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MICROBIOLOGICAL QUALITY OF SPICES MARKETED IN UTTARAKHAND, INDIA

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ABSTRACT

A total of 100 spice samples were investigated for the detection and enumeration of total aerobic mesophilic bacteria, thermotolerant coliforms (TC), Bacillus cereus, Staphylococcus aureus and Salmonella spp. All enumerations were performed according to the International Commission on Microbiological Specifications for Foods (ICMSF). For the total aerobic mesophilic bacteria counted 15% samples were acceptable, 80% were of marginal quality and 5% did not show any mesophilic count. 21% samples did not show any TC, 66% samples were acceptable whereas 13% were found unacceptable according to the limit set by ICMSF. Out of the total, 31%, 89% and 50% samples showed Salmonella spp., B. cereus and Staphylococcus aureus contamination respectively. Mechanical drying, cleaning, microbiological reduction treatment, irradiation, should be applied to spices to reduce microbial load and improve the safety of spices.

Keywords: Bacillus cereus, Mesophiles, Salmonella spp., Staphylococcus aureus, Thermotolerant coliforms

Introduction

Spices are used worldwide to prepare food mainly due to their flavour, colour and aroma. They may be derived from many parts of the plant like bark, buds, flowers, fruits, leaves, rhizomes, roots, seeds, stigmas, style or the entire plant tops (Takeda et al., 2008). In Indian house-holds spices and herbs invariably used for culinary purposes. Some of the most common ones are black pepper, cumin, fenugreek, clove, fennel, cardamom, cinnamon, coriander, paparika, sesame, mustard, bay leaves, garlic, onion. Many of these are grown and harvested in tropical climates where temperature conditions and humidity support the growth of a wide variety of microorganisms. Indian spices exports have more than doubled in between 2008 to 2015 (Babu, 2017). In India's spice production was estimated to amount to 8.1 million metric tons.

Most of the Indian spices are in the effectively dried form to ensure stable stored product. In many of the spicegrowing countries, including India, the drying of the spices is mainly done by spreading them in open fields under the sun which might expose them to microbial contamination (Banerjee and Sarkar, 2003). These are then marketed without any treatment to reduce contamination. Also, during harvesting and processing, spices come in contact with dust, and faeces of rodents, birds and insects (Baneriee and Sarkar, 2003). contaminations ΑII these can transferred to food causing serious health problems in humans.

Reviewing scientific literature shows that several microorganisms including Bacillus cereus and other Bacillus spp., Clostridium Staphylococcus perfringens, Salmonella spp., E. coli, other members of Enterobacteriaceae, Aeromonas veast and mould have been frequently detected in spices (Schwab et al., 1982; Pafumi, 1986: Donia, 2008: Shamsuddeen, 2008; Ahene et al., 2011; Puchianu et al., 2012; Salari et al., 2012; Kimiran & Gürün, 2013; Chaudhary & Singh, 2014; Parveen et al., 2014; Ereifei et al., 2015; Bakobie et al., 2017). Salmonella has been previously isolated in 8.2% of different types of black pepper and B. cereus was isolated in all 33 spice samples studied in Australia (Pafumi, 1986). Mesophilic bacteria were found in ginger, black pepper, and red pepper in India (>106 CFU/g) in 50% of the export quality spice samples (Seenappa & Kempton, 1981).

Data from the above studies show that spices are a potential carrier pathogenic microorganisms which cause diseases in humans. Despite of this fact, there are very few articles that report the evaluation of quality of these spices in where they are extensively produced, exported and consumed in almost all food preparations. More data is therefore required to evaluate microbial quality of these spices so that proper decision about the treatment of these food additives can be taken. The aim of this study was therefore to estimate the microbiological quality of spices offered for sale to consumers in retail stores in Uttarakhand, India.

Materials and Methods Microorganisms used

The reference organisms, used as control in the study, were Bacillus cereus (MTCC 8733), Staphylococcus aureus (MTCC 96), E. coli (MTCC 443) and Salmonella (MTCC 3231) (obtained from Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India).

