointed, long and narrow and rightly called spearmint. Calyx teeth are hirsute or glabrous and corolla is about 3 mm long and whitish purple in colour. *M. spicata* is a natural tetraploid (2n = 48) that originated by chromosome doubling of hybrids between the two closely related interspecific diploids *M. longifolia* (2n = 24) and *M. suaveolens* (2n = 22) (Harley and Brighton, 1977; Tyagi *et al.*, 1992).

31.2 Chemical composition, biosynthesis and genetics of essential oil

31.2.1 Chemical composition

Natural population of M. spicata

As the plant is liable to give hybrids through spontaneous out-crossing, the essential oil constituents (terpenes) in the natural populations frequently fluctuate with the result that a total of nine types of *M. spicata* oil have been reported to date (Hocking, 1949; Bhattacharya and Chakravorty, 1955; Dhingra *et al.*, 1957; Shimadzu and Nagamori, 1961; Baslas and Baslas, 1968; Misra *et al.*, 1989; Garg *et al.*, 2000). These nine types are: (i) carvone and limonene type, (ii) piperitone-oxide type, (iii) piperitenone-oxide type, (iv) menthone and piperitone type, (v) glyoxal and 1, 8-cineole type, (vi) linalool, 1,8-cineole and carvone type, (vii) piperitenone oxide and 1,8-cineole type, (viii) piperitenone and carvone type and (ix) piperitenone and limonene type.

Cultivated varieties

The cultivated varieties and genetic stocks, the essential oils of which are traded in the world, always fall in the carvone and limonene rich category of *M. spicata* (Tucker, 1992). The main constituent on the basis of their relative concentrations in the essential oil of the normal varieties/genetic stocks, are: carvone, limonene, linalool, a terpenic glyoxal $C_{10}H_{14}O_{21}$, peperitenone oxide, peperitone oxide, menthone, 1,8-cineole and carvacrol (Garg *et al.*, 2000) (discussed in detail in 'Quality issues').

31.2.2 Biosynthesis and molecular genetics

The constituents of the essential oil belong to terpenoids. In general, monoterpenes (C_{10}) belong to the large class of isoprenoids and are synthesized from five carbon units of isopentenyl pyrophosphate (IPP) which is produced in plastids by the methylerythritol phosphate pathway (Flesh and Rohmer, 1988; Brun *et al.*, 1991; Litchtenthaler *et al.*, 1997). In contrast to the long-standing misconception (prevalent for about 40 years) that the isoprenoids in living organisms are synthesized only through the single pathway, i.e., acetate/mevalonate pathway of cytoplasm, Litchtenthaler *et al.* (1997) on the basis of extensive inhibitor and precursor studies have discovered that apart from the cytosolic acetate/mevalonate pathway, there exists an alternative novel plastidic pathway (GAP/pyruvate pathway) for the synthesis of terpenoids in higher plants including the medicinal ones, *Taxus chinensis* and *Ginkgo biloba* (see also Schwender *et al.*, 1996).

In mints, including spearmint, monoterpenes are synthesized and accumulated in the secretory cells of glandular trichomes located mainly in leaves (Gershenzon *et al.*, 2000). The biosynthetic pathways leading to different monoterpenes have been well characterized in mints (Fig. 31.1). They are localized in two sub-cellular compartments: limonene is synthesized in the leucoplasts and subsequent biosynthetic transformations occur in the cytoplasm. Diemer *et. al.* (2001) reported that the enzymatic steps are divided into three stages (Gershenzon and Croteau, 1993). The first stage is the condensation of IPP with dimenthylallyl diphosphate yielding geranyl diphosphate (GDP), the universal monoterpene precursor with the interaction of a prenyltransferase (GPP synthase). In the second stage, this acyclic intermediate is transformed by various monoterpenes synthases, such as sabinene synthase, cineole-1, 8 synthase, linalool

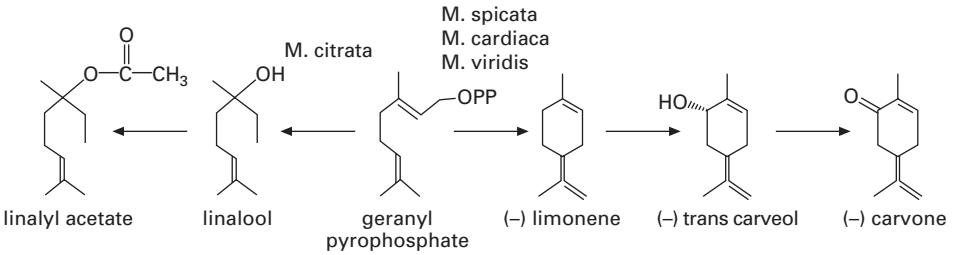

Fig. 31.1 Hypothesized biosynthetic pathway for important monoterpenoids of three *Mentha* species; (*spicata, cardiaca* and *virids*).

