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Food Research International 36 (2003) 469–474

FOOD RESEARCH INTERNATIONAL

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Microbiological quality of some retail spices in India

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Received 26 February 2002; accepted 9 September 2002

Abstract

A total of 154 samples of 27 kinds of spices from retail shops of 20 States of India was investigated to determine their microbial status. As per ICMSF specifications, the total aerobic mesophilic bacteria (TAMB) count showed that 51% of the samples were in the unacceptable level ($>10^6$ cfu g^{-1}). While moulds were detected in 97% of the samples, yeast was found in only one. *Bacillus cereus*, *Clostridium perfringens*, *Staphylococcus aureus* and members of Enterobacteriaceae occurred in 85, 59, 11 and 85%, respectively of the kinds. Whereas black pepper powder, caraway, garlic and red chilli did not contain *B. cereus*, this foodborne pathogen was found in all the samples of ajmud, small cardamom and cumin powder. Coliforms and faecal coliforms were found in 33 and 15%, respectively of the kinds. *Escherichia coli* was detected in only one sample, of garlic. *Salmonella* and *Shigella* were found only in 2.6% of the samples. Although they contained less TAMB, the non-packaged spices had a higher load of moulds, *B. cereus* and Enterobacteriaceae than that of polyethylene-packaged ones.

Keywords: Spices; *Bacillus cereus*; *Staphylococcus aureus*; *Clostridium perfringens*; Enterobacteriaceae

1. Introduction

Spices are used all over the world to prepare foods mainly because of their flavouring properties. However, these are grown and harvested in warm, humid areas of the world where the growth of a wide variety of microorganisms is readily supported. The microbiological quality, the load of total heterotrophs or of Enterobacteriaceae in particular, often acts as an indicator of the hygienic situation of a region where the spices are produced and processed. As many other agricultural commodities, spices are exposed to a wide range of environmental microbial contamination during collection, processing, and in the retail markets by dust, waste water, and animal and even human excreta (De Boer, Spiegelenberg, & Janssen, 1985). 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 sporeforming bacteria in large volumes of foods, such as soups, casseroles, stews and gravies produced by catering establishments; under favourable conditions, they germinate and multiply to infective and toxic levels (Pafumi, 1986).

Previous studies on the microbiology of spices have demonstrated profiles of microorganisms, including total heterotrophs, *Bacillus cereus*, *Clostridium perfringens, Escherichia coli*, *Salmonella* and toxigenic moulds (Baxter & Holzapfel, 1982; Powers, Lawyer, & Masuoka, 1975; Powers, Latt, & Brown, 1976; Schwab et al., 1982). In many of the spice-growing countries, including India, spices after harvesting are often sun-dried by spreading them on open field or tarfelt road, and then sold without any treatment in order to reduce the microbial load. Thus, it is expected that spices sold in these areas contain a more or less "original" microflora. It was found in studies with samples of spices from limited parts of India that those were highly contaminated with any or all of mesophilic sporeformers, coliforms and moulds, and so were of poor quality when compared with international standards (García, Iracheta, Galván, & Heredia, 2001; Geeta & Kulkarni, 1987; Kaul & Taneja, 1989; Krishnaswamy, Patel, Nair & Muthu, 1974; Sha, Wadher & Bhoosreddy, 1996). More data should be made available to have an objective

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0963-9969/03/$-see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0963-9969(02)00194-1

basis for evaluating their quality as well as for the decision whether treatment of these food additives is needed or not. The purpose of this study was, therefore, to determine the occurrence and load of microorganisms, the important foodborne pathogens in particular, in spices offered for sale to consumers in retail stores all over India.

2. Materials and methods

2.1. Microorganisms

The reference organisms, used as control, were *B. cereus* ATCC9139 (courtesy: Dr. M.J.R. Nout, Wageningen University, The Netherlands), and *Staphylococcus aureus* MTCC96, *C. perfringens* MTCC450, *E. coli* MTCC118, *Salmonella typhi* MTCC733 and *Shigella flexneri* MTCC1457 (obtained from Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India).

