2

Review of literature

2.1. Spices

What exactly spices are? Encyclopedia Americana define spices as "products of plant origin for seasoning food to give flavour and aroma". Chambers Dictionary defines spices as "aromatic and pungent vegetable substances used as condiments and for seasoning food". The American Spice Trade Association (ASTA) defines spice as "any dried plant product used primarily for seasoning purposes". The Oxford English Dictionary defines spice as "aromatic or pungent vegetable substance used to flavour food". The word 'spice' is from the Latin *'species'* meaning 'kinds of goods', while 'condiment' comes from Latin *'condire'* meaning 'to preserve'. According to the International Organization for Standardization (ISO), there is no

clear-cut division between spices and condiments, and so they are clubbed together. The term spices or condiments applies to 'such natural plant or vegetable products or mixtures thereof, in whole or ground form, as are used for imparting flavour, aroma and piquancy to and for seasoning food' (De 1999).

There are about 70 species of spices grown in different parts of the world. Many of them are grown in India and other Asian countries. Around 52 of these spices, said to be grown on Indian soil, have been identified by the Indian Spices Board till date (Table 1).

At present, there are as many as 26 kinds of Indian spices that find a ready overseas market. India is reputed for the production of black pepper, cardamom, ginger, turmeric and chillies. Among other spices, the important ones are coriander, cumin , fenugreek, garlic, onion, saffron, clove and nutmeg (Subbulakshmi and Naik 2002). Black pepper is known as the 'black gold of India'. Around 24 types of pepper are grown in southern part of India. The State of Kerala contributes almost 95% of India's total pepper production. Cardamom is known as 'queen of spices'. India is the largest cardamom producing country in the world. Recently, Guatemala has overtaken India in the world export market of spices. Among the spices produced and also consumed in India, chilli or red pepper is now the leading one. Indian ginger is considered second to the Jamaican variety in quality. Turmeric is also cultivated in huge quantities in different parts of India (De 1999).

There was a steady rise in the export of spices in the last decade. It has increased from 109,636 tonnes in 1990-91 to 236,142 tonnes in 1999-2000 to fetch 135 million US$ and 468 million US$ for the respective periods. Latest data made available by the Indian Spices Board in their Trade Highlights showed a decline in spice export (230,000 tones) for the year 2000-2001 (Fig. 1). There was a notable drop in foreign exchange earned during the period, only 352 million US$. Reason stated by the Indian Spices Board is low volume of pepper export coupled with low unit value realization. Still India commands a formidable position in the world spice trade with 46% share in quantity

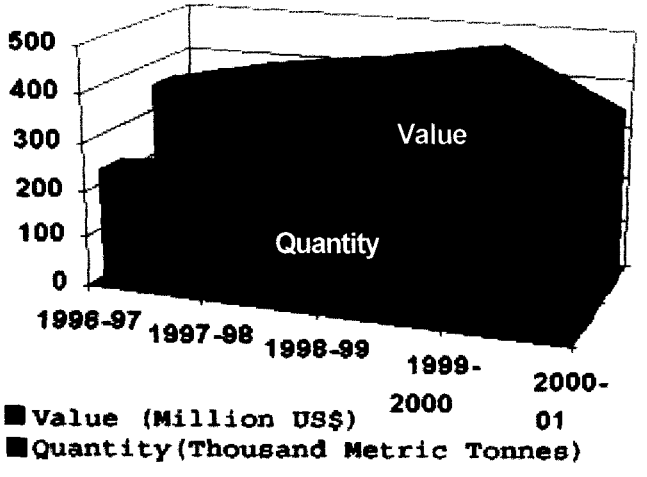

Fig. 1. Trend in India's spice exports

Table 1. List of common Indian spices

English common name	Botanical name	Family	Part used
Ajmud	*Trachyspermum roxburghianum* (DC.) Craib	Apiaceae	Fruit
Allspice/pimento	*Pimenta dioica* (L.) Merr.	Myrtaceae	Fruit & seed
Aniseed/anise	*Pimpinella anisum* L.	Apiaceae	Fruit
Asafoetida	*Ferula asafoetida* L.	Apiaceae	Resin from rhizome
Basil	*Ocimum basilicum* L.	Lamiaceae	Leaf
Bay leaf	*Laurus nobilis* L.	Lauraceae	Leaf
Bishop's weed/carrom	*Trachyspermum ammi* (L.) Sprague	Apiaceae	Fruit & seed
Black cumin	*Nigella sativa* L.	Ranunculaceae	Seed
Black pepper	*Piper nigrum* L.	Piperaceae	Fruit & seed
Cambodge	*Garcinia cambogia* Desr.	Clusiaceae	Pericarp lobes
Caraway	*Carum carvi* L.	Apiaceae	Fruit
Cardamom (small)	*Elettaria cardamomum* Maton.	Zingiberaceae	Fruit & seed
Cardamom (large)	*Amomum subulatum* Roxb.	Zingiberaceae	Fruit & seed
Cassia	*Cinnamomum aromaticum* L.	Lauraceae	Bark
Celery	*Apium graveolens* L.	Apiaceae	Leaf, stem & fruit
Cinnamon	*Cinnamomum zeylanicum* Bregn	Lauraceae	Bark
Clove	*Syzygium aromaticum* (L.) Merr. et Perry	Myrtaceae	Unopened flower bud
Coriander	*Coriandrum sativum* L.	Apiaceae	Leaf & seed
Cumin	*Cuminum cyminum* L.	Apiaceae	Fruit
Curry leaf	*Murraya koenigii* (L.) Spreng.	Rutaceae	Leaf
Dill	*Anethum sowa* L.	Apiaceae	Fruit & seed
Fennel	*Foeniculum vulgare* Mill.	Apiaceae	Fruit
Fenugreek	*Trigonella foenum-graecum* L.	Fabaceae	Seed
Garlic	*Allium sativum* L.	Alliaceae	Bulb
Ginger	*Zingiber officinale* Rosc.	Zingiberaceae	Rhizome
Greater galang	*Alpinia galanga* (L.) Willd.	Zingiberaceae	Rhizome
Horse radish	*Armoracia rusticana* Sch.	Brassicaceae	Rhizome

English common name	Botanical name	Family	Part used
Hyssop	*Hyssopus officinalis* L.	Lamiaceae	All aerial parts
Juniper berry	*Juniperus communis* L.	Cupressaceae	Berry-like cones
Kokam	*Garcina indica* Choisy	Clusiaceae	Peel of fruit
Lovage	*Levisticum officinale* Koch	Apiaceae	Leaf
Mace	*Myristica fragrans* Houtt.	Myristicaceae	Aril of fruit
Marjoram	*Maiorana hortensis* Moench	Lamiaceae	Leaf & flower top
Mint	*Mentha piperita* L.	Lamiaceae	Leaf
Mustard	*Brassica juncea* L.	Brassicaceae	Seed
Nutmeg	*Myristica fragrans* Houtt.	Myristicaceae	Seed
Oregano	*Origanum vulgare* L.	Lamiaceae	Leaf & flower top
Parsley	*Petroselinum crispum* (Mill) A.W. Hill	Apiaceae	Seed
Pepper long	*Piper longum* L.	Piperaceae	Fruit
Pomegranate	*Punica granatum* L.	Punicaceae	Seed
Poppy seed	*Papaver somniferum* L	Papavaraceae	Seed
Red chilli	*Capsicum fruitescens* L.	Solanaceae	Fruit & seed
Rosemary	*Rosmarinus officinalis* L.	Lamiaceae	Leaf
Saffron	*Crocus sativus* L.	Iridaceae	Stigma
Sage	*Salvia officinalis* L.	Lamiaceae	Leaf
Savory	*Satureja hortensis L.*	Lamiaceae	All aerial parts
Sesame	*Sesamum indicum* L.	Pedaliaceae	Seed
Star anise	*Illicium verum* Hooker f.	Illiciaceae	Fruit
Sweet flag	*Acorus calamus L.*	Araceae	Fruit
Tamarind	*Tamarindus indica* L.	Fabaceae	Fruit
Tarragon	*Artemisia dracunculus* L.	Asteraceae	Leaf
Tejpat	*Cinnamomum tamala* Nees at Eberm.	Lauraceae	Leaf & bark
Thyme	*Thymus vulgaris* L.	Lamiaceae	Leaf
Turmeric	*Curcuma longa* L	Zingiberaceae	Rhizome
Vanilla	*Vanilla planifolia* Andrews	Orchidaceae	Pod

*Based on De (1999); Singh and Singh (1996)

and 23% in value (Fig. 2) The Board stressed on quality improvement and technological upgradation by the Indian exporters so that an equal share in terms of value can be obtained (www.indianspices.com/html/s0410trd.htm).