synthase, ocimene synthase and 4-S limonene synthase (4S-LS). The last stage of monoterpenes biosynthesis includes several secondary transformations which start with limonene and lead to a great diversity of final products. Perusal of reviews (Chand *et al.*, 2004) show that very little work has been reported on catabolism of monoterpenes and regeneration of their synthesis. Gershenzon *et al.* (2000) are of the opinion that loss of monoterpenes by catabolism and volatilization occurs at a very low rate.

It has been demonstrated that accumulation of monoterpenes varies during the maturation of leaves. Brun *et al.* (1991) observed that the enzymes are developmentally regulated at the level of gene expression (McConkey *et al.*, 2000). Earlier elegant reviews on the biosynthesis of different monoterpene families are available in Wise and Croteau (1999) and Davies and Croteau (2000).

Recently a family of 40 terpenoid synthase genes was discovered in *Arabidopsis thaliana* by genome sequence analysis (Aubourg *et al.* 2002) and over 30 cDNA encoding plant terpenoid synthases involved in plant primary and secondary metabolism in different plants have been cloned and characterized (Trapp and Croteau, 2001). These terpene synthases were classified into six sub-families based on their sequence homology and into three groups based on the numerical variation in introns. Particularly in *Mentha,* about eight genes concerned with the biosynthesis of secondary metabolites (terpenes) have been cloned (Diemer *et al.*, 2001) and about 1400 nucleotide sequences in the EMBL database, most of which are the EST (expressed sequence tags) sequence from the trichomes. Chand *et al.* (2004) in their review emphasized that ESTs would be of immense help in rapidly constructing the physical mapping of genes for terpenoid biosynthesis and in determining the phylogenetic relationship between species of *Mentha*.

31.3 Cultivation and production

The essential oil, a product obtained from the plant is located in the leaves of the spearmint plant. The vegetative growth for the higher production of leaves can be stimulated by the application of the following improved cultivation practices, enumerated by several workers especially Husain *et al.* (1988), Ram (1999), and Khanuja *et al.* (2004).

31.3.1 Soil and climate

Although spearmint thrives well in the cool climates of hills, it can be profitably cultivated in tropical, sub-tropical plains and foothill areas having sub-tropical agro-

climate. It grows well in soil ranging from sandy loam to clay loam rich in organic matter with a good drainage system. Areas that lie wet in winter will not perform vigorously and the plant may die. Spearmint crop cannot tolerate highly acidic or alkaline soils and performs well under near neutral pH (7.5). Spearmint crop initially needs lower temperatures and later a mean temperature of 20 °C–40 °C is suitable for its main growth period. It is highly successful in humid areas of foothills and in places which receive 100–110 cm of well distributed rainfall.

31.3.2 Land preparation and planting

Spearmint requires a fine seed bed. Soil should be ploughed and harrowed thoroughly in order to achieve this. As spearmint is a perennial crop in most countries, pre-planting weed control is imperative to the long-term viability of the crop. A well planned fallow and weed eradication programme before planting is therefore, strongly recommended. Spearmint is planted by means of underground parts called stolons, aerial runners and plantlets. These planting materials are prepared by fresh nursery planting of rooted whole plants or plantlets drawn from the old (mother) fields. The planting materials in nursery are grown with a plant spacing of 30 cm × 15 cm in August. The nursery grown plants reproduce profuse stolons by the months of December and January.

The ideal time for field planting of spearmint is the second fortnight of December to January end. Regarding ideal time of planting a reference could be made here about the work done by Singh *et al.* (1995) on *M. spicata*. A field experiment was conducted by these authors for two years to study the effect of planting time on plant growth, biomass yield, oil yield and quality of spearmint oil (*M. spicata* L.) in Central Uttar Pradesh, India. Maximum biomass yield (275 Q/ha) and oil yield (175.4 kg/ha) were obtained from the crop planted on December 30, which was due to better crop growth in terms of plant height, leaf area index, dry matter accumulation and oil content. The quality of oil essentially assessed by carvone content was higher in November–December plantings, compared to the late plantings. Planting of spearmint in the second half of December is recommended under the agro-climate conditions of Central Uttar Pradesh, India.

Before planting, the stolons/runners may be treated with 0.2% solution of any contact fungicide like Captan for two minutes. Stolons are planted in shallow furrows (7–10 cm deep) spaced at 45 cm–60 cm apart. After planting the furrows are covered with soil and followed by light irrigation. An approximate quantity of 3–5 quintals of planting material (stolons/runners) is usually needed for raising one hectare of plantation.