2.2. Sampling

A total of 154 random samples of 27 different kinds of spices was purchased from retail outlets scattered over 20 different States of India (Table 1). The general hygienic status of retailers was not satisfactory; several of the spices were kept open in sacks. Those, which were kept inside glass bottles, were picked up by bare hands to weigh for selling. Approximately 150 g of unpacked samples of spices were collected in sterile sampling bags or screw-capped glass bottles. Those as well as packed (in sealed pouches made with low density polyethylene film) samples were transported to the laboratory and analysed as soon as possible.

2.3. Microbiological analysis

Representative 10-g portions of spices were aseptically weighed and homogenized with 90 ml sterile peptone-physiological saline (0.1% w/v neutral peptone, 0.85% w/v sodium chloride, pH 7.2) using a Stomacher lab-blender (Seward Medical, London, UK) at 'normal' speed (1 min for powdered samples, 2 min for whole ones). Serial decimal dilutions were prepared with the same diluent, and duplicate counting plates were prepared using appropriate dilutions. For pour plating, 1 ml of the dilutions were mixed with molten (45 °C) media and poured into plates. For surface seeding, 0.1 ml of the dilutions were spread on the surface of dried plates. After incubation at appropriate temperatures, the colonies that appeared on the selected plates were counted as colony forming units (cfu) per gram fresh weight sample. The representative colonies of each type were picked and diluted by streaking out on plates of appropriate media. After microscopic examination, the purified colonies were grown on slants or in broths of suitable media and stored at 4 °C (FDA, 1984; Speck, 1984).

The standard plate count for total aerobic mesophilic bacteria was carried out in pour-plates of plate count agar (PCA; HiMedia M091), incubated at 35 °C for 18–24 h. In order to estimate mesophilic bacterial spores, 10% (w/v) sample suspensions were treated for 30 min at 80 °C and there upon spread on PCA plates followed by incubation for 72 h at 30 °C (for aerobic sporeformers) and pour-plated with perfringens agar (HiMedia M579, FD011 and FD012) followed by incubation for 48 h at 37 °C in an anaerobic jar (Anaero-HiGas Pack; HiMedia LE002A) (for anaerobic sporeformers).

Enumeration of *B. cereus* was made on spread-plates of *B. cereus* selective agar (HiMedia M833, FD003 and FD045), incubated at 35 °C for 24–48 h. A representative number was purified, and the isolates were maintained on nutrient agar (NA; HiMedia M561) slants. The presumptive identification was confirmed on the basis of endospore formation, glucose fermentation, Voges–Proskauer reaction, nitrate reduction and motility, following standard methods (Claus & Berkeley, 1986).

Selective enumeration of *S. aureus* was carried out on spread-plates of Baird-Parker agar (HiMedia M043, FD047 and FD045), incubated at 35 °C for 24–48 h. Representative colonies were purified, and stored on NA slants. the presumptive colonies were confirmed on the basis of coagulase and acid from mannitol using coagulase mannitol broth base (HiMedia M277) with appropriate addition of sterile pretested coagulase plasma, thermostable DNase using DNase test agar with toluidine blue (HiMedia Mi041) and Voges-Proskauer reaction (HiMedia, 1998).

Selective enumeration of *C. perfringens* was carried out in pour-plates of perfringens agar, incubated at 37 °C in an anaerobic jar for 18–48 h. The representative colonies were purified, and maintained in cooked meat medium (HiMedia M149). Confirmation of the presumptive colonies was made on the basis of motility and nitrate reduction using motility nitrate medium (HiMedia M630I), raffinose fermentation using raffinose gelatin medium (HiMedia M987, substituting lactose with raffinose) and lactose fermentation and gelatin liquefaction using modified lactose gelatin medium (HiMedia M987).