Sampling

Spice samples were selected on the basis of information about the demand of a particular spice, obtained wholesalers of specific spice trading areas. A total of 100 samples of spices were analysed for their microbiological quality. 10 samples (BP 01-BP 10) of black pepper (Piper nigrum), 10 samples (CL 01-CL 10) of clove (Eugenia aromatic), 7 07) (BC 01-BC of samples cardamom (Amomum subulatum), 10 samples (CI 01-CI 10) of cinnamon (Cinnamomum zeylanicum), 10 samples (CU 01-CU 10) of cumin (Cuminum cyminum), 7 samples (BL 01-BL07) of Indian bay leaves (Cinnamomum tamala), 10 samples (CM 01-CM10) of cardamom areen (Elettaria cardamomum), 8 samples (FE 01-FE 08) of fenugreek (Trigonella foenum- graecum), 8 samples (KA 01-KA 08) of kalonji (Nigella sativa), 10 samples (DH 01-DH 10) of coriander (Coriandrum sativum) and 10 samples (GM 01-GM 10) of garam masala were collected between October 2018 and March 2019 from Uttarakhand, India. Samples were brought to the laboratory and analysed as soon as possible.

Microbiological analysis

Represented spices weighed at five grams (powdered samples), were homogenized in 45 ml of buffered

sterilized water having neutral pH by vortexing at normal speed for 1 minute. Serial decimal dilutions were prepared with the same diluent, and triplicate counting plates were prepared using appropriate dilutions. 0.1 ml of the dilutions were spread on the surface of dried nutrient agar plates for colony forming unit (CFU) count. Determination of the most probable number (MPN) of TC was carried out according to the American Public Health Association quideline, using three-tube serial dilution. The MPN was calculated based on gas production in tubes of E. coli broth. The colonies were tested for detection of articulate pathogen coliform by the spread plate method on Eosin Methylene Blue agar (Hi Media M317) medium plates (Kornacki & Johnson, 2001).

The pour plate method was used for mesophilic bacterial enumeration. One millilitre dilution of the sample was dispensed in a dish and mixed in 20 ml nutrient agar (Hi Media M001). After spreading, the plate was kept in an incubator at 37°C for 24 hours for CFU count. In order to calculate the final concentration, the number of CFU was multiplied by the inverse of the dilution factor of the respective plate (Morton et al., 2001).

For *S. aureus* enumeration, serial dilutions of food homogenates were plated on Baird-Parker agar (Hi Media M043) with 5% egg yolk tellurite emulsion, and incubated at 35°C for 48 hours. Characteristic colonies were counted and transferred to tubes containing nutrient agar. Random colonies were tested by Soyabean Casein Digest Agar (Hi Media M290) for catalase production

and coagulase test was performed (Lancette et al., 2001).

For B. cereus enumeration, 0.1 ml serial dilutions of food homogenates were plated on the Mannitol Yolk Polymixin Agar and incubated at 37°C for 48 hours. After incubation, colonies were counted and random colonies were tested by Gram staining, Soyabean Casein Digest Agar (Hi Media M290) for catalase production and Simmons Citrate Agar (Hi Media M099) for citrate production (Tallent et al., 2012).

For detection and enumeration Salmonella, represented spices weighed at five grams, were homogenized in 45 ml of buffered peptone broth by vortexing at normal speed for 2 minutes and incubated for 18 to 20 hours at 37°C. After incubation, 0.1 ml of the homogenate was transferred to 10 ml of Rappaport-Vassiliadis broth (Hi Media MH1491), and 1 ml of the homogenate was transferred to 10 ml of tetrathionate brilliant green broth (Hi Media M1255). suspensions were incubated at 42°C and 35°C for 24 hours, respectively. After incubation, a loopful of each suspension was plated onto xylose-lysine-desoxycholate agar (Hi Media M031) and Salmonella-Shigella agar (Hi Media M108D). After incubation at 35°C for 24 hours, colonies were counted and random colonies from plates were biochemically tested using triple sugar iron agar (Hi Media MM021) (Andrews & Sillikar, 2001).

Statistical analysis

All experiments were performed in triplicates. Data were analysed by determining standard error of the mean,

two-way analysis of variance and simple correlation. Interpretation of results was done according to International Commission on Microbiological Specifications for Foods (ICMSF), 2005.

