



The evaluation of microbiological profile of some spices used in Turkish meat industry

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Abstract

Beside meat products, spices are used in preparation of various foods for seasoning, flavouring and imparting aroma in all over the world. Because of the warm and humid climate, poor collection conditions, unpretentious production process and extended drying times, spices are exposed to a wide range of microbial contamination. In addition, spices can be contaminated through dust and waste water in unpackaged spices which are sold in markets and bazaars. Our study is a comprehensive research that aimed to determine the microbiological quality of spices used in meat industry and it was conducted with a large number of samples sold in Istanbul, Turkey. A total of 420 samples of 7 different kinds of spices (black pepper, red pepper, white pepper, cumin, coriander, allspice and ginger) were collected from markets and bazaars. The counts of total mesophilic aerobic bacteria, yeasts, moulds, *S. aureus*, *E. coli*, sulphite-reducing anaerobic bacteria, *B. cereus* and the presence of *Salmonella* spp. were analyzed. The results were evaluated according to Turkish Food Codex. The results of this paper demonstrate that spices may contain pathogenic bacteria which are potential health threat to consumers. Use of sterilised spices in meat industry will be useful to minimise the hygiene and health risks.

Key words: Spices, meat products, meat, pathogens, microbiological quality.

Introduction

In Turkish meat industry fermented sausage (sucuk), salami and sausage are well-known and very popular meat products. These products are produced from meat, fat or tail fat, additives and spices, such as black pepper, red pepper and cumin, which give their characteristic flavor ¹⁻³. Beside meat products, spices are used in preparation of various foods for seasoning, flavouring and imparting aroma in all over the world.

The spices are produced from root, bark, leaves, bulb, stems, flowers and seeds of certain plants and mostly grown and harvested in warm, humid areas of the world ⁴. Because of the warm and humid climate, poor collection conditions, unpretentious production process and extended drying times, spices are exposed to a wide range of microbial contamination. In addition, spices can be contaminated through dust, waste water and animal/human excreta in unpackaged spices which are sold in markets and bazaars ^{5,6}. Therefore, spices are the primary sources of alimentary intoxication when added to foods in which pathogen growth is favorable. The possibility of pathogen growth is higher until consumption when spices are used in food without subsequent preservation, and this may lead to serious health hazards ⁷. Several studies have shown that spices contain various types of microorganisms such as pathogenic bacteria and toxigenic moulds ⁸⁻¹¹. In our country the microbiological quality of spices was evaluated by a number of researchers ¹²⁻¹⁴. However, more data should be obtained for evaluating the microbiological quality of spices which are used in meat industry.

Our study is a comprehensive research that aimed to determine the microbiological quality of spices used in meat industry and it was conducted with a large number of samples sold in Istanbul, Turkey.

Materials and Methods

Samples: A total of 420 samples of 7 different kinds of spices (black pepper, red pepper, white pepper, cumin, coriander, allspice and ginger) were randomly collected from various markets and bazaars located in Istanbul. Samples were transported under proper conditions from their place of collection to the laboratory.

Microbiological analyses: Ten-g samples of spices were transferred to a sterile bag with 90 ml of sterile peptone water (Oxoid, CM0009) and homogenized for 90 s using a stomacher (Lab Blender 400, Model BA 6021, Steward Lab., London, UK) and then serially diluted in sterile 0.1% peptone water.

Number of *S. aureus* was determined by duplicate spread-plating on Baird-Parker agar (BPA - Oxoid CM0275) supplemented with egg yolk-tellurite emulsion (E-Y-T Emulsion - Oxoid SR0054) followed by incubation at 35°C for 24 h. Colonies with typical *S. aureus* morphology were subjected to Gram staining, examined microscopically, tested for catalase and coagulase reactions and confirmed with DNase agar (DNase - Oxoid CM0321) following 18-24 h of incubation at 35°C ¹⁵. Total mesophilic aerobic bacteria (TMAB) was determined on Plate Count Agar (Oxoid CM0463) followed by 48 h of incubation at 35°C whereas *E. coli* was quantified on TBX Agar (TBX - Oxoid, CM0945), followed by 24 h of incubation at 44°C ¹⁶.