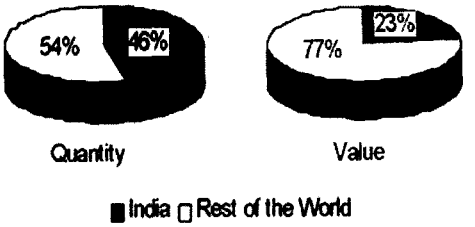

Fig. 2. India's share in world trade of spices during 2000-2001

For the export of spices and spice products, the exporting countries have to comply with the specifications laid down by the regulatory agencies in importing countries. Before the liberalization, exporters had to comply with the pre-shipment inspection and quality control as per the AGMARK (Agricultural Produce Grading and Marketing Act) grade specification prescribed by the Directorate of Marketing and Inspection (DMI). Export Inspection Council of India also have the mandate for pre-shipment inspection and quality control certification. With the liberalization pre-shipment inspection and withdrawal of quality control the exporters are free to export the spices as per the specifications prescribed by the importing countries. The most popular worldwide specification for spices is the ASTA Cleanliness Specification. Major producing countries have built up their facilities to meet the requirements as per ASTA Cleanliness Specification. Countries like UK, Germany and the Netherlands have laid down cleanliness specification for spices. European Spice Association (ESA) has come out with the "quality minima for herbs and spices". In addition to the cleanliness specification, importing countries are cautious on the microbial contaminants in spices at the time of import. Almost all the importing countries have fixed the limit for *Salmonella* as absent in 25 g of spices (http://www.indianspices.com/html/s1490qua.htm). Different countries have laid down specified norms depending on microbial parameters such as total aerobic mesophilic bacteria, *Escherichia coli*, coliforms etc. while importing (Table 2). However, there are no separate Indian standards for microbial specifications of spices (Spices Board of India, personal communication).

Table 2. Bacteriological Standard Specifications of spices

Bacteria	Germany*	Netherlands*	ESA†	ICMSF‡
Total aerobic mesophilic bacteria	10^5 g^{-1}	10^6 g^{-1}		10^4 g^{-1}
Bacillus cereus	10^4 g^{-1}	Nil $(20\ g)^{-1}$		
Clostridium perfringens	10^4 g^{-1}	Nil $(20\ g)^{-1}$		
Staphylococcus aureus	10^2 g^{-1}	Nil $(20\ g)^{-1}$		
Coliform		10^2 g^{-1}		
Escherichia coli	Nil	Nil $(20\ g)^{-1}$	10^2 g^{-1}	10 g^{-1}
Salmonella	Nil $(25\ g)^{-1}$	Nil $(20\ g)^{-1}$	Nil $(25\ g)^{-1}$	

*http://www.indianspices.com/html/s1493qua.htm
†European Spice Association (http://www.indianspices.com/html/s1490qua.htm)
‡International Commission on Microbiological Specifications for Foods (ICMSF 1974)

2.2. Foodborne pathogenic bacteria

2.2.1. *Bacillus cereus*

An early report on food poisoning was obtained as early as in 1906 in a hospital outbreak of acute gastritis, where 300 patients and hospital staff were the victims. Although Lubenau (1906) named the organism *Bacillus peptonificans*, the properties he described resemble those of *B. cereus*. It was not conclusively established as a cause of food poisoning until 1950, when the taxonomy of the genus had been clarified. Hauge (1950, 1955) described four outbreaks in Norway involving 600 people. An incriminated food vehicle, vanilla sauce, stored at room temperature for 24 h, contained 10^7-10^8 *B. cereus* ml^{-1}. This classic report and many of the early ones from Europe described an illness in which diarrhoea was the predominant syndrome (Adams and Moss 1995).

In Hungary, it was ranked as the third most common cause of food poisoning during the period 1960-1966 (Nikodemusz 1968). It is interesting that the high incidence of *B. cereus* food poisoning in meat was attributed to the Hungarian custom of abundantly seasoning meat dishes with spices which often contain large number of aerobic sporeformers (Ormay and Novotny 1968, 1970). Numerous reports of *B. cereus* foodborne illness in Europe have been cited by Goepfert *et al.* (1972). For these outbreaks the levels of *B. cereus* in the food remnants were found to be 10^6-10^9 cfu g^{-1} or ml^{-1} (Gilbert 1979).

Bacillus cereus is a Gram-positive aerobic sporeformer whose cells are large rods (1.0 μm x 3.0-5.0 μm in chains) and whose spores do not swell the sporangium. It grows over a temperature range of 8-50 °C, optimally at 28-35 °C (Gordon *et al.* 1973; Adams and Nout 2001).

The heat resistance of *B. cereus* spores is a factor of primary concern to the food industries and has received considerable attention. Since spore inactivation is the principal concern in producing appertized foods, much higher temperatures are used in appertization processes and in the measurement of spore D-values. The D-value (decimal reduction time) is defined as the time at a given temperature for the surviving population to be reduced by 1 log cycle, and *z*-value is defined as the temperature change which results in a 10-fold (1 log) change in D (Adams and Moss 1995).

Mol (1957) demonstrated that D-values at 85, 90, 95 and 100 °C in phosphate buffer (pH 7.0) were 220, 71, 13 and 8 min, respectively. In contrast, the $D_{121°C}$-values in soybean oil and olive oil were 30 and 17.5 min, respectively (Molin and Snygg 1967). Ingram (1969) estimated the $D_{100°C}$-value in low acid foods (pH <4.5) to be 5 min. The $D_{100°C}$-value of 2.7-3.1 min for *B. cereus* in skimmed milk was reported by Mikolajcik (1970). A strain with a $D_{121°C}$ of 2.4 min was isolated from spoiled canned soup by Bradshaw *et al.* (1975). Parry and Gilbert (1980) showed that spores of serotype I, which is most commonly implicated in food poisoning in UK generally have a higher heat resistance (mean $D_{95°C}$ of 24 min) than spores of

other serotype (mean $D_{95°C}$ of 3 min), indicating that the cooking procedure might select certain serotypes. In rice broth, $D_{100°C}$ and $D_{92°C}$-values for various spore suspension of *B. cereus* (rice isolates) ranged from 4.2 to 6.3 min and from 16 to 36 min, respectively (Chung and Sun 1986).

Behaviour of seven strains of *B. cereus* was studied by Rajkowski and Mikolajcik (1987). In demineralized water $D_{100°C}$-values ranged from 0.6 to 27 min, and *z*-values ranged from 7.4 to 14.5°C. In 0.067 M phosphate buffer (pH 7.0), $D_{100°C}$-values ranged from 0.9 to 6.9 min, and *z*-values from 6.5 to 11.0°C. Another study showed great differences in the thermal resistance of two enterotoxigenic strains isolated from cooked chilled foods; the $D_{90°C}$-values were 4.04 and 39 min (Fernandez *et al.* 1999).