Results and Discussion

According to the International Commission on Microbiological Specifications for Foods (ICMSF, 2005), the acceptable limit for total aerobic mesophilic bacteria is set at <104 cfu/g and limit for marginal quality is 104-106 cfu/g. Above 106 cfu/g it is not acceptable for consumption. The limit for coliforms is set at 10⁴ cfu/g. The load of total aerobic mesophilic bacteria was found maximum (7.52×10⁵ cfu/a) in black pepper and minimum (2.6×10³ cfu/g) in cloves. In our study one sample each of kalonji, bay leaf, garam masala, black cardamom and clove did not show any mesophilic bacteria. Mesophilic bacteria were found in black pepper in India (>106 cfu/g) in 50% of the export quality spice samples (Seenappa & Kempton, 1981). In black pepper the counts of total aerobic mesophilic bacteria were found to range 104 108 between and cfu/a al., 1974). (Krishnaswamy et Thermotolerant coliforms were found maximum (7.68×10⁵ cfu/g) in kalonji and minimum (3.0×10³ cfu/g) in fenugreek. 4 samples of bay leaves, 7 samples of garam masala, 1 sample of fenugreek, 6 samples of green cardamom and 3 samples of black cardamom did not thermotolerant anv coliform. show Banerjee and Sarkar, 2003 showed that 51% samples out of 154 had unacceptable levels of TAMB (total aerobic mesophilic bacteria), coliforms



and faecal coliforms were found in 33 and 15%. Bacillus cereus, Staphylococcus members aureus and of Enterobacteriaceae occurred in 85, 59, 11 and 85% respectively of the samples. Our study showed a high load of Salmonella $(4.0 \times 10^3 - 6.76 \times 10^5 \text{ cfu/g})$, B. cereus $(2.0 \times 10^3 - 7.0 \times 10^5 \text{ cfu/g})$ and S. aureus $(4.0 \times 10^3 - 7.12 \times 10^5 \text{ cfu/g})$ in the spice samples. 31/100 of the studied spice samples showed Salmonella, 89/100 showed B. cereus and 50/100 showed S. aureus contamination. Sagoo et al., 2009 detected Salmonella spp. in both 1% of dried spices and herbs sampled at retail and production. A small proportion of herbs and spices contained high count of B. cereus (1%, ≥10⁴ cfu/g). Moreira et al., 2009 analysed samples for Bacillus cereus, Staphylococcus aureus, and Salmonella. Their study showed that 5.6% of these samples were not in agreement with the standards of Brazilian law in highest Zweifel and Stephan, 2012 quality. assessed spices and herbs as source of

Salmonella-related foodborne diseases. Their study showed that the proportion of Salmonella-positive samples ranged from 0% to 8.4%. **Bedada et al.**, **2018** found *S. aureus* in 11.7% of 162 spice samples tested whereas Salmonella spp. was not noticed in any of the samples. The bacterial count in different spices studied is shown in Table 1. Number of samples within ICMSF range for total aerobic mesophilic bacteria and coliforms are shown in Table 2. The number of samples contaminated with Salmonella, B. cereus and S. aureus are shown in Table 3.

The computed analysis of variance showed no significant correlation (p \leq 0.05) between total aerobic mesophilic bacteria, Salmonella, B. cereus, S. aureus and thermotolerant coliforms. Cumin and coriander were found loaded with significantly (p \leq 0.05) high doses of all the contaminants studied. On the other hand, Indian bay leaf and green cardamom were significantly (p \leq 0.05) less contaminated.

Table 1: Microbiological count of unprocessed spices from India

Spices	No. of	Range of microbial count (cfu/g)			