Sulphite-reducing anaerobic bacteria (SRA) were determined on Perfringens selective agar (SPS - Merck, 1.10235, Darmstadt, Germany) and roll tube method was used for incubation at 35°C for 24 h ¹⁷. Yeasts and moulds were determined on Yeast Extract Glucose Chloramphenicol Agar (YGC - Merck 1.16000) spread plates incubated at 25°C for 3-5 days ¹⁵. Polymyxin-pyruvate-

egg yolk–mannitol–bromothymol blue agar (PEMBA; Oxoid CM617) with 50,000 IU of polymyxin per litre and egg yolk emulsion (Oxoid SR47) was used for *B. cereus* counts after incubating at 30°C for 24–48 h. All other further analyses were performed according to Holbrook and Anderson¹⁸.

Isolation of *Salmonella* spp. was carried out at four steps. Buffered peptone water (BPW - Oxoid, CM0509) (225 ml) was added to 25 g meatball sample of each group, and incubated at 35–37°C for 16–20 h for pre-enrichment step, and 0.1 and 1 ml of the homogenate was transferred to Rappaport-Vassiliadis medium (RV - Oxoid, CM0669) and tetrathionate broth (TT - Oxoid, CM0671) for selective enrichment with an incubation period of 42 and 43°C for 24 h, respectively. After incubation, a loopful from each tube was streaked on bismuth sulfite agar (BS - Oxoid, CM0201), xylose lysine desoxycholate agar (XLD - Oxoid, CM0469) and Hectoen enteric agar (HE - Oxoid, CM0419) and incubated for 20–24 h at 35°C. Typical colonies were checked and selected for growing on nutrient agar (NA - Oxoid, CM0003) at 35°C for 18–24 h and identified by triple sugar iron agar (TSI - Oxoid, CM0277), lysine iron agar (LIA - Oxoid, CM0381) fermentation tests, urease test (urea broth - Oxoid, CM0071) and Voges-Proskauer, indol, O-, Vi- and H-antigen tests (Murex Salmonella Polyvalent Agglutinating Sera)¹⁹.

All microbiological tests were carried out in duplicate and the results were expressed as log₁₀ cfu/g.

Results and Discussion

The results of microbiological analysis of 420 samples of spices are shown in Tables 1 and 2. The maximum rates for *E. coli*, *S. aureus*, *B. cereus*, SRA and yeasts and moulds were 28.3% (in red pepper), 21.6% (in red pepper and ginger), 25% (in ginger), 51.6% (in black and red pepper) and 100% (in coriander), respectively. *Salmonella* spp. were detected in 12 out of 420 (2.9%) spices samples (2 black pepper, 1 red pepper, 2 cumin, 3 coriander, 4 ginger) whereas TMAB were positive in all samples (Table 1). TMAB count was found maximum (8.8x10⁶ cfu/g) in red pepper and minimum (3x10¹ cfu/g) in allspice. According to Turkish Food Codex²⁰, 87 spices samples were unacceptable in terms of TMAB counts (>10⁶ cfu/g).

S. aureus is considered the third most important cause of disease in the world amongst the reported food-borne illnesses^{21, 22}. Staphylococcal food poisoning is a persistent cause of gastroenteritis worldwide, especially in developed countries²³. In this study, while *S. aureus* could not be isolated in 365 out of 420 (86.9%) spices samples, maximum *S. aureus* count was detected in ginger (5.2x10⁴ cfu/g) and minimum count in black pepper, red pepper, white pepper, ginger and allspice samples (1.0x10¹ cfu/g). According to these results 4 black pepper, 5 red pepper, 3 cumin and 6 ginger samples exceeded the maximum limits of *S. aureus* (1.0x10³ cfu/g) set in Turkish Food Codex²⁰.

In our study the maximum *B. cereus* count was also detected in ginger (5.3x10⁵ cfu/g) and minimum count in red pepper, white pepper, coriander and allspice samples (1.0x10¹ cfu/g). In 362 out of 420 (86.2%) samples *B. cereus* could not be detected. Only four samples (black pepper, red pepper, coriander and ginger) were unacceptable (>10⁵ cfu/g) according to codex²⁰.

