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Microbiological Quality and *Salmonella* spp.,/*Listeria monocytogenes* of Spices in Turkey

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ABSTRACT

Spices contaminated with pathogens may contaminate the food to which they are added and therefore, they pose a great risk to public health. In this study, 250 spices samples (50 samples each of red sweet pepper, red chili pepper, red pepper flakes, cumin and black pepper) were evaluated for Total Aerobic Mesophilic Bacteria (TMAB), *Staphylococcus*/*Micrococcus*, Coagulase (+) *Staphylococcus*, Yeast/Mold, Enterobacteriaceae, *Coliform* Bacteria, *E. coli*, *Enterococcus* spp., *B. cereus*, *Salmonella* spp. and *Listeria monocytogenes*. The means of total aerobic mesophilic bacteria (TMAB), *Staphylococcus*/*Micrococcus*, Coagulase (+) *Staphylococcus*, Yeast/Mold, Enterobacteriaceae, *Coliform* Bacteria, *E. coli*, *Enterococcus* spp. and *B. cereus*, counts were detected 5.50, 2.03, <log 2.00, 3.98, 3.57, 3.07, <log 2.00, 3.32 and 2.79 log CFU g^{-1}, respectively. *Salmonella* spp. and *L. monocytogenes* were detected 2.8 and 1.2%. We also determined the antimicrobial resistance of detected *Salmonella* spp. and *L. monocytogenes*. *Salmonella* spp. was resistant to Gentamicin/Ampicillin/Streptomycin, Nalidixic Acid and Tetracycline, while *L. monocytogenes* was resistant to Gentamicin/Amoxicillin and Ampicillin/Tetracycline/Penicillin. Based on these results, we recommend that spices be packaged with suitable packaging material, preserved in adequate conditions and that all HACCP regulations be strictly obeyed at every stage from production to consumption.

Key words: Spices, *Salmonella* spp., *Listeria monocytogenes*, antimicrobial resistance

INTRODUCTION

Spices are additives that are produced by drying the seed, bud, kernel, fruit, crust, root, stem, node, leaf, stalk and onion parts of plants. They can be used whole, crumbled and/or ground. Spices and herbs are very popular for their aroma and flavor and they often add desirable color to food, as well (TFC., 2013; Elmali and Yaman, 2005). The most commonly used spices in Turkey include red pepper, black pepper, cumin, pimento, cinnamon and ginger (Vural, 2004). Because, they are phytogenetic, spices may become contaminated, especially if they are produced under unhealthy conditions in wet/hot climates and if they are stored under inappropriate conditions (Schwab *et al*., 1982). Spices are exposed to a wide range of environmental microbial contamination during collection and processing and they are further exposed in retail markets by dust, wastewater and

animal and even human excrement. The microbiological quality of Enterobacteriaceae is often used as an indicator of the hygienic situation of a region, where spices are produced and processed (De Boer *et al*., 1985). Contaminated spices may affect the microbiological quality of the end product and may cause infections and toxicity in humans upon consumption (Elmali and Yaman, 2005). In addition, contaminated spices also increase the microbial load of the food to which they are added, which may cause food spoilage. Spices containing pathogen bacteria may be a potential danger to public health (Coskun, 2010). When added during cooking, spices often undergo sufficient heating to eliminate vegetative pathogens, such as *Salmonella*. However, when spices are added to pre-cooked dishes as a final flavoring or decoration, they could create a potential health hazard (D'Aoust, 1994). In this study, we investigated the microbiological quality of some spices (red sweet pepper, red chili pepper, red pepper flakes, cumin and black pepper) that are unpackaged in the Afyonkarahisar province.

MATERIALS AND METHODS

Sample collection: A total of 250 dried spices (50 samples each of red sweet pepper, red chili pepper, red pepper flakes, cumin and black pepper) were collected from 20 different herbalist markets between November, 2012 and April, 2013 in the Afyonkarahisar province of Turkey. The spices were collected aseptically in sterile bags (Inter science, 132025) and were analyzed the same day.

Enumeration of microorganisms: Ten grams (10 g) of each spice was homogenized in a sterile stomacher bag (Bag Mixer) with 90 mL of sterile Buffered Peptone Water (BPW). Serial dilutions up to 10^{-9} were prepared from the homogenized samples. Prepared dilutions were inoculated and incubated as shown in Table 1.