	samples	Total aerobic mesophilic count	Salmonella	B. cereus	S. aureus	Coliforms
Black Pepper	10	$\begin{array}{c} 1.32(\pm0.13) \\ \times 10^4 \\ 7.52(\pm0.01) \\ \times 10^5 \end{array} -$	3.04(±0.07) ×10 ⁴ -7.04 (±0.02) ×10 ⁴	$\begin{array}{c} 2.40(\pm 0.06) \\ \times 10^3 \text{-} 6.80 \\ (\pm 0.02) \times 10^3 \end{array}$	$1.68(\pm 0.19) \\ \times 10^4 - 6.52 \\ (\pm 0.04) \times 10^4$	$1.72(\pm 0.15) \\ \times 10^4 - 3.74 \\ (\pm 0.06) \times 10^4$
Cumin	10	5.60(±0.20) ×10 ⁵ -5.72(±0.33) ×10 ⁵	$1.48(\pm 0.09) \\ \times 10^{4} - 5.44 \\ (\pm 0.44) \times 10^{4}$	$2.0(\pm 0.63) \times 10^3 - 3.84 (\pm 0.47) \times 10^4$	$4.0(\pm 0.09) \\ \times 10^3 - 4.64 \\ (\pm 0.05) \times 10^4$	$1.52(\pm 0.37) \\ \times 10^{4} - 3.20 \\ (\pm 0.10) \times 10^{4}$
Cinnamon	10	$\begin{array}{ccc} 5.0(\pm0.82) \\ \times 10^3 - & 7.04 & (\pm \\ 0.08) \times 10^5 \end{array}$	$4.0(\pm 0.38) \times 10^{3}$ -2.52 $(\pm 0.16) \times 10^{5}$	$\begin{array}{c} 8.0(\pm 0.11) \\ \times 10^3 - 8.40 \\ (\pm 0.05) \times 10^4 \end{array}$	$8.0(\pm 0.41) \times \\ 10^3 - 4.0 \\ (\pm 0.23) \times 10^4$	$1.04(\pm 0.08) \\ \times 10^4 - 3.36 \\ (\pm 0.17) \times 10^5$
Kalonji	8	$ \begin{vmatrix} 1.6 & (\pm & 0.19) \\ \times 10^4 - 3.84 & (\pm \\ 0.08) \times 10^5 \end{vmatrix} $	$2.04(\pm 0.26) \\ \times 10^5 - 6.76 \\ (\pm 0.09) \times 10^5$	$\begin{array}{c} 4.8(\pm 0.34) \\ \times 10^4 \text{-} 7.0 \\ (\pm 0.31) \times 10^5 \end{array}$	$\begin{array}{c} 2.84(\pm0.48) \\ \times 10^5 \text{-} 7.12 \\ (\pm \ 0.33) \times 10^5 \end{array}$	$1.36(\pm 0.06) \\ \times 10^4 - 7.68 \\ (\pm 0.01) \times 10^5$
Bay Leaves	7	$\begin{array}{c} 4.0(\pm0.08) \\ \times 10^3 \text{-} 1.88 (\pm \\ 0.03) \times 10^4 \end{array}$	nil	$ \begin{vmatrix} 1.6(\pm 0.05) & \times \\ 10^4 - 4.56 \\ (\pm 0.01) \times 10^5 \end{vmatrix} $	$4.0(\pm 0.38) \times \\ 10^{3} - 7.6 \\ (\pm 0.19) \times 10^{4}$	$3.2(\pm 0.04) \times 10^{4}$ - 9.84 $(\pm 0.82) \times 10^{4}$
Garam Masala	10	$\begin{array}{c} 4.0(\pm 0.62) \\ \times 10^3 - 9.6 \\ 0.37) \times 10^4 \end{array} (\pm$	$ \begin{array}{l} 4.0(\pm 1.1) \\ \times 10^3 2.0 \\ (\pm 0.83) \times 10^4 \end{array} $	$ \begin{vmatrix} 1.2(\pm 0.17) \\ \times 10^4 & -6.72 \\ (\pm 0.08) \times 10^5 \end{vmatrix} $	$\begin{array}{l} 4.0(\pm 0.09) \times \\ 10^3 \text{-} 4.16 \\ (\pm \ 0.13) \times 10^5 \end{array}$	$9.2(\pm 0.46) \times 10^{4}$ -1.16 $(\pm 0.03) \times 10^{5}$
Coriander	10	$\begin{array}{c} 1.12(\pm 0.01) \\ \times 10^5 - 3.96 \ (\pm \\ 0.05) \times 10^5 \end{array}$	$1.96(\pm 0.09) \\ \times 10^5 - 6.72 \\ (\pm 0.04) \times 10^5$	$5.2(\pm 0.14) \\ \times 10^4 - 5.96 \\ (\pm 0.18) \times 10^5$	$4.0(\pm 0.93) \times \\ 10^3 - 1.88 \\ (\pm 0.10) \times 10^5$	$4.0(\pm 0.05) \times 10^{3}$ -7.52 $(\pm 0.13) \times 10^{5}$
Fenugreek	8	$ \begin{vmatrix} 4.0(\pm 0.72) \\ \times 10^3 - 8.8 \\ 0.08) \times 10^4 \end{vmatrix} (\pm$	$ \begin{vmatrix} 4.0(\pm 0.73) \\ \times 10^3 - 2.32 \\ (\pm 0.04) \times 10^5 \end{vmatrix} $	10^4 -2.16	$4.0(\pm 0.51) \times 10^3$ -3.88 (± 0.18) $\times 10^5$	$3.0(\pm 0.09) \times 10^{3}$ -5.6 $(\pm 0.02) \times 10^{4}$
Green Cardamom	10	$\begin{array}{ccc} 4.0(\pm 0.28) \\ \times 10^3 - & 2.56 & (\pm \\ 0.12) \times 10^4 \end{array}$	nil	$8.0(\pm 0.35) \\ \times 10^{3} - 2.6 \\ (\pm 0.61) \times 10^{5}$	$\begin{array}{l} 4.0(\pm 0.97) \times \\ 10^3 \text{-} 1.6 \\ (\pm 0.48) \times 10^4 \end{array}$	$ \begin{vmatrix} 8.0(\pm 0.05) & \times \\ 10^3 - 3.2 \\ (\pm 0.08) \times 10^4 \end{vmatrix} $
Black Cardamom	7	$ \begin{vmatrix} 1.6(\pm 0.62) \\ \times 10^4 - & 1.92 \\ 1.21) \times 10^5 \end{vmatrix} (\pm$	nil	$ \begin{vmatrix} 4.8(\pm 0.93) & \times \\ 10^4 - 1.84 \\ (\pm 0.47) \times 10^5 \end{vmatrix} $	$4.0(\pm 0.80) \times \\ 10^{3} - 2.8 \\ (\pm 0.86) \times 10^{4}$	$4.0(\pm 0.39) \times 10^{3}$ -6.4 $(\pm 0.09) \times 10^{4}$
Cloves	10	$\begin{array}{ccc} 2.6(\pm 0.74) \\ \times 10^3 - & 4.64 & (\pm \\ 0.43) \times 10^4 \end{array}$	$4.0(\pm 0.42) \\ \times 10^{3} - 1.2 \\ (\pm 0.76) \times 10^{5}$	$2.0(\pm 0.78) \\ \times 10^3 - 2.32 \\ (\pm 0.84) \times 10^4$	$1.40(\pm 0.91) \\ \times 10^4 - 3.16 \\ (\pm 0.40) \times 10^5$	$3.40(\pm 0.21) \times 10^3$ - 3.44 $(\pm 0.18) \times 10^5$