31.3.3 Nutrient management

The essential oil yield of spearmint largely depends upon its growth, especially of the vegetative parts or foliage. To ensure better vegetative growth, application of sufficient amounts of essential plant nutrients to the soil or directly to the plant is highly desirable. Sufficient amounts of organic manures (FYM, vermi-compost, etc.) have to be integrated with inorganic fertilizers to improve the crop productivity as well as the health of soil. In this respect FYM (10–15 t/ha) or vermi-compost (5 t/ha) may be applied to the soil before planting. The requirement for the inorganic fertilizer essentially depends on the fertility status of the soil. For soils with high organic matter and

medium available NPK usually 100 kg N, 50 kg P_2O_5 and 50 kg K_2O/ha are applied for standard oil yields. The entire quantity of the phosphorus and potash along with 50 kg N/ha is applied as a basal dose at the time of planting. Remaining quantity of N is applied in three equal parts. The first dose is applied after first weeding (5–6 weeks after planting) and second dose after 9–10 weeks of planting and third dose after first harvest.

Working on the different species of *Mentha* Singh *et al.* (1989) reported that herbage and oil yields of *Mentha arvensis* (Japanese mint), *M. piperita* (Peppermint) and *M. spicata* (Spearmint) increased significantly with N fertilization up to 100 kg N/ha and those of *M. citrata* (Bergamot mint) with up to 150 kg N/ha. Plant height, leaf:stem ratio and leaf area index increased with N application and oil content decreased in all the species. Economic optimum doses of N for *M. arvensis, M. piperita* and *M. spicata* were 167, 157 and 145 kg/ha, respectively and their oil yields expected from the response equation were 190, 103 and 50 kg/ha, respectively.

Field investigations were carried out by Randhawa *et. al.* (1984) to ascertain the optimum row spacing and nitrogen requirements of *M. spicata*, at Punjab Agricultural University, Ludhiana, India. The treatments consisted of all combinations of three row to row (30, 45 and 60 cm) spacing and four nitrogen (0, 50, 100 and 150 kg/ha) levels. The results of three years study showed that in order to get higher herb and oil yields this crop should be spaced at 30 cm between rows and supplied with 100 kg N/ha.

31.3.4 Irrigation and drainage

The water requirement of this crop is very high (above 1000 mm/annum). During the winter months (December–March) irrigations are generally required at 10–15 day intervals whereas during summer months (April–June) irrigations may be applied at an interval of 7–10 days. Waterlogging nevertheless, has to be avoided by providing adequate drainage both for irrigation and rain water.

Ram *et al.* (1992) investigated the effect of irrigation on the yield and oil quality of mints including spearmint. The result of a field experiment conducted by these authors on spearmint var. MSS-5, and *M. arvensis* var. Hybrid-77 and CIMAP/MAM-11 under five levels of irrigation (0.4, 0.6, 0.8, 1.0 and 1.2 IW:CPE ratio), revealed that both the *Mentha* species, regardless of their varieties, produced maximum herb and essential oil yields at 1.2 IW:CPE ratio. While the carvone content of spearmint var. MSS-5 remained almost constant, the menthol content of the essential oil of the two *M. arvensis* varieties considerably increased with irrigation levels up to 1.2 IW:CPE ratio during the first harvest. At the second harvest the menthol content of both the varieties of the *M. arvensis* decreased with the irrigation levels. The carvone content of MSS-5 variety of spearmint was maximum at 0.8 IW:CPE ratio.

31.3.5 Interculture and weed control

Like all other mints, the fields of spearmint are also affected by weed infestation and competition. The weeds, if not controlled in time can even cause a 60–80% reduction in yields. The critical period of weed interference in spearmint is found to be between 30–50 days after planting and 15–30 days after first harvest. Usually 2–3 manual weedings are needed to keep the weed growth under check. The weed menace can be minimized by resorting to suitable rotation involving crops like paddy. When paddy

is taken as a preceding crop the weed infestation in spearmint is found to be reduced by at least 30%.

The chemical control of weeds has not become popular in spearmint, even though some chemical herbicides, if applied 2–3 days after planting, (pre-emergence spray) have been found effective. These herbicides include oxyflourefen (at 0.5 kg a.i./ha), pendimethalin (at 0.75 kg a.i./ha) and diuran (at 0.5 kg a.i./ha). However, one should bear in mind that no single weedicide can control all types of weeds and the optimal rate of weedicides may vary with soil type and organic matter content of the soil. Considering the fact that its oil is used in edible confectionery and general health care, it is preferable to avoid the use of chemical herbicides in spearmint from a safety viewpoint as well as the higher commercial value of organic products.