Data on other factors affecting the growth of *B. cereus* are scant. For example, the effect of pH has received little attention. In laboratory media, the minimum and maximum pH permitting growth were reported as 4.9 and 9.3, respectively (Fluer and Ezepchuk 1970; Kim and Goepfert 1971a). Other factors that have been shown to exert an inhibitory effect on the growth of *B. cereus* include 2 g sorbic acid kg^{-1} rice filling of Karelian pastry (Raevuori 1976), 500 (pH 6.3) μg benzoic acid ml^{-1} (Lueck 1980) and 100 g garlic extract l^{-1} (Saleem and Al-Delaimy 1982). Application of nisin at levels of 2.5 and 5 mg l^{-1} has been shown to act as an effective preservative giving significant increase in shelf-life and providing protection against the growth of psychrotrophic *B. cereus* (Delves-Broughton *et al.* 1992). Addition of nisin to a batter of crumpets at levels of 3.75-6.26 μg g^{-1} effectively prevented the growth to levels capable of causing food poisoning (Jenson *et al.* 1994).

The antibiotic susceptibility was tested for 66 isolates of *B. cereus* from rice in Taiwan against 12 different antibiotics. The isolates were 100% susceptible to chloramphenicol (30 μg), erythromycin (15 μg) and streptomycin (10 μg), and 92.4% sensitive to gentamicin (10 μg). However, they were 100% resistant to penicillin G (10 units) and polymyxin B (300 units); 98.5% resistant to ampicillin (10 μg) and carbenicillin (100 μg) and 88% resistant to cephalothin (30 μg) (Chung and Sun 1986). Shah *et al.* (1996), isolated *B. cereus* from about 300 samples of a variety of foods in which *B. cereus* was found in 20% spices. The antibiogram pattern of *B. cereus* was obtained with 50 isolates against nine antibiotics. All the isolates were resistant to ampicillin (10 μg). A high resistance was found against trimethoprim (5 μg) (92%), colistin (10 μg) (86%) and rifampicin (5 μg) (92%). All the isolate were sensitive to chloramphenicol (30 μg) and ciprofloxacin (5 μg) and 88% sensitivity was seen to streptomycin (10 μg) and vancomycin (30 μg).

Both the spores and vegetative cells of *B. cereus* have a ubiquitous distribution in the environment and can readily be isolated from air, soil, dust, natural waters, vegetation and many kinds of food, including milk and dairy products, spices, cereals and cereal products, meat and poultry (Goepfert *et al.* 1972; Norris *et al.* 1981). Nygren (1962), e.g., reported an isolation frequency of 47.8% from 3888 samples of food and food ingredients in Sweden.

The presence of a large number of *B. cereus* (>10^5 cfu g^{-1}) in a food is indicative of active growth and proliferation of the organism and is consistent with a potential hazard to health (Adams and Moss 1995). The presence of >10^5 *B. cereus* cells g^{-1} food is an acceptable epidemiologic evidence for confirmation of foodborne outbreaks due to *B. cereus* (CDC 1979). Another suggested criterion is the comparison of *B. cereus* counts isolated from stools of healthy and ill people. Injested *B. cereus* may be shed in the feaces at levels reflecting the number consumed (Ghosh 1978).

There are two types of *B. cereus* food poisoning syndromes caused by two separate toxins. Till 1972 there was no answer to questions on pathogenicity as no suitable assay system for enterotoxin was developed (Goepfert *et al.* 1972). Several attempts have been made to isolate the enterotoxin (Spira and Goepfert 1972, 1975; Ezepchuk *et al.* 1979; Turnbull 1981), however only recently were homogeneous enterotoxin preparations were obtained (Guaycurus *et al.* 1993). The diarrhoeal toxin is a heat labile protein with a molecular mass of around 50 kDa. It is sensitive to proteolytic enzymes and produced by the organism during the late exponential phase of growth. The onset of illness is about 8-16 h, lasts for 12-24 h, and is characterized by abdominal pain, profuse watery diarrhoea and rectal tenesmus. Nausea and vomiting are less frequent (Kramer and Gilbert 1989; Adams and Moss 1995). It is commonly associated with reheated, spiced, meat-casserole dishes. Spices may be heavily contaminated with heat-resistant spores which survive during cooking.

The emetic toxin (<5 kDa) is heat-resistant (stable at 126°C for 90 min) and resistant to proteolytic enzymes and low pH. Production of the toxin occurs in the food in late exponential to stationary phase of growth. It causes nausea and vomiting 1-5 h after eating the contaminated food, frequently rice (Gilbert and Taylor 1976). Cereals other than rice have been implicated and other vehicles are pasta, milk pudding and pastry (Kramer and Gilbert 1989; Adams and Moss 1995).

The ubiquitous nature of *Bacillus* species ensures that such organisms inevitably contaminate our food supply to some extent. However, their presence in small numbers is usually not a problem, and the ingestion of low levels is not harmful. Consequently, effective prevention and control measures depend on the control of spore germination, and prevention of the multiplication of vegetative cells in cooked, ready-to-eat foods. Suitable heat or irradiation treatment should be used where complete destruction of the organism is warranted (Kramer and Gilbert 1989).

2.2.2. *Clostridium perfringens*

Clostridium perfringens (previously known as *Cl. welchii*) was first described in detail by Welch and Nuttall (1892). It was recognized as a cause of foodborne illness as early as in 1895 (Klein 1895) and the link was established firmly through epidemiological studies (McClung

1945). The identification of an enterotoxin was reported by Duncan and Strong (1969). In addition to enteritis, *Cl. perfringens* is responsible for necrotizing tissue infections. Historically, the organism is best known for its association with gas gangrene.

Clostridium perfringens occurs in a variety of foods and causes food poisoning (De Boer and Boot 1983; Labbé 1991). From 1973 to 1979, 63 (23%) of the 271 general outbreaks of food poisoning reported in Scotland occurred in hospitals and 37 (62%) were caused by *Cl. perfringens*. In the USA, from 1970 to 1978, *Cl. perfringens* was involved in 87 (9.5%) of the 916 outbreaks reported and in 40 (14.5%) of the 275 outbreaks in Canada from 1973 to 1976. In England and Wales, 18,970 cases of food poisoning were caused by *Cl. perfringens* between 1970 and 1980 (Stringer 1985; Labbé 1989; Todd 1991). The poisoning is generally a self-limiting, nonfebrile illness characterized by nausea, abdominal pain, diarrhoea, and less commonly vomiting. An onset is usually 8-28 h after consumption of food. Spices and herbs containing *Cl. perfringens*, even in low numbers ($<10^2$ cfu g^{-1}), may play an important role in contributing to the microbial load of prepared foods (Powers *et al.* 1975; De Boer *et al.*1985). Although foods related to food poisoning outbreaks caused by *Cl. perfringens* usually contain large numbers (10^5-10^6 cfu g^{-1}) of this organism (Labbé 1991), spores, even at low levels, could germinate and the organism could grow to reach significant numbers (Nakamura and Kelly 1968). Duration of illness is short, usually 12-24 h. Symptoms associated with the disease are caused by an enterotoxin that is expressed during sporulation of enterotoxigenic strains (Czeczulin *et al.* 1993; Heredia *et al.* 1994). Ingested vegetative cells that survive the stomach's acidity pass to the small intestine where they grow, sporulate and release enterotoxin. Most cases are associated with meat products, e.g., stew, meat gravies, roast, joints and pies. Many surveys have shown that *Cl. perfringens* is found in raw and processed food, most notably, raw meat products and spices (Labbé 2000).