Estimation of Enterobacteriaceae was carried out by mixing 1.0 ml of appropriate dilutions of spices with tryptone soya agar (TSA; HiMedia M290) and incubating the plates for 1–2 h at room temperature (27 °C) followed by a thick overlay of violet red bile glucose agar without lactose (HiMedia M581) and incubated at 35 °C for 18–24 h. The representative colonies were purified on TSA and stored on NA slants. Confirmation

Table 1
Sampling of retail spices from different States of India

Spice	Botanical name	States[a] of collection	No. of samples analysed	
			Total	Packed[b]
Ajmud	*Trachyspermum roxburghianum*	B, O, T_2, W	6	L-2, B-0
Allspice	*Pimenta dioica*	D, U_2, W	6	L-2, B-0
Aniseed	*Pimpinella anisum*	A_3, M_1, P_1, W	5	L-2, B-0
Asafoetida	*Ferula asafoetida*	A_2, A_1, C, D, P_2, W	6	L-2, B-4
Bishop's weed	*Trachyspermum ammi*	A_3, C, D, M_1, P_1, W	6	L-1, B-1
Black cumin	*Nigella sativa*	A_1, M_1, P_1, W	8	L-3, B-0
Black pepper	*Piper nigrum*	M_3, M_1, P_1, T_1, W	6	L-2, B-0
Black pepper powder		D, U_2, W	5	L-1, B-3
		D, U_2, W	5	L-1, B-0
Caraway	*Carum carvi*	M_2, M_1, S, W	5	L-0, B-0
Cardamom (small)	*Elettaria cardamomum*	C, D, P_2, S, W	5	L-1, B-0
Cardamom (large)	*Amomum subulatum*	K, M_1, T_1, W	5	L-1, B-0
Cinnamon	*Cinnamomum zeylanicum*	M_3, M_1, P_1, T_1, W	6	L-2, B-0
Clove	*Syzygium aromaticum*	A_3, K, M_1, P_1, T_1, W	7	L-2, B-0
Coriander	*Coriandrum sativum*	C, D, T_1, W	5	L-2, B-3
Coriander powder		A_3, K, P_1, T_1, W	7	L-2, B-1
Cumin		D, P_2, W	6	L-2, B-2
Cumin powder	*Cuminum cyminum*	A_3, M_1, P_1, W	6	L-2, B-0
Fenugreek	*Trigonella foenum-graecum*	C, D, G, O, P_2, W	6	L-0, B-0
Garlic	*Allium sativum*	A_1, C, D, O, P_2, W	6	L-0, B-0
Ginger	*Zingiber officinale*	M_3, M_1, P_1, W	5	L-2, B-0
Mustard seed	*Brassica juncea*	K, M_1, P_1, S, W	6	L-2, B-0
Poppy seed	*Papaver somniferum*	M_2, T_2, U_1, W	5	L-0, B-0
Red chilli	*Capsicum frutescens*	C, D, T_1, W	6	L-3, B-3
Red chilli powder		A_2, B, D, U_2, W	5	L-0, B-0
Tejpat	*Cinnamomum tamala*	G, M_2, M_1, U_1, W	5	L-0, B-0
Turmeric	*Curcuma longa*	C, D, T_1, W	5	L-2, B-3
Turmeric powder				

[a] A_1, Andaman and Nicobar Islands; A_2, Andhra Pradesh; A_3, Assam; B, Bihar; C, Chandigarh; D, Delhi; G, Gujarat; K, Kerala; M_1, Madhya Pradesh; M_2, Maharashtra; M_3, Meghalaya; O, Orissa; P_1, Pondicherry; P_2, Punjab; S, Sikkim; T_1, Tamil Nadu; T_2, Tripura; U_1, Uttaranchal; U_2, Uttar Pradesh; W, West Bengal.

[b] L, locally packed; B, branded.

of the presumptive isolates was made on the basis of cytochrome oxidase using oxidase disc (HiMedia DD018) and glucose fermentation in stab cultures of purple agar base (HiMedia M098) supplemented with 1% w/w D(+) glucose (Merck 17809).

Coliforms were confirmed by inoculating confirmed Enterobacteriaceae isolates into brilliant green bile broth, 2% (BGBB; HiMedia M121) with inverted Durham tubes, incubating those at 37 °C, for 24–48 h, and examining for growth and gas formation (Adams & Moss, 1995; Nout, Bakshi & Sarkar, 1998). For tests of faecal coliforms, inoculated BGBB tubes were incubated at 44±0.5 °C for 24 h. The presence of *E. coli* was confirmed on the basis of indole production by using tryptone water (HiMedia M463I) and Kovac's reagent strip (HiMedia DD019).