31.3.6 Crop rotations

Continuous cropping of spearmint in a field is not advisable as it leads to considerable increase in weed population, soil-borne diseases and insects. One of the potent methods of weed control is by growing the crops in sequences. Transplanting of paddy in a crop rotation system not only minimizes weed interference but helps in reducing the soil-borne diseases. The following rotations have been found quite economical and are suggested for adopting.

1. Maize – potato – spearmint.
2. Early paddy – potato – spearmint.
3. Late paddy – pea – spearmint.
4. Maize – 'Lahi' (*Brassica*) – spearmint.
5. Arhar (*Cajanus*) – spearmint.
6. Paddy – spearmint.

31.3.7 Harvesting

Spearmint should be harvested in bright and sunny weather. The crop planted in December becomes ready for first harvest during the last week of April in about 100–110 days, the second harvest is taken in some varieties like Neer kalka (the most popular Indian spearmint variety) between 60–70 days following the first harvest. After harvesting, the green herbage may be spread under shade for a day for obtaining good oil recovery (Singh *et al.*, 1990; Singh and Naqvi, 1996). The yield of fresh herb essentially depends upon the crop growth. A good crop of spearmint can give 20–30 t of fresh herb/ha. The yield of essential oil of spearmint ranges from 100–175 kg/ha depending on the crop growth and the cultivars used.

31.3.8 Organic cultivation

Organically grown spearmint oil is high value oil which finds wide uses in food, flavour and aroma therapy. The right quantity of organic manure is an important nutrient supplement component of organic farming system. The requirement of organic manure depends upon the inherent properties of the soil, especially the organic matter content of the soil. For instance, the forest soil being rich in organic matter (more than 1.5%), it requires lower quantities of organic manures from external source. The areas which are poor in organic matter should be provided adequate amount of organic manures. Among the organic manures so far used in organic

farming, vermicompost has emerged as a best source of organic nutrients. To ensure optimum growth and yield of oil of high quality, vermicompost (@10 t/ha) is recommended for peppermint (*M. piperita*) (Khanuja *et al.*, 2004). Because essentially there is no difference between *M. piperita* and *M. spicata* for their cultivation and organic farming, the same recommendation (10 t/ha vermicompost) is suggested for also *M. spicata*.

31.3.9 Important Indian varieties

The use of its high-yielding varieties is an essential prelude to achieving success in commercial cultivation of spearmint. Indian spearmint now has a couple of high-yielding varieties which are described in brief in Table 31.1. Among the varieties discussed above, the interspecific hybrid Neer kalka (Fig. 31.2) has emerged as the most popular variety for large-scale commercial cultivation in view of its high yield potential and profuse stolon reproductivity that is usually not observed in other varieties.

Table 31.1 Characteristics and origin of spearmint cultivars

S. no.	Cultivars	Characteristics	Origin
1.	MSS-5	Medium tall; growth erect; stem medium hard, largely green, lower portion magenta coloured, 4.6 mm thick; leaf:stem ratio 0.58; leaves elliptic ovate, green 6.4 cm^2 size; inflorescence raceme of verticillasters, upper cymes condensed and lower cymes lax, sessile; flowers white, medium in fertility; essential oil content in fresh shoot herb 0.62%; oil carvone rich (65%); medium in yield.	Clonal selection in the accession MSS-1
2.	Arka	Medium tall; growth erect, vigorous; stem hard, largely green, lower portion magenta coloured, 4.2 mm thick; leaf:stem ratio 0.60; leaves elliptic-ovate, green, 6.8 cm^2 size; inflorescence similar to that of cv. MSS-5, early flowering; essential oil content in fresh shoot herb 0.65%; oil carvone rich (68%); medium in yield.	Clonal selection of cv. MSS-5
3.	Neera	Medium tall; growth semi-erect; stem medium hard, largely green, lower portion light magenta coloured, 4.1 mm thick; leaf:stem ratio 0.54; leaves elliptic, green, 4.5 cm^2 size; inflorescence raceme of verticillasters, cymes condensed, sessile; flowers purplish white, low fertile; essential oil content in fresh shoot herb 0.45%; oil carvone rich (58%); oil yield low; unique odour and flavour.	Unknown geneology
4.	Neer kalka	Tall; growth erect; stem soft, largely green, purple pigmented at base, 5.5 mm thick; leaf:stem ratio 0.61; leaves ovate-elliptic, green, 6.9 cm^2 size; inflorescence racemose of axillary verticillasters; flowers whitish purple, fertile; essential oil content in fresh shoot herb 1.0%; oil rich in carvone (72%) and limonene (9.7%); high oil yield (growth properties mostly like that of its female parent *M. arvensis* cv. Kalka and oil quality mostly like that of its male parent *M. spicata* cv. Neera.	F_1 hybrid from the cross: ♀*M. arvensis* cv. Kalka X ♂*M. spicata* cv. Neera

Source: (Bahl *et al.*, 2000 and Patra *et al.* 2001).