Clostridium perfringens is a Gram-positive, anaerobic, sporulating bacillus (relatively large rods, 1 μm x 3-9 μm), unusual amongst the clostridia in being nonmotile. It has the ability to multiply over a temperature range of 15-50 °C, and optimal temperature being about 45 °C. Willardsen *et al.* (1978) listed one strain (NCTC 8238) as having a slightly shorter generation time (7.1 min) at 41 °C than at 45 °C. *Clostridium perfringens* grows best at pH value of 6.0-7.0, the same pH range as many meat and poultry products. Growth is severely limited at pH 5.0 and 8.3 (Smith 1972). A level of 70-80 g sodium chloride l^{-1} is required to prevent the growth of most strains of it, although some inhibition occurs at 50-60 g sodium chloride l^{-1} (Labbé 1989). Tompkin *et al.* (1974) found an inhibitory effect of sorbic acid on *Cl. perfringens*. Control of *Cl. perfringens* was achieved in Italian wirstel sausage by incorporation of 5μg nisin g^{-1} (Caserio *et al.* 1979).

Roberts (1968) reported that spores of heat-resistant strains had $D_{90°C}$-values of 15-145 min and *z*-values of 9-16 °C, whereas spores of heat-sensitive strains had $D_{90°C}$-values of only 3-5 min and *z*-values of 6-8 °C. Furthermore, spores of heat-resistant strains required heat

activation at 78-80°C, whilst up to 50% of heat-sensitive strains grew without heat activation (Crowther and Baird-Parker 1984).

Spores of *Cl. perfringens* which have a $D_{99°C}$-value at 99°C of 17.3-19.8 min can be expected to survive while cooking (Juneja and Majika 1995). Inadequate cooling practices have been attributed as a major cause of food poisoning with *Cl. perfringens* (Bean and Griffin 1990). D-values of spores at 100°C showed a wide interstrain variation with recorded values of 0.3-38 min (Adams and Moss 1995).The $D_{90°C}$-values for the spores can be as high as 200 min (Labbé 2000). The heat resistance could be a part of the explanation of the association of *Cl. perfringens* with chromosomal copies of *cpe* in food poisoning outbreaks (Sarker *et al.* 2000).

The enterotoxin has been shown to be the major virulence factor in the common form of food poisoning. Stark and Duncan (1971) first showed that all clinically significant properties were linked to the enterotoxin. Human volunteer studies strengthened the theory (Skelkvåle and Uemura 1977), and gene deletion studies (Sarker *et al.* 2000) gave the definitive proof that the effects seen are solely due to the production of enterotoxin (Brynestad and Granum 2002).

Although *Cl. perfringens* can produce over 13 different toxins, each bacterium produces only a subset of these (Petit *et al.* 1999). Strains of *Cl. perfringens* are subdivided into five types, A, B, C, D and E depending on presence or absence of four major toxins namely, α, β, ϵ and ι; genes for three of these are located in plasmid except α toxin in chromosome (Canard *et al.* 1992; Katayama *et al.* 1996). Apart from these, *Cl. perfringens* also produces eight other minor toxins. Enterotoxin responsible for food poisoning in human is produced by type A strains which contain α toxin. Rarely, type C strains which contain α and β toxins may cause a much more severe necrotizing enteritis which may be fatal. The toxin is a protein of 35 kDa. Its action is to damage epithelial cells on the villi and inhibit absorption of glucose. There is an efflux of sodium chloride and water into the gut lumen causing diarrhoea.

Multiple antibiotic-resistant *Cl. perfringens* (tetracycline, erythromycin, lincomycin and clindamycin) were isolated from porcine faeces (Rood *et al.* 1978). Tetracycline resistance is the most common resistance phenotype (Lyras and Rood 1996). Antibiotics are not used in cases of human food poisoning because no overt infection occurs. There are reports of antibiotic-associated diarrhoea caused by *Cl. perfringens* (Borriello *et al.* 1984). Metronidazole was reported to be an effective treatment in at least one such case (Borriello and Williams 1985).

In virtually all outbreaks the principal cause is failure to refrigerate properly previously cooked foods, specially when prepared in large portions. Rapid, uniform cooling of foods is therefore imperative. Gravies, broths, and large pieces of meat should be cooled to <10°C within 2-3 h. Cooked, chilled foods should be reheated to a minimal internal temperature of

75 °C immediately before serving to destroy vegetative cells. Cooked meat should be kept above 60 °C or below 10 °C. As most people harbor *Cl. perfringens* in their intestinal tract, preventing carriers from handling food is rather impossible. Similarly, the organism is present in a wide variety of foods. So, education of the foodhandlers remains a critical aspect of *Cl. perfringens*-related food poisoning control (Labbé 1989).

2.2.3. *Staphylococcus aureus*

The staphylococci were first described by Ogston (1881) as a cause of a pyogenic infection in humans. He gave them the name staphylococcus after their appearance as bunch of grapes under the microscope. Dack *et al.* (1930) showed that staphylococcal poisoning was caused by a filterable enterotoxin. Approximately 60% of the human biotype of *Staph. aureus* and a smaller proportion of other biotypes are enterotoxigenic. For these reasons, *Staph. aureus* is important to the food manufacturer both as a marker for good hygienic practices and as an organism responsible for food poisoning (Adams and Moss 1995).

Staphylococcus aureus is of Gram-positive, spherical to ovoid cells of about 1 μm in diameter. Some strains are capable of producing a heat-stable protein toxin that causes illness in human. It is a typical mesophile with a growth temperature range between 7 and 48 °C and an optimum of 37 °C. The range of temperature over which enterotoxin is produced is narrower by a few degrees and has an optimum at 35-40 °C. Growth occurs optimally at pH values of 6-7 with minimum and maximum limits of 4.0 and 9.8-10.0, respectively. Lahellec *et al.* (1981) reported that the growth of *Staph. aureus* in brain heart infusion (pH 5.0) was inhibited by 10 g sorbate l^{-1}, but at pH 7.0 the organism grew in the presence of 50 g sorbate l^{-1}. Inhibition of *Staph. aureus* by sorbic acid in cooked uncured sausage was also reported by Tompkin *et al.* (1974). A characteristic feature of *Staph. aureus,* which is particularly an important consideration in some foods, is its tolerance of salt. It grew readily in media containing 50-70 g sodium chloride l^{-1} and some strains are capable of growth in up to 200 g sodium chloride l^{-1} (Adams and Moss 1995). The bacterium was sensitive to nisin (Thomas *et al.* 2000).

Staphylococcus aureus showed around 50% resistance to penicillin and ampicillin in all the tested isolates. Resistance level to erythromycin, kanamycin, neomycin, oxacillin, cephalexin, sulfonamides/trimethoprim, enrofloxacin and tetracycline had been reported to be in the range of 2-20% (Owens and Watts 1988; Myllys *et al.* 1998; Vasil 1998; Teuber 1999).

Food poisoning is characterized by a short incubation period, typically 2-4 h. Nausea, vomiting, stomach cramps, retching and prostration are the predominant symptoms, although diarrhoea is often reported. Recovery is normally complete within 1-2 d. The short incubation period is characteristic of an intoxication where illness is the result of ingestion of a pre-formed toxin in the food. *Staphylococcus aureus* produces seven protein exotoxins, designated A, B,

C_1, C_2, C_3, D and E. Toxin types A and D, either singly or in combination, are more frequently implicated in outbreaks of food poisoning. Foods that are frequently incriminated include meat products, poultry products, salads, bakery products, and milk and dairy products. Foods that require considerable handling during preparation and that are kept at slightly elevated temperatures after preparation are frequently involved in staphylococcal food poisoning (Adams and Moss 1995).

At homes and in public caterings the major factors contributing to staphylococcal food poisoning are temperature abuse of foods at risk and poor hygiene. Education on correct handling, preparation and storage of cooked and processed foods is considered to give the greatest opportunity to reduce the incidence of *Staph. aureus* food poisoning. Application of the factors inhibiting the growth of *Staph. aureus* and the use of predictive growth models together with good manufacturing practices to reduce its occurrence and control its incidence can contribute to minimizing the risk in food manufacturing operations (Gilbert 1983).