Detection of *Salmonella* spp. and *Listeria monocytogenes*: Isolation and identification of *Salmonella* spp. was performed according to ISO (2002) protocol. Briefly, samples (25 g) were weighed into sterile stomacher bags, diluted and homogenized with 225 mL BPW and incubated at 37°C for 18±2 h. Following the incubation, 0.1 mL sample was taken from the culture, inoculated in 10 mL of Rappaport-Vassiliadis enrichment broth (Oxoid) and incubated at 42°C for 24 h. After incubation, one loop cultures were streaked onto XLD Agar (Oxoid) and incubated at 37°C for 24 h. Five suspected colonies per plate were lifted and re-streaked onto Tryptone Soya Agar (TSA, Oxoid, CM131) to purify the colonies. The isolated colonies were biochemically characterized with a Microbact™ TM 24E Gram-Negative Identification System (Oxoid, MB1074A) according to the manufacturer's instructions.

Table 1: Methods of microbiological analysis

Microorganisms	Mediums	Incubation Temperature (°C)	Incubation time	Methods
Total aerobic mesophilic bacteria	Plate count agar	30	72 h	ISO. (2003)
Enterobacteriaceae	Violet red bile glucose agar	30	24/48 h	ISO. (1993)
Coliform	Violet red bile agar	30	24/48 h	ISO. (1991)
Escherichia coli	Tryptone bile X-glucuronide	43-44	18/24 h	ISO. (2001)
Enterococcus spp.	Slanetz and bartley agar	30	24/48 h	Domig *et al.* (2003)
Staphylococcus/*Micrococcus*	Baird parker agar	37	24/48 h	ISO. (1999)
Yeast/Molds	Potato dextrose agar	22	4/5 day	Tournas *et al.* (2001)
Bacillus cereus	Mannitol-egg-yolk-polymyxin agar	30	18/24 h	Rhodehamel and Harmon (2012)

We detected *L. monocytogenes*, according to ISO (1996) protocol. Samples (25 g) were weighed into sterile stomacher bags, diluted and homogenized with 225 mL Half Fraser broth (Oxoid, SR142) and incubated at 30°C for 24 h. After the incubation, 1 mL sample was taken from the culture, inoculated in 10 mL of Fraser broth (Oxoid, SR143) and incubated at 30°C for 24 h. After incubation, one loop cultures were streaked onto Oxford Agar (Oxoid, CM 856) and incubated at 30°C for 48 h. The isolated colonies were biochemically characterized with a Microbact™ TM 12L *Listeria* identification system (Oxoid, MB1128) according to the manufacturer's instructions.

Antimicrobial susceptibility tests: Antimicrobial susceptibility was evaluated with a disk-diffusion test (Kirby-Bauer Method), which was performed on Mueller-Hinton agar from a bacterial suspension with turbidity equal to McFarland 0.5. The interpretation was made according to the criteria provided by the CLSI. (2012).

All *Salmonella* isolates and 3 *L. monocytogenes* isolates from the recovered spices were evaluated for their resistance to 12 antimicrobial agents by the disk diffusion method on Mueller-Hinton agar plates. The standard procedure of CLSI. (2012) (formerly NCCLS) was strictly followed throughout. We included the quality control strains *S. typhimurium* (ATCC-14028) and *L. monocytogenes* (ATCC 7644) in each run. The concentrations of antimicrobial agents on the disks (Oxoid, Australia) were as follows: Chloramphenicol (30 µg, Oxoid, CT0013B), Ampicillin (10 µg, Oxoid, CT0003B), Gentamicin (10 µg, Oxoid, CT0024B), Streptomycin (10 µg, Oxoid, CT0047B), Vancomycin (30 µg, Oxoid, CT0058B), Amoxicillin (10 µg, Oxoid, CT0161B), Erythromycin (5 µg, Oxoid, CT0066B), Trimethoprim (5 µg, Oxoid, CT0076B), Kanamycin (30 µg, Oxoid, CT0026B), Nalidixic Acid (30 µg, Oxoid, CT0031B), Tetracycline (30 µg, Oxoid, CT0054B) and Penicillin G (10 U, Oxoid, CT0043B). We determined the resistance of the isolates according to the zone diameter interpretative standards recommendations by CLSI (2012).