(a)

(b)

Fig. 31.2 (a) Spearmint variety 'Neer kalka' (before flowering); (b) Spearmint variety 'Neer kalka' (after flowering).

31.4 Diseases, pests and their control

31.4.1 Fungal and viral diseases

Spearmint, like other mints, is susceptible to a variety of diseases. These diseases are major bottlenecks in production that affect both yield and overall quality of the essential oil. Some of these diseases reduce the yield of spearmint crop, especially when virulent forms of one or more diseases attack the monoclonal spearmint crops spread over wide areas. The most economically threatening diseases of spearmint are caused by the fungi *Puccinia menthae* (rust), *Sphaceloma menthae* (anthracnose), *Rhyzoctonia solani* (aerial blight) and *R. bataticola* (stolon rot), (reviewed by Kalra *et al.*, 2004).

Rust (P. menthae)

This disease has been reported from all mint growing countries. *P. menthae*, a macrocyclic autocious organism produces uredosori on leaves, stems and stolons. The uredospores (17–28 μm × 14–19 μm) of the disease are borne singly. The aerial stage, though observed in other countries, has not been observed in India (Gangulee and Pandotra, 1962). Teliospores (37 μm × 20 μm) are brown, 2-celled, pedicellate, obtuse to slightly pointed.

A high degree of physiologic specialization has been observed in *P. menthae* (Walker and Corroy, 1969; Bruckner, 1972). Rust isolates from *M. spicata* have been observed to infect *M. cardiaca*, but not *P.* × *piperita*. The biotypes that infected *M.* × *piperita* were avirulent on *M. spicata* (Roberts and Horner, 1981). In France, eight races of *P. menthae* were identified during the early part of the last century (Cruchet, 1907). A total of six races were detected in the north-eastern United State (Neiderhauser, 1945), nine races were detected in England (Fletcher, 1963), and three races were detected in New Zealand (Breesford, 1982). In the United States, 15 physiologic races have been observed on mints (Baxter and Cummins, 1953). In another study, a high degree of physiological specialization was observed on mint hosts with 17 collections of *P. menthae* (Johnson, 1965).

Rust has been noted to persist as uredospores on the stolons of the host (Wheeler, 1969). Maximum germination of uredospores occurs at 20 °C (El-Zayat *et al.*, 1994) and the bottom and middle leaves of the plant are most prone to rust disease (Bhardwaj *et al.*, 1995). The disease increases in severity when cultivation is continued in the same area for several years (Kral, 1977). Rust can be avoided by using the disease-free planting material (stolons). Sometimes stolons are treated with hot water at 112 °F for ten minutes (Staniland, 1947) or at 45.4 °C for ten minutes to obtain rust-free planting material (Ogilivie and Brian, 1935; Neiderhauser, 1945). However, planting clones resistant to rust is the most economical and environmentally friendly approach to control the disease (Kalra *et al.*, 1997).

Application of nickel chloride (Molnaz *et al.*, 1960), tebuconazole, belixasol (Margina and Zheljazkov, 1994), mancozeb (Melian, 1967; Bhardwaj *et al.*, 1995), plantovax (Mancini *et al.*, 1976), propiconazole, and diclobutazol (Nagy and Szalay, 1985) offer reliable protection against rust. Good control of rust has also been obtained from spraying the soil surface with denitroamine (Campbell, 1956) at pre-emergence and Krezonit-E (DIVOC) at shoot emergence (Suab and Nagy, 1972).

Anthracnose (S. menthae)

Anthracnose disease is a common disease of spearmint grown on a large scale in areas of the United States and Yugoslavia. It causes stunting, defoliation and economic

loss in spearmint as well as the other species *M. piperita* (Baines, 1938; Dermelj, 1960). The anthracnose fungus grows well at temperature ranging from 4–28 °C, while the most favourable temperature for development of the disease is about 21 °C. Saturation of the atmosphere for 48 h at a temperature of >15 °C, enhanced infection that did not occur at a relative humidity of 80% (Dermelj, 1960). Overwintering of the fungus is on infected mint debris (Baines, 1938). The use of planting materials from healthy crops helps prevent anthracnose. Application of ferbam and copper oxychloride controls the disease to some extent (Dermelj, 1960).