2.2.4. Enterobacteriaceae

The history of the hygienic significance of groups or individual members of the Enterobacteriaceae is almost as old as the science of microbiology itself, and the topic has been the subject of many reviews including, amongst others, that of Mossel (1967). Bacteria belonging to the Enterobacteriaceae commonly form a part of microbiological criteria and their presence is traditionally related to hygiene and safety of foods. Test of this kind was first applied to milk and ice-cream and later extended to all foods, particularly with foods of unknown origin (Ayers and Johnson 1915; Shippen 1915; Finkelstein 1919; Weinzirl and Harris 1928; Tanner and Windsor 1929).

Members of the family are Gram-negative, straight rods, 0.3-1.5 μm in diameter; facultative anaerobes, oxidase-negative and have a respiratory and fermentative metabolism. They ferment D-glucose to give acid and often gas. They often utilize glucose as sole source of carbon and grow well on peptone and meat extracts. They are non-sporeforming, non-acid fast and non-halophilic but tolerate the presence of bile salts (Brenner 1984).

Traditionally the group (designated as coliforms) has been chosen as an indicator of faecal pollution. These organisms are capable of fermenting lactose in the presence of bile at 37°C. This group includes not only most strains of *Escherichia coli* but also organisms such as *Citrobacter* and *Enterobacter* which are not predominantly of faecal origin. The faecal coliforms, a more restricted group of organisms, are those coliforms which can grow and produce gas at 44-45°C in suitable selective media (ICMSF 1978). One criticism of using coliforms and faecal coliforms is that their absence could give a false reassurance of safety when lactose-negative organisms predominate. The lactose-negative organisms include not only *Salmonella* and *Shigella*, but also enteropathogenic strains of *E. coli* itself such as O124.

For this reason, tests for the whole of Enterobacteriaceae are increasingly being used. The Enterobacteriaceae includes even more genera of non-faecal origin than the coliforms, such as species of *Erwinia* and *Serratia* which are predominantly plant associated. So, Enterobacteriaceae counts are used more generally as an indicator of hygienic quality rather than of faecal contamination and, therefore, say more about general microbiological quality than possible health risks posed by the product (Adams and Moss 1995).

Some important foodborne pathogens of this group, namely *E. coli, Salmonella* and *Shigella* are reviewed here.

2.2.4.1. *Escherichia coli*

Since 1885, when it was first isolated from children's faeces and described by Theodor Escherich, scientific attention has been lavished on *Escherichia coli.* Its common occurrence in faeces, ready culturability, generally nonpathogenic character, and survival characteristics in water led to the adoption of *E. coli* as an indicator of faecal contamination and the possible presence of enteric pathogens such as *Salmonella typhi* in water. This usage has been transferred to foods where greater circumspection is required in interpreting the significance of positive results (Adams and Moss 1995).

Escherichia is the type genus of the Enterobacteriaceae family, and *E. coli* is the type species of the genus. It is a catalase-positive, oxidase-negative, fermentative, short, Gram-negative, non-sporing rod. *Escherichia coli* is a typical mesophile growing from 7°C up to 50°C with an optimum around 37°C. A near-neutral pH is optimal for growth. Effect of 7.5 g sorbic acid l^{-1} (pH 5) on *E. coli* was reported by Doell (1962). Minimum inhibitory concentration of 50-100 μg sorbic acid ml^{-1} (pH 5.2-5.6) and 50-120 μg benzoic acid ml^{-1} (pH 5.2-5.6) were cited by Lueck (1980).

Kaul and Taneja (1989) studied antibiotic resistance patterns of *E. coli* isolates of spices from Chandigarh against six antibiotics. They showed maximum resistance (77%) towards penicillin, while all the isolates were sensitive towards chloramphenicol. The frequency of multidrug resistance was fairly high (39.4%). Majority of the resistant strains were from asafoetida followed by fenugreek and small cardamom. Among 20 human and cattle isolates of *E. coli*, 80% were resistant to at least one antibiotic. Seventy percent were resistant to streptomycin, 65% to sulfonamide, 50% to tetracycline, 25% to ampicillin, 20% to sulfonamide/trimethoprim, 10% to chloramphenicol, 8% to cephalothin and 5% to gentamicin (Farina *et al.* 1996). Antibiotic resistance of 80% level for tetracycline, chloramphenicol, streptomycin and sulfonamide in some *E. coli* strains from mastitis infections in cows was reported by Teuber (1999).

Important measures to prevent food poisoning include educating food workers in safe food handling techniques and proper personal hygiene, properly heating foods to kill

pathogens, and holding foods under appropriate conditions to avoid bacterial multiplication. Additionally, untreated human sewage should not be used to fertilize vegetables and crops used for human consumption nor should unchlorinated water be used for cleaning food processing equipment and food contact surfaces (Doyle and Padhye 1989).

2.2.4.2. *Salmonella*

Most salmonellas are regarded as human pathogens, though they differ in the characteristics and the severity of the illness they cause. The typhoid bacillus was first observed by Eberth (1880). The paratyphoid bacilli were first isolated by Achard and Bensaud (1896), and confirmed as culturally and serologically distinct from the typhoid bacilli by Schottmüller (1901). Other salmonellas were isolated during the same period (Adams and Moss 1995).

Salmonellas are now established as one of the most important causes of foodborne illness worldwide. It is estimated that 2-4 million cases of salmonellosis occur in USA annually. More probably, the cause of *Salmonella* diarrhoea is multifactorial. Evidence has been obtained that diarrhoeagenic *Salmonella* also produce enterotoxin which stimulates fluid secretion.

Salmonellas are Gram-negative, non-sporeforming rods (typically 0.5 μm x 1-3 μm) which are facultatively anaerobic, catalase-positive, oxidase-negative and generally motile with peritrichous flagella. Growth has been recorded from 5 to 47°C with an optimum at 37°C. Salmonellas are heat-sensitive and are readily destroyed by pasteurization temperatures (Adams and Moss 1995). The optimal pH for salmonella growth lies between 6.5 and 7.5, with possibilities for growth at pH values ranging from 4.5 to 9.0 and slow death at more extreme conditions (Bryan *et al.*1979). The growth of salmonella is generally inhibited in the presence of 30-40 g sodium chloride l^{-1}. Higher temperature facilitated initiation of growth in medium of greater salinity, high concentrations of sodium chloride protracted the lag periods and decreased the rate of growth (D'Aoust 1989). Similar results were reported in a study of 23 strains of salmonella grown at 10-30°C in the presence of 20-80 g sodium chloride l^{-1} (Alford and Palumbo 1969). Inactivation of *Salmonella* by 7.5 g sorbic acid l^{-1} (pH 5.0) was reported by Doell (1962). Park and Marth (1972) found that 3 g sorbic acid l^{-1} (pH 5.0) inactivated *S. typhimurium* in 12 h in nutrient broth at 37°C. Inhibition by sorbate was also reported by Tompkin *et al.* (1974).