Data represented as Mean \pm SE (n=3)

Table 2: ICMSF range for total aerobic mesophilic bacteria and coliforms

Spices	No. of	No. of samples within ICMSF range	Coliforms	
_	samples	(Total aerobic mesophilic bacteria)		

		<10 ⁴ cfu/g (Acceptable)	10 ⁴ – 10 ⁶ cfu/g (Marginal)	>10 ⁶ cfu/g (Unacceptable)	>10 ⁴ cfu/g (Unacceptable)
Black Pepper	10	0	10	0	0
Cumin	10	0	10	0	0
Cinnamon	10	4	6	0	3
Kalonji	8	1	7	0	4
Bay Leaves	7	2	5	0	0
Garam Masala	10	2	8	0	1
Coriander	10	0	10	0	4
Fenugreek	8	5	3	0	0
Green Cardamom	10	2	8	0	0
Black Cardamom	7	1	6	0	0
Cloves	10	3	7	0	1

Table 3: Spice samples contaminated with Salmonella, B. cereus and S. aureus

Spices	No. of samples	Number of contaminated spice samples			
		Salmonella	B. cereus	S. aureus	
Black Pepper	10	5	4	4	
Cumin	10	4	7	5	
Cinnamon	10	4	10	3	
Kalonji	8	5	8	8	
Bay Leaves	7	0	7	4	
Garam Masala	10	2	10	5	
Coriander	10	4	10	4	
Fenugreek	8	4	8	4	
Green Cardamom	10	0	10	4	
Black Cardamom	7	0	7	4	
Cloves	10	3	8	5	

Conclusion

As per the specifications of the ICMSF, our study indicates high level of microbial

contamination in spices which are extensively used in the Indian cuisines. This microbial contamination may have an



adverse effect on health depending on the method of preparation of food and the time of addition of the spices to food. Steps need to be taken to improve the quality of spices before they are marketed. Mechanical drying, cleaning, microbiological reduction treatment, irradiation, can reduce microbial load and therefore can improve the safety of spices.

Declarations

Conflict of Interest: The authors declare that they have no conflict of interest.

Authors' contributions: The 1st author contributed to the concept, work plan, analysis of data and writing of manuscript. 2nd author did the survey, sample collection and lab work. Both authors read and approved the final manuscript.

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