For qualitative detection of *Salmonella* and *Shigella*, 25-g samples were used for pre-enrichment, followed by enrichment and isolation in appropriate media (HiMedia) (Adams & Moss, 1995). The isolates were maintained on NA slants. The presumptive isolates were confirmed on the basis of acid and gas production by using triple sugar iron agar (HiMedia M021), lysine iron agar (HiMedia M377), motility by using motility nitrate medium (HiMedia M630I), production of acid from glucose by using MRVP medium (HiMedia M070), and indole production by using tryptone water and Kovac's reagent strip.

Enumeration of yeasts and moulds was carried out in pour-plates of potato dextrose agar (HiMedia M096). The plates were incubated at 28 °C for 2–5 days. The representative colonies were checked for purity before counting.

2.4. Moisture content

Moisture content was determined by drying approximately 10-g samples of spices at 105 °C to constant weights (Nout et al., 1998).

2.5. Statistical analysis

Data were analyzed by determining standard error of the mean, two-way analysis of variance and simple

correlation after converting the microbial counts to a logarithmic scale (Snedecor & Cochran, 1989).

3. Results and discussion

The results of moisture and microbial analysis of 154 samples of spices are summarized in Tables 2 and 3. The moisture profile conforms well with the specifications of European Spice Association (ESA), British Standards Institute (BSI) and International Standards Organization (ISO) (http://www.indianspices.com/html/s1490qua.htm) for spices.

Although some variations occur, specifications for microbial parameters in spices have been laid out by several countries. However, there are no separate Indian standards for microbial specifications of spices (Spices Board of India, personal communication). International Commission on Microbiological Specifications for Foods (ICMSF, 1974) set up maximum limits of 10^6, 10^4, 10^4 and 10^3 cfu of total aerobic mesophilic bacteria (TAMB), yeasts and moulds, coliforms and *E. coli*, respectively, per gram spice. In Germany, however, the standard values for TAMB, *B. cereus* and *S. aureus* are 10^5, 10^4 and 10^2 cfu, respectively, per gram spice. *E. coli* should be absent, and salmonella count should be zero in 25-g sample (http://www.indianspices.com/html/s1493qua.htm). Considerable variations were observed in the microbial counts, even between samples of the same kind. Hence, the distribution was contagious (Jarvis, 1989).

As per ICMSF specifications, TAMB count of $<10^4$ g^{-1} is of acceptable quality and 10^4–10^6 g^{-1} is of marginal quality. Our results indicate a high level of contamination; 51% (78/154) of the samples were in the unacceptable range ($>10^6$ cfu g^{-1}). The mean load of TAMB was found maximum (8×10^7 cfu g^{-1}) in black pepper and minimum (5×10^3 cfu g^{-1}) in garlic. Krishnaswamy et al. (1974) observed that in black pepper the counts of TAMB ranged between 10^4 and 10^8 cfu g^{-1}. Samples of black pepper, collected from warehouses in selected spice-trading areas of India, contained 10^4–10^7

Table 2
Moisture content and percentage of samples containing total aerobic mesophilic bacteria (TAMB), mesophilic bacterial spores (MBS) and moulds in retail spices of India