A report of antibiotic resistance patterns of 21 *Salmonella* isolates of spices from Chandigarh against six antibiotics showed maximum resistance (93%) towards penicillin, while chloramphenicol was the antibiotic to which least resistance (18%) was shown. The frequencies of multidrug resistance were fairly high (39%). Majority of the resistant strains were from asafoetida followed by fenugreek and small cardamom (Kaul and Taneja 1989). Antibiotic resistance in epidemic isolates of *S. typhimurium* DT104 has approached a 100%

level for tetracycline, chloramphenicol, streptomycin and sulfonamide (Teuber 1999). A total of 502 isolates of *Salmonella* recovered from various food products, was tested for susceptibility to 12 antimicrobial agents. All the isolates were found to be resistant to rifampin (5 μg), whereas 245 of the isolates were resistant or intermediate to additional antimicrobial agents. Most of the isolates were resistant or intermediate to streptomycin (132 isolates), sulfisoxazole (122 isolates) and tetracycline (93 isolates). No isolates were resistant to norfloxacin, however 15 were resistant to ampicillin and nalidixic acid, 11 to chloramphenicol, 6 to trimethoprim, one to ciprofloxacin. Of the 247 isolates exhibiting resistance to one or more antimicrobial agents, 168 were resistant or intermediate to one, 33 to two, 25 to three, 7 to four, 8 to five, and 2 each to six and seven antimicrobial agents (Kiessling *et al.* 2002)

An infective dose is as few as 15-20 cells, depending on age and health of the host and strain differences among the members of the genus. Acute symptoms are nausea, vomiting, diarrhoea, fever and headache. Since birds, insects, handlers of infected food can all contaminate foods directly or indirectly, potential food vehicles for *Salmonella* are numerous. Meat, milk, poultry and eggs are primary vehicles; they may be undercooked allowing the *Salmonella* to survive or they may cross-contaminate other foods which are consumed without further cooking. New knowledge that a single *Salmonella* cell can be infectious emphasizes the need for a greater stringency in food quality assurance programmes (D'Aoust 1985; Adams and Moss 1995).

2.2.4.3. *Shigella*

The genus *Shigella* was discovered as the cause of bacillary dysentery by Shiga (1898). It consists of four species, namely *Sh. dysenteriae, Sh. flexneri, Sh. boydii* and *Sh. sonnei,* all of which are regarded as human pathogens, though they differ in the severity of illness they cause. In USA, the annual reports of shigellosis over recent years have ranged between 3 and 4.5 lacs.

Shigellas are nonmotile, non-sporeforming, Gram-negative rods which are catalase-positive (with the exception of Shiga's bacillus, *Sh. dysenteriae* serotype I), oxidase-negative and facultative anaerobes. They are typical mesophile with a growth temperature range between 10 and 45 °C. They grow best in the pH range of 6-8 and do not survive well below pH 4.5 (Adams and Moss 1995).

The first description of transmissible multidrug resistance in the 1950s was reported in *Sh. flexneri* isolated in Japan (Wantanabe 1963). Antibiotic therapy reportedly reduces the length of illness and of the carrier state for shigellas (Van Gelde *et al.* 1947). A 1983 report was particularly troublesome. Investigators in Japan have found that approximately 66% of shigellas isolated between 1971 and 1979 were resistant to multiple antibiotics. Furthermore, 97.5% of these strains were able to transfer their resistance to sensitive recipient bacterial

strains (Tanaka *et al.* 1983).

Shigellas cause bacillary dysentery in humans and primates. Studies with human volunteers have indicated that the infective dose is low; in the order of 10-100 cells. The incubation period varies between 7 h and 7 d, although foodborne outbreaks are commonly characterized by shorter incubation periods of up to 36 h. Some strains produce Shiga-toxin (like verotoxin of *E. coli* O157:H7). Symptoms are abdominal pain, vomiting and fever accompanying diarrhoea. Milder forms of illness are self-limiting, but *Sh. dysenteriae* infections often require fluid and electrolyte replacement and antibiotic therapy. In fooodborne cases, the source of the organism is normally a human carrier involved in preparation of food. Uncooked foods which may have received extensive handling such as prawn cocktail or tuna salad have been implicated in a number of outbreaks (Adams and Moss 1995).

The best preventive measures for foodborne acquisition would be good personal hygiene and health education for foodhandlers. Proper treatment (chlorination) of water and sanitary disposal of sewage would prevent some foodborne as well as waterborne outbreaks of shigellosis (Doyle *et al.*1985).

2.3. Microbiology of spices

The ancient Aryans considered spices as a powerful remedy for various disorders in human beings. Even today in Unani, Homeopathy and Ayurvedic systems of medicine, most of the spices are used as an ingredient in medicinal drug preparations. They are also famous as folk medicine in different parts of the country. Spices have been used since ages to preserve food, specially meat or fish for long periods, when there was no refrigeration. Its use for stability and safety of foodstuffs, supports the antimicrobial properties of spices. Some spices, like clove, garlic, and mustard possess strong antimicrobial properties (Singh and Singh 1996). Other spices, aniseed, caraway, cardamom, cinnamon, coriander, ginger, pepper and turmeric are known to possess antimicrobial activity towards various foodborne pathogens (Frazier and Westhoff 1978; Huhtanen 1980; Shelef *et al.* 1980, 1984; Deans and Richie 1987; Beuchat and Golden 1989). The antimicrobial activity of spices is principally due to their essential oils (Davidson 1997), such as anethole, a major constituent of aniseed and eugenol, a major constituent of clove oil (Karapinar and Aktuğ 1987).

Garlic (*Allium sativum* L.), even from aoristic, has been used by the people of Babylonia, China, Egypt and India not only as a spice but also for treatment of many diseases (Jain 1977). Its uses have been described on the walls of ancient Egyptian places of worship and Pyramids. Its importance has often been highlighted by the scriptures (Pruthi 1980). The use of garlic was described by Virgil as a treatment for snake bite and by Hippocrates as a treatment for pneumonia and suppurating wounds. The use of garlic extract for treating such ailments as dysentery, typhoid, and cholera still continues in some areas of the world (Wills

1956). Insecticidal, hypoglycaemic, anti-tumour, and anti-atherosclerotic properties have also been noted (Lau *et al.* 1983). Deshpande *et al.* (1993) observed the inhibitory effect of garlic on pulmonary infection caused by the *Mycobacterium avium* complex which may be life-threatening in non-immunocompromised patients in early stages of immune deficiency diseases such as AIDS.

Garlic, which is commonly used in culinary practices, has been reported to possess a broad spectrum antibacterial property (Datta *et al.* 1948; Wills 1956; Al-Delaimy and Ali 1970; Sharma *et al.* 1977; Mantis *et al.* 1978; Srivastava *et al.* 1982; Sato *et al.* 1990; Waqar *et al.* 1994; Kumar and Berwal 1998). The antibacterial studies have revealed varying magnitude of its effects depending on the organism, growth medium and garlic preparation used (Al-Delaimy and Ali 1970; De Wit *et al.* 1979; Srivastava *et al.* 1982). *Staphylococcus aureus* (Datta *et al.* 1948; Mantis *et al.* 1978; Dababneh and Al-Delaimy 1984), *B. cereus* (Saleem and Al-Delaimy 1982), *Cl. botulinum* (De Wit *et al.* 1979), *Cl. perfringes* (Mantis *et al.* 1979), *S. typhimurium* (Johnson and Vaughn 1969; El-Khateib and El-Rahman 1987) and *E. coli* (Datta *et al.* 1948; Johnson and Vaughn 1969; Sharma *et al.* 1977; Kumar and Sharma 1982) are all shown to be adversely affected by garlic extracts.

The principal antimicrobial component of garlic is allicin, a diallyl thiosulfinate (2-propenyl-2-propenethiol sulfinate) (Cavallito and Baily 1944). Intact garlic bulbs do not contain allicin in its active form, but contain its precursor, alliin (S-allyl-L-cysteine-S-oxide). Upon crushing garlic bulbs, alliin is enzymatically hydrolyzed to allicin, pyruvate and ammonia by the action of phosphopyridoxal enzyme alliinase (Stoll and Seebeck 1948). Allicin is an inhibitor of respiratory SH-group enzymes (Beuchat and Golden 1989) and of acetyl-CoA synthetase (Focke *et al.* 1990).

Another antimicrobial component containing no sulphur or metal in its structure and chemical tests for allicin and acrolein were negative, is known as garlicin (Almeida Machado *et al.* 1948). Unfortunately, the original garlicin substance was not defined by the modern chemical analysis and no elucidation of its structure has been reported (Koch 1993).