Aerial blight (R. solani)
Although aerial blight has been reported in several species of *Mentha*, maximum loss of herb due to this disease has been reported in *M. spicata* as well as other species, for example, *M. arvensis.* The disease is particularly damaging after the first plant harvest (Bhardwaj *et al.*, 1980) and when the crop is closely planted (Bhardwaj and Garg, 1986). The disease first appears on leaf margins as faded patches that gradually extend inwards under moist and humid conditions. Later, the blight broadens towards twigs (stems) causing necrosis of above-ground parts (Bhardwaj *et al.*, 1996). In India, early planting of the crop before the rainy months reduces the losses during crop maturation. One or two applications of mancozeb can also restrict aerial blight.

Stolon rot (multiple agents)
Stolon rot (also known as stolon decay) caused by *R. bataticola* was first recorded on *M. cardiaca* (Green, 1961) and subsequently recorded on *M. arvensis* and *M. spicata* (Husain and Janardhanan, 1965). That the stolon rot is, indeed, a complex of *R. solani* and *R. bataticola*, was later reported by Singh (1991). The initial symptom of the disease is a yellowing of the foliage with eventual death of the whole plant. Underground stolons show pinkish brown lesions in the early stages of the disease, which gradually turn to dark brown or black patches. The patches increase in size to finally result in decay of a portion or entire stolon. The use of healthy planting material and practices such as deep summer ploughing and crop rotation can control the disease (Jain, 1995). Treatment of the stolons with Zineb, Mancozeb or Captan before planting can also effectively control the disease (Sastry, 1969). To check the spread of the disease, healthy stolons need to be grown in a disease-free plot.

Viral disease: tobacco ring spot virus
Severely stunted and deformed leaves are characteristic of spearmint plants infected with a strain of tobacco ring spot virus (Stone *et al.*, 1962). In China, two cucumo virus, cucumber mosaic and tomato aspermy, have been isolated from spearmint plants displaying mosaic symptoms and distorted leaves (Zhou *et al.* 1990).

31.4.2 Pests

Both aerial and underground parts of spearmint are attacked by insects. The aerial part is affected by leaf folder (*Syngamia abrutails*), hairy caterpillar (*Spilosoma obliqua*), bug (*Nisia atrovenosa*) and whitefly (*Bemisia tabaci*) whereas the underground part is damaged by white grub (*Holotrichia consaguinea*) and termites (*Microtermes obesi*) (Husain *et al.*, 1988). Insecticides like dimenthoate (0.05%), quinalphos (0.05%) and chloropyriphos (0.05%) successfully manage the pests of this crop (Khanuja *et al.*, 2004).

31.5 Food uses

Spearmint is widely valued world wide as a culinary herb. The leaves have a strong spearmint flavour and they are used in flavouring salads or cooked foods (Hedrick, 1972; Grieve, 1984; Mabey, 1974; Facciola, 1990). In European counties, the leaves find frequent use in preparing sauces for desserts, fruit, soup, split pea soup, lamb stew and roast, fish, poultry, sweet dishes, vegetables, mint jelly, symps, fruit, compotes, devils food cake, ice cream, herbal teas and mint tea. The carvone-rich essential oil hydro-distilled from the above-ground part of *M. spicata* plants, is used for flavouring sweets, chewing gums. toothpastes, etc. (Facciola, 1990). According to Duke and Ayensu (1985), the nutritive composition of fresh leaves of spearmint is as given below:

Leaves (fresh weight) in grammes per 100 g of leaves

1.	Water	83.0
2.	Protein	4.8
3.	Fat	0.6
4.	Carbohydrate	8.0
5.	Fibre	2.0
6.	Ash	1.6

In milligrammes per 100 g weight

1.	Calcium	200.0
2.	Phosphorus	80.0
3.	Iron	15.0
4.	Niacin	0.4

31.6 Medicinal uses

Spearmint is commonly used as a domestic herbal remedy. A tea made from the leaves has traditionally been used in the treatment of fevers, headaches, digestive disorders and various minor ailments (Foster and Duke, 1990). The herb is antiemetic, antispasmodic, carminative, diuretic, restorative, stimulant and stomachic (Lust, 1983; Grieve, 1984; Duke and Ayensu, 1985). The leaves should be harvested at the time of flower initiation of the plant and can be dried for later use (Grieve 1984). The essential oil of the plant is antiseptic, though it is toxic in large doses (Foster and Duke, 1990). The essential oil and the aerial stems are often used in folk remedies for cancer and a poultice prepared from the leaves (macerated leaves) is said to remedy tumours (Duke and Ayensu, 1985).