The presence of *E. coli*, indication of fecal contamination and the possible presence of enteric pathogens, could not be found in 375 samples (89.2%). The count of this pathogen was maximum

(5.2x10³ cfu/g) in red pepper. One black pepper, 10 red pepper, 2 cumin, 5 coriander and one allspice samples exceeded the maximum limits of *E. coli* (1.0x10² cfu/g) set in Turkish Food Codex²⁰.

Yeast and mould contamination rate on examined spices samples was fairly high. No yeast and mould could be detected in 59 spices samples (14.0%). All coriander samples (100%) were contaminated with yeasts and moulds. Maximum count was detected in red pepper (8.0x10⁶ cfu/g) and minimum count in black pepper (1.0x10¹ cfu/g). According to these results, 191 spices samples were unacceptable (>10⁴ cfu/g) according to codex²⁰.

SRA could not be isolated in 238 out of 420 samples (56.7%). Cumin and ginger had the maximum SRA count (2.0x10⁶ cfu/g), while black pepper, red pepper, white pepper and allspice had the minimum count (1.0x10¹ cfu/g); 16 red pepper, 11 cumin, 4 coriander, 2 allspice and 21 ginger samples (totally 54 samples) exceeded the maximum limits of SRA (1.0x10⁴ cfu/g) set in Turkish Food Codex²⁰.

Salmonella spp. could not be detected in 408 samples (97.1%). This pathogen was positive only in 2 black pepper, 1 red pepper, 2 cumin, 3 coriander and 4 ginger samples, and these samples were unacceptable according to food codex²⁰.

There are several studies on microbiological quality of various spices in Turkey. Uner and Ergun²⁴ found TMAB counts as 4.1x10⁵ cfu/g (5.61 log), 7.0x10⁶ cfu/g (6.85 log) and 8.9x10⁷ cfu/g (7.95 log), *B. cereus* 1.8x10² cfu/g (2.26 log), 3.8x10² cfu/g (2.58 log) and 4.0x10² cfu/g (2.60 log) and yeasts and moulds 1.1x10⁵ cfu/g (5.04 log), 1.1x10³ cfu/g (3.04 log) and 3.7x10⁴ cfu/g (4.57 log) in cumin, black pepper and red pepper samples (each of 10), respectively. Similarly, Elmali and Yaman²⁵ analyzed 15 black pepper, 15 red pepper and 15 cumin samples. They found TMAB, *B. cereus*, yeast and mould counts as 7.23, 3.0 and 5.58 log cfu/g in black pepper, 6.43, 1.09 and 5.07 log cfu/g in red pepper and 5.61, 2.84 and 4.62 log cfu/g in cumin, respectively.

In a study conducted by Vural¹⁴, the average results for TMAB, *E. coli*, SRA and yeast-moulds were found as 4.8x10⁶ cfu/g, 5.4x10² cfu/g, 1.7x10¹ cfu/g and 1.5x10⁴ cfu/g in 15 cumin samples, respectively. Also the average counts were 5.5x10⁶ cfu/g (6.74 log), 4.2x10² cfu/g (2.62 log), 2.3x10² cfu/g (2.30 log) and 1.9x10³ cfu/g (3.28 log) in 15 allspice samples. Erol *et al.*¹¹ determined the mean TMAB counts as 6.8x10⁶ cfu/g (6.83 log), 3.1x10⁶ cfu/g (6.49 log), 9.2x10⁴ cfu/g (4.96 log) in black pepper, red pepper and cumin (each of 25), respectively. *B. cereus* was isolated from 80% of black pepper, 44% of red pepper and 28% of cumin, while none of the examined samples contained *S. aureus* and *E. coli*.

In a study conducted by Vural¹⁴, the average results for TMAB, *E. coli*, SRA and yeast-moulds were 4.8x10⁶ cfu/g, 5.4x10² cfu/g, 1.7x10¹ cfu/g and 1.5x10⁴ cfu/g in 15 cumin samples, respectively. Also the average counts were 5.5x10⁶ cfu/g (6.74 log), 4.2x10² cfu/g (2.62 log), 2.3x10² cfu/g (2.30 log) and 1.9x10³ cfu/g (3.28 log) in 15 allspice samples. Erol *et al.*¹¹ determined the mean TMAB counts as 6.8x10⁶ cfu/g (6.83 log), 3.1x10⁶ cfu/g (6.49 log) and 9.2x10⁴ cfu/g (4.96 log) in black pepper, red pepper and cumin (each of 25), respectively. *B. cereus* was isolated from 80% of black pepper, 44% of red pepper and 28% of cumin, while none of the examined samples contained *S. aureus* and *E. coli*.