All the studies referred above demonstrate various antimicrobial properties of different spices. These studies only support and strengthen the popular belief that spice environment is not suitable for growth and multiplication of micro-organisms. However, available literatures not necessarily always corroborate the same. Following are some examples, those hit the common belief that spices are free from pathogenic bacteria. Jessen *et al.* (1934) succeeded to pinpoint that the sporeforming micro-organisms present in a spice mixture containing coriander and white pepper were responsible for swelling cans of chopped hams. Being stimulated by this report, scientists at the National Canners Association in Washington investigated 127 samples of spices. Black pepper was the spice most heavily contaminated, the total count for seven samples ranged from 10^6 to 10^7 cfu g^{-1}, and thermophilic aerobic spores were relatively low. Putrefactive anaerobes were not found in any sample. Peppers, coriander

and marjoram varied widely in the counts of the aerobic sporeformer. Cloves with a maximum count of 12,000 cfu g^{-1} were least contaminated. However, cleaning processes reduce the number and ethylene oxide eliminates most viable organisms in these products (Yesair and Williams 1942).

Bacteriological studies on a limited number of spice samples by Castell (1944) and Karlson and Gunderson (1965) showed that the predominant bacteria were aerobic sporeformers. The total number of viable aerobic organisms varied considerably depending on the spice source and numbers may range from several thousand to several million per gram (Julseth and Deibel 1974; Pruthi 1980).

Hall (1938) and James (1938) in their separate studies showed that many spices were heavily contaminated at import. Julseth and Deibel (1974) failed to demonstrate *E. coli* in spices as imported, though it was occasionally found in spices on the retail shelves. In the analysis of 11 samples of black pepper obtained from retail stores, interstate carriers and food service establishments, Christensen *et al.* (1967) found aerobic plate count (APC) values which ranged from 10^7 to 10^9 cfu g^{-1}, with a mean of 10^8 cfu g^{-1}.

In two different studies during 1970s spices were found to contain a large number of micro-organisms, mainly bacilli, whereas anaerobic sporeformers, thermophilic anaerobes and aerobes, enterococci and members of Enterobacteriaceae were found occasionally. The spores of *Cl. perfringens* were present at low level. *Salmonella, Shigella* and coagulase-positive staphylococci were rarely found in spices (Guarino and Peppler 1976; Frazier and Westhoff 1978)

In a study of microbiology of processed spices, 114 samples were collected from 16 armed force bases located in different geographical areas of USA. In these samples, the aerobic plate count (APC) varied from <100-10^7 cfu g^{-1}. However with the exception of cayenne pepper, half or more of all the spice samples had APC's less than 10^5 cfu g^{-1}. The incidence of *Cl. perfringens* in the spices analyzed was 15%; the count ranged from 50 to 2850 cfu g^{-1}. The organism was found in four types of spices. Low numbers of coliforms were found in cayenne pepper, cinnamon and garlic, representing 9% of the samples, faecal coliforms were not found. Coagulase-positive staphylococci, found in only one sample (oregano), and at a very low level (30 cfu g^{-1}) (Powers *et al.* 1975).

In another significant study Powers *et al.* (1976) tested for incidences and levels of *B. cereus* in retail spices sold in US defense barracks. One hundred and ten processed spice samples were analyzed, where 58 samples (53%) contained *B. cereus;* the count ranged from 50 to 8500 cfu g^{-1}, however 59% had counts less than 100 cfu g^{-1}. Only 13.6% had counts greater than 1000 cfu g^{-1}. Eighty-nine percent of them were toxigenic which occurred in each kind of spice. Schwab *et al.* (1982) examined microbiological quality of some spices in retail markets. They conducted a national level survey in USA where they have determined microbiological quality of 10 varieties of spices. The APC values for the products ranged from

<100 to 10^8 cfu g^{-1}; mean values of the individual spices ranged from 1400 to 10^6 cfu g^{-1}. Coliform count ranged from <3 to 10^6 cfu g^{-1}. However, mean values were <20 cfu g^{-1} for all products. *Escherichia coli* counts ranged from <3 to 2300 cfu g^{-1}. Except for celery seed which had a mean *E. coli* value of 7 cfu g^{-1}, all the mean values were <3 cfu g^{-1}.

In a total of 36 spices and food additives from various sources in South Africa, Baxter and Holzapfel (1982) found a high total viable number of sporeformers. The total aerobic count varied between 200 cells g^{-1} in cardamom and several million g^{-1} in the case of black pepper. Exceptionally high contamination (>10^6 cfu g^{-1}) was found in black pepper, coriander, paprika, mace, pimento and white pepper. The presence of *B. cereus* was confirmed in 7 out of 19 samples.

In an investigation, the microflora of 20 retail samples of ground cinnamon sold in Izmir, Turkey was examined by Karapinar and Aktuğ (1986) to exhibit various microbial loads including sporeforming bacteria and spoilage organisms. The APC ranged from 5200-10^5 cfu g^{-1}, although none of the samples had APC's greater than 10^6 cfu g^{-1}. Low numbers of coliforms were found and the counts exceeded 10 cfu g^{-1} in only five samples. However, *E. coli* was not found in any of the samples tested. The total numbers of anaerobes varied between zero and 95 cfu g^{-1}, and the counts exceeded 50 cfu g^{-1} in only two samples. None of the samples contained *Cl. perfringens*. *Bacillus cereus* was found in all the samples, ranging from 40 to 6800 cfu g^{-1} and *Staph. aureus* was not detected in any of the samples tested.

Malmsten *et al.* (1991) mentioned their findings on 200 dried spice and herb samples tested. In this study APC ranged from 10^4 to 10^7 organisms g^{-1} dried material, where marjoram showed the highest count. The incidence of coliform and fecal coliform were low (3-11,000 cfu g^{-1}). Counts for faecal streptococci ranged from 5 to 1000 cfu g^{-1}. Aerobic sporeformers were present in 100% of the samples with a load of 50-18,000 cfu g^{-1}. *Bacillus cereus* was present in most of the samples; counts ranging from 50-3900 cfu g^{-1}. Anaerobic sporeformer count was very low (5-30 cells g^{-1} dried material).

A total of 160 different samples of 55 different spices collected from six different suppliers and retailed outlets in Vienna were screened for their microbiological quality, based on plate count and selective identification techniques. Although some spices, namely black pepper, chillies and China spice had total aerobic mesophilic bacteria of >10^7 cfu g^{-1}, more than 50% of the samples contained between 10^4 and 10^6 cfu g^{-1}. Bacilli were found in nearly all samples, showing >10^5 cfu g^{-1} in almost 40% of the products. Anaerobic sporeformers were detected only in three products. Although several samples gave colonies indicating the presence of coagulase-positive staphylococci, representative isolates were not identified as *Staph. aureus*. Some samples, especially of ginger and curry powder had *B. cereus* count as high as 10^5 cfu g^{-1}. On the contrary, *Cl. perfringens* was detected in only one caraway sample (Kneifel and Berger 1994).

Several reports on the microbiology of spices indicate the presence of *Cl. perfringens*. Rodríguez-Romo *et al.* (1998) analyzed 380 samples of widely used spices in Mexico for the presence of *Cl. perfringens*. The count varied from <100 to 500 cfu g^{-1} in garlic powder, <100-200 cfu g^{-1} in black pepper, <100-433 cfu g^{-1} in cumin seed, <100-340 cfu g^{-1} in oregano and <100-450 cfu g^{-1} in bay leaves.