31.7 Functional benefits

31.7.1 Antimicrobial activity

The essential oils obtained from *M. spicata* and *M. pulegium* exhibit antimicrobial properties against eight strains of Gram-positive and Gram-negative bacteria (Sivropoulou *et al.*, 1995). These authors ascertained that the main p-menthane components of the essential oils exhibit a variable degree of antimicrobial activity not only between different bacterial strains but also between different strains of the

same bacteria. Likewise, Torres *et al.* (1996) reported the antimicrobial activity of spearmint oil against *Staphylococus aureus* and *E. coli.*

31.7.2 Insecticidal and genotoxic activities

The essential oils (EOs) extracted from mint species, *M. spicata* and *M. pulegium*, together with their main constituents, carvone, pulegone and menthone, were tested for insecticidal and genotoxic activities on *Drosophila melanogaster* (Franzios *et al.*, 1977). The EOs of both aromatic plants showed strong insecticidal activity, while only the oil of *M. spicata* exhibited a mutagenic one. Among the constituents studied by these authors, the most effective insecticide was found to be pulegone while the most effective for genotoxic activity was menthone. Data revealed that both toxic and genotoxic activities of the EOs of the two studied mint plants are not in accordance with those of their main constituents, pulegone, menthone and carvone. Whereas pulagone is significantly more effective (×9) as an insecticide, menthone and carvone are less effective (×6 and ×2, respectively) insecticides when used in their authentic forms.

31.7.3 Nematicidal activities

Walker and Melin (1996) investigated the nematicidal activities of six spearmint and six peppermint accessions. They inoculated the accessions with *Meloidogyne incognita* race 3 and *M. arenaria* race-2 under greenhouse conditions. No nematode galls formed on roots of any of the plants inoculated with 1,800 eggs/pot. Fewer than two galls per root system formed on three accessions of peppermint inoculated with *M. incognita* at 5,400 eggs/pot. Only one peppermint accession developed galls when inoculated with *M. arenaria*, whereas none of the spearmint accessions was susceptible to this species. Plant dry weight was in general unaffected by infection with root-nematodes at these densities. Growing spearmint and peppermint accessions for eight or 12 weeks in *M. arenaria*-infested soil before tomato cultivation resulted in a 90% reduction in root galls compared with tomato following tomato.

31.7.4 Fungicidal activities

Working with several essential oil bearing plants including *M. spicata*, Yegen *et al.* (1992) reported the remarkable fungicidal activities of their essential oils and oil constituents. These authors investigated the fungitoxicity of these essential oils against four phytopathogenic fungi. The essential oils were more toxic against *Phytopathogenic capsici* than the fungicide carbendazen and pentachlor nitro benzene. The investigations with thin-layer chromatography implicated carvacrol of *M. spicata* as one active compound having significant fungicidal property.

Adam *et al.* (1998) reported the significant antifungal properties of the essential oils of various aromatic plants including *M. spicata* against human pathogens, *Malassezia furfur*, *Trichophyton rubrum* and *Trichosporon beigelii*. Their results demonstrated that among the main components of the essential oils, carvacrol of *M. spicata* and Thymol of the other plants exhibited the highest levels of antifungal activity. Furthermore, the studied essential oils when tested with the Ames test did not exihibit any mutagenic (carcinogenic) activity.

31.7.5 Antioxidative properties

The kinetics of peroxide accumulation during oxidation of sunflower oil at 100 °C in the presence of different concentrations of hexane, ethyl acetate and ethanol extracts of *Melissa officinalis*, *Mentha piperita*, *M. spicata*, *Ocimum besilicum*, *Origanum vulgare*, and *Saturejae hertensis* were studied by Marinova and Yanishlieva (1997). It has been established that the extracts from *O. basilicum* and *Origanum vulgare* do not improve the oxidation stability of sunflower oil. The ethanol extracts from the other four species including *M. spicata* have proved to be the most active in retarding the auto-oxidation process for stabilization of sunflower oil.