RESULTS AND DISCUSSION

The microbiological qualities of the spices collected from the markets are presented in Table 2 and 3. The concentrations of Total Aerobic Mesophilic Bacteria (TAMB), *Staphylococcus*/*Micrococcus*, Coagulase Positive *Staphylococcus*, Yeast/mold, Enterobacteriaceae, *Coliform* bacteria, *E. coli*, *Enterococcus* spp. and *B. cereus* were 5.50, 2.03, <log 2.00, 3.98, 3.57,

Table 2: Microbiological quality of spices

Parameters	TAMB	*Staphylococcus*/ *Micrococcus*	Coagulase (+) *Staphylococcus*	Yeast/ mould	Enterobacteriaceae	*Coliform* bacteria	*E. coli*	*Enterococcus* spp.	*B. cereus*
Red sweet pepper (n = 50)									
Mean	5.35	3.66	1.24	3.96	4.55	4.11	1.59	3.83	2.56
Min	5.08	<log2.00	<log2.00	3.00	3.60	4.60	<log2.00	2.30	2.04
Max	6.51	5.53	4.00	4.62	5.40	5.24	5.12	4.53	3.48
Red chili pepper (n = 50)									
Mean	5.67	2.01	1.17	3.60	4.55	4.02	1.59	4.09	2.77
Min	5.05	<log2.00	<log2.00	3.40	2.70	<log2.00	<log2.00	3.08	2.08
Max	6.63	2.60	3.43	3.88	5.62	5.31	5.11	4.79	3.38
Red pepper flakes (n = 50)									
Mean	5.38	2.73	1.54	4.43	1.76	1.68	<log2.00	3.01	2.94
Min	4.40	<log2.00	<log2.00	4.08	<log2.00	<log2.00	<log2.00	2.30	2.30
Max	6.51	4.66	3.46	4.73	5.28	5.10	<log2.00	3.54	3.51
Cumin (n = 50)									
Mean	5.85	1.11	0.67	4.42	4.16	3.28	<log2.00	2.50	2.52
Min	5.09	<log2.00	<log2.00	4.08	<log2.00	<log2.00	<log2.00	2.08	2.00
Max	6.65	4.64	3.46	4.81	5.74	5.51	<log2.00	3.38	3.32
Black pepper (n = 50)									
Mean	5.01	0.57	0.42	3.31	2.63	2.10	<log2.00	3.00	3.04
Min	4.15	<log2.00	<log2.00	3.04	<log2.00	<log2.00	<log2.00	2.40	2.30
Max	5.89	3.78	3.18	3.65	4.81	4.34	<log2.00	3.65	3.72
Total (250)									
Mean	5.50	2.03	1.00	3.98	3.57	3.07	0.63	3.32	2.79

Table 3: *Salmonella* spp. and *Listeria* spp. in spices

Parameters	No.	*Salmonella* spp.	*Listeria monocytogenes*
Red sweet pepper	50	*ND	ND
Red chili pepper	50	2% (1)	2% (1)
Red pepper flakes	50	ND	ND
Cumin	50	6% (3)	4 % (2)
Black pepper	50	6% (3)	ND
Total	250	2.8% (7)	1.2% (3)

*ND: Not detected

3.07, <log2.00, 3.32 and 2.79 log CFU g^{-1}, respectively (Table 2). According to the Turkish Food Codex Regulation on Microbiological Criteria (TFC., 2013), the standard values for coagulase-positive *Staphylococcus* and *B. cereus* should be between 10^3-10^4 CFU^{-1} of spices and should be zero for *Salmonella* spp. in 25 g samples.

Contaminated spices may cause microbiological problems and may pose a risk to public health because they are often added to foods (Banerjee and Sarkar, 2003). In this study, the average values of TAMB were 5.35, 5.67, 5.38, 5.85 and 5.01 log CFU g^{-1} in red sweet pepper, red chili pepper, red pepper flakes, cumin and black pepper, respectively. Vural (2004) detected TAMB levels of 6.81, 6.68, 7.00, 6.92 and 6.59 log CFU g^{-1} in black pepper, cumin, chili pepper, red pepper flakes and black pepper flakes, respectively. Elmali and Yaman (2005) reported average TAMB levels of 7.23, 6.43, 7.20 and 5.61 log CFU g^{-1} in black pepper, powdered red pepper, granulated red pepper and cumin, respectively. Shamsuddeen (2009) reported TAMB levels in spice mixture samples, which were produced in Kilishi, as 9.47 log CFU g^{-1}. Therefore, TAMB levels in our study were lower than those previously reported.