In another Mexican market survey 304 samples of spices (garlic powder, cumin seeds, black pepper, oregano and bay leaves), nonpackaged and packaged in polyethylene bags and glass containers were analyzed. High levels (10^5-10^7 cfu g^{-1}) of mesophilic aerobic micro-organisms were found in most of the samples of garlic powder, cumin seed and black pepper. Lower levels (<10^2 cfu g^{-1}) were found in oregano and bay leaves. Levels of total and faecal coliform were dependent on the type of packaging. More than 70% of the polyethylene-packaged samples had <10^3 cfu g^{-1} of micro-organisms. Glass and nonpackaged spices showed lower levels of these micro-organisms. On the other hand, <30 cfu g^{-1} of fecal coliform was present in 74% of the nonpackaged samples. Seventy-eight percent of the polyethylene-packaged samples had fecal coliform less than 10^3 cfu g^{-1}, whereas all glass-packaged samples had less than 30 cfu g^{-1}. *Bacillus cereus* was present in 32 samples, of which most were in polyethylene-packs. The other pathogenic bacteria such as *Salmonella* and *Shigella* were not detected (García *et al.* 2001)

There is a dearth of literature on the microbiology of processed spices commonly used by the mass in India. However, available literatures in the field have been mentioned here.

Krishnaswamy *et al.* (1971), in their study on microbiological quality, analyzed several spices and spice mixtures collected from leading manufacturers and exporters of these commodities in Madras, Mangalore and Cochin. They observed that the total microbial load ranged from 10^4 to 10^7 cfu g^{-1}, turmeric showing the maximum. Coliforms were present in black pepper, coriander, mustard, fenugreek, cumin, fennel and curry powder; coriander having the maximum load (2400 cfu g^{-1}) and fenugreek the minimum (45 cfu g^{-1}). Aerobic thermophiles ranged from 10^3 to 10^6 cfu g^{-1}. Aerobic mesophile counts were highest in turmeric (10^6 cfu g^{-1}) and lowest in fennel (10^3 cfu g^{-1}). Incidences of mesophilic putrefactives ranged from 26 cfu g^{-1} (fenugreek) to 920 cfu g^{-1} (coriander and fennel). Thermophilic flat-sours (not producing hydrogen sulphide) were present in limited numbers (40 cfu g^{-1}) in cumin. Non-coagulase types of staphylococci were present in minimum numbers in some of the spices, e.g. black pepper, turmeric, coriander, mustard and fenugreek. Of those, the highest number was found in mustard (111,000 cfu g^{-1}) and the lowest in fenugreek (1300 cfu g^{-1}). *Clostridium perfringens* count was maximum in mustard and turmeric (700 cfu g^{-1}) and minimum in fenugreek (30 cfu g^{-1}). Sulphide-stinkers and salmonella were absent.

Microbial contamination of black pepper, dry ginger, cardamom and dehydrated onion have been reported in a different study by Krishnaswamy *et al.* (1974). In this study, the standard plate count ranged from 10^3 cfu g^{-1} in cardamom to 10^8 cfu g^{-1} in black pepper. The

density of coliform ranged from zero to 2400 cfu g^{-1}. The incidence of aerobic thermophiles ranged from zero (cardamom and dehydrated onion) to 10^6 cfu g^{-1} (dry ginger). Contamination with aerobic sporeformers was from zero (cardamom) to 10^7 cfu g^{-1} (dry ginger), while mesophilic putrefactives ranged from zero (cardamom and black pepper) to 10^7 cfu g^{-1} (dry ginger). *Salmonella* was not recovered.

To quantify the magnitude of contamination at the point of export, Seenappa and Kempton (1981), analyzed 100 samples of unprocessed Indian spices comprising 25 each of black pepper, red pepper, ginger and turmeric. Samples were collected from warehouses in selected spice-trading areas of Kerala and Karnataka States of India. All the samples were aseptically transported to the University of Waterloo, Canada for bacteriological analysis. Nature of this study was separate from general studies of retail level problems in an importing country (Christensen *et al.* 1967) and specific post-processing problems (Seenappa and Kempton 1980) by obtaining samples at the point of export in India. Results showed a high level of contamination; 50% of the black pepper and ginger samples were in the unacceptable range (>10^6 cfu g^{-1}). *Bacillus cereus* was detected in more samples of these spices than any other sporeforming aerobes.

A detailed evaluation of the microbial profile of cumin seeds and chilli powder, sold in retail shops in the city of Mumbai, revealed high aerobic plate counts ranging from 10^6 to 10^8 cfu g^{-1} for chilli powder and 10^4 to 10^8 cfu g^{-1} for cumin. Among the bacteria present, 50-90% constituted sporeformers, which included amylolytic and proteolytic bacilli in both the spices. Eighty percent of the chilli powder samples contained *B. cereus* with counts up to 10^2 cfu g^{-1}. *Escherichia coli* was found in all the samples of chilli powder; counts ranged from 10 to 10^4 cfu g^{-1}, whereas cumin seed contained *E. coli* in insignificant numbers. The enteric pathogens like *Salmonella* and *Shigella* could not be detected in any of the samples. *Clostridium* was detected in only 20% of the samples of chilli powder, but in cumin seeds it was below the detection limit. Presence of *Staph. aureus* was insignificant, being <10 cfu g^{-1} in both the samples (Bhat *et al.* 1987).

Geeta and Kulkarni (1987) demonstrated microbiological quality of whole black pepper and turmeric powder sold in retail shops in Mumbai. Microbiological analysis of loosely packed samples obtained from retail shops revealed that the samples of both spices were highly contaminated. Aerobic plate counts of black pepper ranged from 10^8 to 10^{10} cfu g^{-1} and that of turmeric powder from 10^7 to 10^{10} cfu g^{-1}. In both the spices, mesophilic sporeformers like *Bacillus* occurred. Coliforms, that ranged in counts from 10^2 to 10^3 cfu g^{-1}, were further identified as *Enterobacter aerogens* and *E. coli*. *Staphylococcus, Salmonella* and *Shigella* were absent in all the samples analyzed. Anaerobic organisms were not found in any of the samples. The extent of contamination was slightly greater in pepper than in turmeric, although both the spices were of poor quality when compared with international standards.

In one report of microbial quality and sterilization of pre-packed ground Indian spices, which included pepper, turmeric, chilli and coriander, they were found to be heavily contaminated with bacteria. The total bacterial counts ranged between 10^5 and 10^7 cfu g^{-1}. The heaviest load of bacteria was found in chilli followed by pepper, turmeric and coriander. The aerobic spore counts corresponded well with total bacterial counts (Munasiri *et al.* 1987).

Singh *et al.* (1988) obtained black pepper, turmeric and chilli powder from the local market of Mysore in India and analyzed to determine microbiological quality. They showed that the total plate count was 10^6 g^{-1} turmeric, 10^5-10^7 g^{-1} black pepper and 10^6 g^{-1} chilli. Coliform counts were lowest (10^2 g^{-1}) in chilli, however in pepper and turmeric those were 10^5 g^{-1}. Mesophilic and thermophilic spores were present in pepper in the range of 10^2-10^5 g^{-1}. Turmeric powder harboured 10^5 g^{-1} spores, but in chilli powder the spores ranged from 10^2-10^5 g^{-1}. Moisture content in the samples of black pepper, chilli powder and turmeric powder ranged from 6.5 to 7.0, 8.2 to 8.3 and 9.9%, respectively.

Microbiological quality of seven spices, obtained from different grocery stores of Chandigarh in India, were evaluated by Kaul and Taneja (1989). Five to six samples of each spice were taken. The counts varied among different samples of the same spice. The total bacterial load ranged from 10^4 cfu g^{-1} in asafoetida to 10^7 cfu g^{-1} in fenugreek. *Escherichia coli* and *Salmonella* were found in all the seven spices.

Potential hazards of food poisoning due to usages of contaminated spices triggered the zeal for investigating the Indian retail level scenario of spice trade. A need for establishing Indian standards for microbial specification of spices was felt, as nothing such exists.