31.7.6 Role in augmenting N-uptake of plants

Kiran and Patra (2002) conducted a field study to compare efficiency of Dicyandiamide (DCD)-coated urea with some natural essential oils and their derivatives, viz., *M. spicata* oil, dementholized oil (DMO) and terpenes-coated urea on wheat (*Triticum aestivum* L.) yield, nitrogen (N) uptake and apparent N recovery on a sandy loam soil of Central Uttar Pradesh, India, where excessive loss of N due to NO3 leaching is a serious problem due to its light texture. A significant increase in grain and straw yield, N-uptake and apparent N-recovery was observed on application of these nitrification inhibitors. However, their performance varied with the percentage used; it was higher with the higher level of application. All three natural coating materials retarded nitrification significantly, throughout the growth period of wheat as compared to materials at a 0.50% (V/W) level of coating on urea were 29.6%, 27.2% and 22.7% with DMO, *M. spicata* oil and terpenes, respectively. Corresponding values at a 1.00% (V/W) level of coating were 4.0%, 38.6%, 23.2%, respectively. With DCD coated urea (at a 1.00% level of coating, W/W basis) it was 33.1%, while the corresponding value with uncoated urea was 22.7%.

Economic analyses were done for the use of these natural coating materials for cultivation. Benefits obtained from the use of DMO, *M. spicata* oil and terpenes at a 0.50% level of coating were Rs. 5,210/ha, Rs. 1,400.00/ha and Rs. 450.00/ha, respectively while at a 1.00% level of coating, corresponding profits were Rs. 9,040.00/ha, Rs. 4,570.00/ha and 575.00/ha, respectively. The benefit obtained from the use of DCD at a 1.00% level of coating was Rs. 1,005.00/ha.

31.7.7 Role as intercrop in pest management

The efficacy of intercropping cabbage with other vegetables and herbs including *M.*

Table 31.2 Comparative chemical composition (%) of Indian spearmint oil produced from plants of the variety Arka, harvested after 100 days of planting

Sr. no.	Compounds	Relative concentration (%)
1.	Carvone	62.1
2.	Limonene	16.2
3.	1,8 Cineole	2.0
4.	3-octanol	0.4
5.	β-bourbonene	0.9
6.	β-Caryophyllene	0.9
7.	Sabinene hydrate	1.5

Data source: Bahl *et al.* 2000.

Table 31.3 Effect of development stage on oil content (%) and percent content of major important terpenoids in *M. spicata* cultivars

Name of cultivar	Mode of planting	Period between planting & harvesting	Oil content %	Percent contents of major oil components										
				Carvone	Limonene	1,8-Cineole	3-Octonol	Menthone	Isomenthone	Neomenthol	Menthol	β-Bourbonene	β-Caryophyllene	Sabinene hydrate
Arka	By stolons	(i) 70 DAP	0.40	72.3	8.7	3.1	0.8	–	–	–	–	1.2	0.9	0.70
		(ii) 100 DAP	0.55	62.1	16.2	2.0	0.4	–	–	–	–	0.9	0.9	1.50
Neera	-do-	(i) 70 DAP	0.25	67.5	4.9	4.7	0.3	–	0.8	–	0.7	4.1	–	–
		(ii) 100 DAP	0.34	44.2	25.4	6.5	0.4	–	0.5	–	0.1	3.4	–	–
MSS-5	-do-	(i) 70 DAP	0.38	80.2	1.2	0.6	0.6	–	–	–	–	1.2	2.4	0.8
		(ii) 100 DAP	0.58	58.3	18.9	2.2	0.6	–	–	–	0.1	0.5	0.2	0.1
Neer-kalka	-do-	(i) 70 DAP	0.35	71.6	9.6	0.2	0.5	0.4	0.5	0.9	0.2	–	–	–
		(ii) 100 DAP	0.58	47.4	35.4	0.4	0.1	0.2	0.8	0.5	0.6	–	–	–

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

spicata as a management tool in mitigating insect pest problems of cabbage was investigated by Timbilla and Nyako (2001) in the field at Kwadaso, Kumasi during a three-season period in the forest region of Ghana. The results showed that *Plutella xylostella* could be effectively controlled when cabbage is intercropped with spearmint, onion and tomato.

31.8 Quality issues

Like other mint oil, spearmint oil is a complex mixture of terpenic hydrocarbons and aromatic compounds with carvone and limonene as major oil constituents. Because the latter two major components have many industrial applications in a variety of food and cosmetic products, their separation in pure forms through fractional distillation under a specific temperature and pressure from the raw oil is imperative prior to their industrial uses. In accordance with the traded international oil quality standard an ideal spearmint variety should contain the oil of the composition shown in Table 31.2.

The quality of essential oil nevertheless depends on the genetic makeup, geographical and ecological conditions and stages of plant growth. In this particular regard, working on four popular Indian cultivars of *M. spicata,* Bahl *et al.* (2000) have reported that to obtain carvone and limonene rich herbage, the spearmint crop should be harvested after 100 days of planting (DAP), and according to them early harvesting (i.e., 70 DAP) causes enhancement in carvone content in exchange for a decrease in limonene content (Table 31.3).

31.9 References

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