Agaoglu *et al.*¹² examined 10 black pepper, 10 cumin and 10 red pepper and reported that average *B. cereus* counts were 1.0x10³ cfu/g (3.0 log) in black pepper, 3.9x10² cfu/g (2.59 log) in cumin and 6.0x10² cfu/g (2.78 log) in red pepper. These findings were lower than ours. Temelli and Anar¹³ determined mean *B. cereus*

Table 1. Microbial evaluation and distribution of spices.

Spice	Microorganism	ND	10 to < 10 ²	10 ² to < 10 ³	10 ³ to < 10 ⁴	10 ⁴ to < 10 ⁵	10 ⁵ to < 10 ⁶	10 ⁶ to < 10 ⁷
Black pepper	APC	0	0	0	11	22	22	5
	<i>E.coli</i>	56	3	1	0	0	0	0
	<i>S.aureus</i>	51	2	3	1	3	0	0
	<i>B.cereus</i>	53	1	3	2	0	1	0
	SRA	29	18	10	3	0	0	0
	Yeasts/Moulds	30	4	10	12	1	2	1
<i>Salmonella</i> spp.	58				2			
Red pepper	APC	0	0	0	5	7	31	17
	<i>E.coli</i>	43	7	6	4	0	0	0
	<i>S.aureus</i>	47	5	3	4	1	0	0
	<i>B.cereus</i>	49	4	2	3	1	1	0
	SRA	29	10	4	1	12	4	0
	Yeasts/Moulds	3	0	8	9	22	11	7
<i>Salmonella</i> spp.	59				1			
White pepper	APC	0	0	0	18	14	17	11
	<i>E.coli</i>	56	4	0	0	0	0	0
	<i>S.aureus</i>	55	5	0	0	0	0	0
	<i>B.cereus</i>	55	3	2	0	0	0	0
	SRA	35	9	5	1	0	0	0
	Yeasts/Moulds	7	1	12	11	15	10	4
<i>Salmonella</i> spp.	60				0			
Cumin	APC	0	0	0	0	8	30	22
	<i>E.coli</i>	54	4	2	0	0	0	0
	<i>S.aureus</i>	52	1	4	3	0	0	0
	<i>B.cereus</i>	55	1	1	1	2	0	0
	SRA	39	3	4	3	5	5	1
	Yeasts/Moulds	2	0	1	5	25	19	8
<i>Salmonella</i> spp.	58				2			
Coriander	APC	0	0	0	10	14	19	17
	<i>E.coli</i>	50	5	5	0	0	0	0
	<i>S.aureus</i>	58	1	1	0	0	0	0
	<i>B.cereus</i>	52	2	2	3	0	1	0
	SRA	37	12	5	2	1	3	0
	Yeasts/Moulds	0	4	14	8	15	12	7
<i>Salmonella</i> spp.	57				3			
Allspice	APC	0	1	0	15	26	15	3
	<i>E.coli</i>	56	3	1	0	0	0	0
	<i>S.aureus</i>	55	5	0	0	0	0	0
	<i>B.cereus</i>	53	7	0	0	0	0	0
	SRA	39	8	9	2	2	0	0
	Yeasts/Moulds	9	1	18	17	8	6	1
<i>Salmonella</i> spp.	60				0			
Ginger	APC	0	0	1	15	6	26	12
	<i>E.coli</i>	60	0	0	0	0	0	0
	<i>S.aureus</i>	47	3	4	2	4	0	0
	<i>B.cereus</i>	45	5	4	3	2	1	0
	SRA	30	5	2	2	11	8	2
	Yeasts/Moulds	8	3	13	9	9	6	2
<i>Salmonella</i> spp.	56				4			

counts as 8.5×10^3 cfu/g (3.93 log) in black pepper, 4.8×10^2 cfu/g (2.68 log) in cumin and 2.9×10^5 cfu/g (5.46 log) in red pepper samples. When the results were compared to ours, the *B. cereus* counts were lower in black pepper and cumin samples (Table 2).