Spice	Moisture (g 100 g^{-1}) Mean±SE	Log cfu g^{-1} fresh weight									
		TAMB				MBS		Moulds			
		3.0–3.9	4.0–5.0	5.1–6.0	6.1–9.0	2.0–5.0	5.1–7.0	<DL	1.0–3.0	3.1–4.0	4.1–6.0
Ajmud	8.51±0.32				100		100		17	66	17
Allspice	12.34±0.48			17	83	50	50		100		
Aniseed	8.47±0.85		20	20	60	40	60		60	40	
Asafoetida	14.05±0.59		100			100			67	33	
Bishop's weed	9.35±0.78		50	17	33	50	50		100		
Black cumin	8.61±0.60				100		100		25	63	12
Black pepper	11.25±0.82			17	83		100		67	33	
Black pepper powder	11.24±0.92				100	100			80	20	
Caraway	10.27±0.41			60	40	80	20	20	80		
Cardamom (small)	11.87±0.71		20	20	60	40	60		100		
Cardamom (large)	21.06±1.66		80		20	80	20	60	40		
Cinnamon	13.15±0.92			20	80	40	60		80	20	
Clove	19.33±1.30		50		50	50	50	17	83		
Coriander	9.98±0.80		28	15	57	43	57		58	28	14
Coriander powder	11.62±1.50			20	80	20	80		80		20
Cumin	9.46±0.96		28	28	44	57	43		86	14	
Cumin powder	11.64±1.03		17	33	50	50	50		83	17	
Fenugreek	10.63±0.72		33	67		83	17		83		17
Garlic	61.34±0.67	83	17			100			33	67	
Ginger	90.35±1.25			83	17	50	50		67	33	
Mustard seed	6.65±0.48		20	40	40	60	40		40	60	
Poppy seed	5.94±0.42		17		83	17	83		83	17	
Red chilli	11.61±0.35		100			100			60	40	
Red chilli powder	13.77±1.85		17	17	66	33	67		17	50	33
Tejpat	11.32±0.76		40	60		60	40		100		
Turmeric	9.40±0.28		80		20	80	20		100		
Turmeric powder	13.57±0.79			20	80	20	80		60	40	

DL (detection limit) was log 1 cfu g^{-1} fresh weight.

Table 3
Percentage of samples containing foodborne bacterial pathogens in retail spices of India

Spice	Log cfu g^{-1} fresh weight												
	Bc				*Cp*			*Sa*		Enterobacteriaceae			
	<DL	2.0–4.0	4.1–5.0	5.1–6.0	<DL	1.0–2.0	2.1–3.0	<DL	2.0–3.0	<DL	1.0–3.0	3.1–5.0	5.1–8.0
Ajmud		100			100			100		50	17	33	
Allspice	67	33			100			100		67		33	
Aniseed	20	60	20		80	20		100		60		40	
Asafoetida	83	17			100			83	17	100			
Bishop's weed	17	83			67	33		100		83	17		
Black cumin	37	37	13	13	50	50		100		62	13		25
Black pepper	50	50			83	17		100		50	33	17	
Black pepper powder	100				60	20	20	100		20	20	40	20
Caraway	100				80	20		100		40	20	40	
Cardamom (small)		100			100			60	40	60	20	20	
Cardamom (large)	60	20	20		100			100		80	20		
Cinnamon	40	60			60	40		100		80			20
Clove	50	50			83	17		100		100			
Coriander	14	72	14		72	28		100		43	43	14	
Coriander powder	60	40			60	20	20	100		80		20	
Cumin	14	86			72	28		100		86			14
Cumin powder		100			67	33		100		66	17	17	
Fenugreek	17	33	50		83	17		100		83	17		
Garlic	100				100			83	17	83	17		
Ginger	33	17	50		83	17		100		100			
Mustard seed	20	40	40		80	20		100		60	40		
Poppy seed	67	33			100			100				83	17
Red chilli	100				100			100		80		20	
Red chilli powder	66	17			100			100		50		50	
Tejpat	40	60			80	20		100		60	40		
Turmeric	40	40	20		100			100		100			
Turmeric powder	60	40			100			100		60		40	

DL (detection limit) was log 2 cfu g^{-1} for *Bc* (*Bacillus cereus*), however log 1 cfu g^{-1} for *Cp* (*Clostridium perfringens*), *Sa* (*Staphylococcus aureus*) and Enterobacteriaceae.

TAMB g^{-1} (Seenappa & Kempton, 1981). The TAMB counts in whole black pepper and turmeric powder samples, collected from retail shops in the city of Mumbai in India, were 1×10^8–8×10^9 g^{-1} and 4×10^7–7×10^9 g^{-1}, respectively (Geeta & Kulkarni, 1987). Mesophilic bacterial spores (MBS) constituted 0.3–90% of the TAMB population. Baxter and Holzapfel (1982) observed that MBS accounted for 50–95% of TAMB in spices. Potential for the presence of *B. cereus* was also high; except four, all the kinds of spices contained this aerobic sporeformer. It occurred in all the samples of ajmud, small cardamom and cumin powder. The anaerobic sporeformer, *C. perfringens* was found in 59% (16/27) of the kinds and 17% (26/154) of the total samples analysed. The frequency of occurrence of *S. aureus* was relatively low; it was found in only a few samples of asafoetida (1), small cardamom (2) and garlic (1). It would be of interest to understand the reason for its incidence when the frequency of associated microflora is remarkably low.

Yeasts were detected in one sample, of whole cumin seeds (398 cfu g^{-1}), while moulds occurred in 97% (150/154) of the samples. The unacceptable ($>10^4$ cfu g^{-1}) percentage of the samples was 4.5. In the cumin samples of the city of Mumbai in India the yeast cell count ranged between 10 and 10^4 g^{-1} (Bhat, Geeta & Kulkarni, 1987). The maximum mould count was in red chilli powder. A similar observation was made by Christensen, Fanse, Nelson, Bates, and Mirocha (1967) and Bhat et al. (1987).

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 & Moss, 1995). The members of Enterobacteriaceae occurred in 23 out of 27 kinds and 32% of the samples of spices. When the counts of different shops were compared, the most unhygienic shops showed the highest counts of TAMB and Enterobacteriaceae. The counts decreased proportionally with an increase in hygienic condition of the environment. Coliforms were found to occur in 12 samples representing nine kinds (data not shown in Tables). A high level ($>10^4$ cfu g^{-1}) occurred in some (14–20%) of the samples of allspice, black pepper powder, whole cumin and turmeric powder. Faecal coliforms were found in four

kinds, namely large cardamom (63 cfu g^{-1}, one sample), coriander seeds (100 cfu g^{-1}, one sample), garlic (233 cfu g^{-1}, one sample) and turmeric powder ($5–13\times10^3$ cfu g^{-1}, two samples). The presence of *E. coli*, indicative of faecal contamination and the possible presence of enteric pathogens, occurred in only one sample (of garlic) at a load of 233 cfu g^{-1} and its presence may be due to unhygienic handling methods. Indeed, the level of sanitation in the sampling site of this garlic was poorer than the other places of collection. *Salmonella* was found only in two samples, one each of ginger and poppy seeds. *Shigella* was also found in two samples, of Bishop's weed and tejpat. These results agree with those of others (Baxter & Holzapfel, 1982; Julseth & Deibel, 1974; Schwab et al., 1982) who reported that *E. coli*, salmonella and shigella in spices are apparently rare and sporadic. Powers et al. (1975) detected coliforms in only three samples (one of each kind), but no faecal coliforms, in a total of 114 samples of spices tested.

To determine significance of the differences, analysis of variance was computed using log cfu values of TAMB, MBS, moulds, *B. cereus* and Enterobacteriaceae. There was no significant ($P\leqslant0.05$) correlation between TAMB, moulds, *B. cereus* and Enterobacteriaceae. Results of *C. perfringens*, *S. aureus*, *E. coli*, *Salmonella* and *Shigella* were not considered because of their low incidence in spices. Black cumin was found loaded with significantly ($P\leqslant0.05$) high doses of all those contaminants. On the contrary, asafoetida and garlic were significantly ($P\leqslant0.05$) less contaminated. No significant ($P\leqslant0.05$) difference was found between the levels of contaminants in spices collected from the eastern and western zones or in the summer and winter seasons of India.

The number of packaged kinds of spices containing TAMB was 54% higher than that of non-packaged ones. On the other hand, the respective numbers of non-packaged kinds containing moulds, *B. cereus* and Enterobacteriaceae were 85, 69 and 62% higher than the packaged kinds.

Considering the ICMSF specifications as a guide, our results indicate a high level of microorganisms in spices that may be a source of contamination in the kitchen. However, it is difficult to select a single microbial index for quality determination of these food additives because they are used as ingredients in a variety of products prepared in different ways. The desired index selected should reflect the use and method of preparation of the food product.

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