There are several studies conducted in countries other than Turkey and various results were established on the microbiological quality of spices. In India, a study performed by Banerjee and Sarkar⁸, 6 allspice, 5 black pepper, 7 coriander, 6 cumin and 5 ginger samples were examined. According to results, *S. aureus* could not be detected in all samples. *Salmonella* spp. was found in only one ginger sample. *B. cereus* counts ranged between 2.0 and 4.0 log cfu/g in cumin, <2.0 and 5.0 log cfu/g in ginger, <2.0

and 4.0 log cfu/g in coriander, whereas the organism could not be detected in black pepper samples. It was reported that the maximum mean load of TMAB (8.0×10^7 cfu/g) was found in black pepper. Similarly, Krishnaswamy *et al.*²⁶ determined the counts of TMAB ranging between 10^4 and 10^8 cfu/g in black pepper. These TMAB counts were higher than ours (Table 2). In Egypt, Abou Donia²⁷ analyzed 10 cumin, 15 coriander and 5 black pepper samples and found the mean counts of TMAB as 2.8×10^6 , 4.0×10^5 and 4.0×10^3 cfu/g in cumin, coriander and black pepper, respectively. However, none of the examined samples contained *S. aureus* and *Salmonella* spp., and the mean yeast counts were 3.1×10^3 cfu/g in cumin, 1.2×10^3 cfu/g in coriander and 3.4×10^2 cfu/g in black pepper samples.

Table 2. Minimum, maximum and average microbial counts (log CFU/g) and percentage (%) of positive samples.

Spice	APC			E. coli			S. aureus			B. cereus			Yeast/Moulds			SRA		
	Min	Max	Avg	Min	Max	Avg	Min	Max	Avg	Min	Max	Avg	Min	Max	Avg	Min	Max	Avg
Black Pepper	3.0	6.77	5.61	1.0	2.38	1.89	1.0	4.23	3.68	1.34	5.25	4.43	1.0	6.23	4.76	1.0	3.18	4.04
Red Pepper	3.30	6.94	6.18	1.0	3.64	3.04	1.0	4.15	3.28	1.0	5.15	4.23	2.0	6.90	5.60	1.0	5.48	4.53
White Pepper	3.04	6.76	5.80	1.0	1.48	1.23	1.0	1.48	1.20	1.0	2.48	1.96	1.90	6.48	5.38	1.0	3.0	2.11
Cumin	4.23	6.90	6.17	1.0	2.25	1.86	1.30	3.54	3.04	1.48	4.71	4.18	2.48	6.52	5.59	1.0	6.30	5.17
Coriander	3.0	6.83	6.08	1.0	2.70	2.17	1.30	2.0	1.78	1.0	5.08	4.15	1.60	6.63	5.62	1.30	5.18	4.41
Allspice	1.48	6.60	5.40	1.30	2.26	1.67	1.0	1.48	1.20	1.0	1.70	1.30	1.30	6.20	4.93	1.0	4.18	1.73
Ginger	2.0	6.94	5.97	-	-	-	1.0	4.72	3.87	1.30	5.72	4.55	1.90	6.07	4.89	1.30	6.30	5.28
		100%		6.6%			10.0%			15%	11.6%		50%			51.6%		
		100%		28.3%			3.3%			21.6%	18.3%		95%			51.6%		
		100%		6.6%			8.3%			8.3%	8.3%		88.3%			41.6%		
		100%		10.0%			13.3%			8.3%	8.3%		96.6%			35%		
		100%		16.6%			3.3%			13.3%	13.3%		100%			38.3%		
		100%		6.6%			8.3%			11.6%	11.6%		85%			35%		
		100%		-			21.6%			25%	25%		86.6%			50%		

In conclusion, the results of this paper demonstrate that spices may contain pathogenic bacteria which are potential health threat to consumers. After utilisation of contaminated spices in meat products, there is the risk of early spoilage, food-borne infections and intoxications. In order to prevent the health risk, a number of methods (the use of ethylene oxide, microwave treatments, UV, infrared or gamma irradiation) exist to reduce or eliminate pathogens from spices. Use of sterilised spices in meat industry will be useful to minimise the hygiene risks and to prevent consumer's health.

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