Levels of *Micrococcus* and *Staphylococcus* (*S. aureus*) are indicative of personal hygiene used during the preparation of spices (Elmali and Yaman, 2005). In this study, the average levels of *Micrococcus*/*Staphylococcus* were 3.66, 2.73, 1.11 and 0.57 CFU g^{-1} in red pepper, red pepper flakes, cumin and black pepper, respectively. Coagulase-Positive *Staphylococcus* were detected at average levels of 1.00 log CFU g^{-1} in all spices. According to the Turkish Food Codex, the maximum level of Coagulase-positive *Staphylococcus* in spices should be between 3-4 log CFU g^{-1} (TFC., 2013). In this study, 12 samples had levels between 3-4 log CFU g^{-1}, including 4 red sweet pepper, 2 red chili pepper, 4 red pepper flakes, 1 cumin and 1 black pepper. Erol *et al.* (1999) reported levels of *Micrococcus*/*Staphylococcus* as 3.07, 3.42, 2.58 and 2.48 CFU g^{-1} in red pepper, red pepper flakes, black pepper and cumin, respectively, in Ankara. Similarly, Elmali and Yaman (2005) reported *Micrococcus*/*Staphylococcus* levels as 2.88, 2.95, 3.47 and 3.92 CFU g^{-1} in red pepper, granulated red pepper, cumin and black pepper, respectively. The *Micrococcus*/*Staphylococcus* levels in the red pepper in our study were higher than those detected by Erol *et al.* (1999) and by Elmali and Yaman (2005). However, our results in the red pepper flakes, cumin and black pepper were lower than those of the others (Erol *et al*., 1999; Elmali and Yaman, 2005).

Levels of yeast/mold were 3.96, 3.60, 4.43, 4.42 and 3.31 log CFU g^{-1} in red sweet pepper, red chili pepper, red pepper flakes, cumin and black pepper, respectively. Vural *et al.* (2004) reported that there was a high amount of yeast contamination (2.00-5.00 log CFU g^{-1}) in spice samples. Shamsuddeen (2009) found the fungal count of spice mixture samples to be 5.01 log CFU g^{-1}. Abou Donia (2008) reported that these levels are 1.00-4.00 CFU g^{-1} in spices in Egypt. The levels of yeast/mold detected in our study are similar to those reported by Abou Donia (2008) but they are lower than those reported by Vural *et al.* (2004) and Shamsuddeen (2009).

Enterobacteriaceae is an important bacterial group used in the evaluation of the manufacturing processes and include bacteria such as; *Coliform*, *E. coli* and *Enterococcus* spp. (Erol *et al*., 1999). Enterobacteriaceae counts are generally used as indicators of hygienic quality and therefore,

indicate the general microbiological quality of the product (Adams and Moss, 1995). The levels of Enterobacteriaceae in our study were 4.55, 4.55, 1.76, 4.16 and 2,63 log CFU g^{-1} in red sweet pepper, red chili pepper, red pepper flakes, cumin and black pepper, respectively. Elmali and Yaman (2005) reported Enterobacteriaceae levels between 3.04-4.91 log CFU g^{-1} and Ulukanli *et al.* (2005) reported levels between 2.00-7.00 log CFU g^{-1}. The levels of Enterobacteriaceae detected in our study were similar to those of these previously published studies (Elmali and Yaman, 2005; Ulukanli *et al*., 2005).

Coliform bacterial level was on average 3.07 log CFU g^{-1} for all spices. *E. coli* was not found in the red pepper flakes, cumin, or black pepper, although it was detected in seven red sweet pepper samples (3.30-5.12 log CFU g^{-1}) and eight red chili pepper samples (2.53-5.11 log CFU g^{-1}). *Enterococcus* spp. levels were on average 3.32 log CFU g^{-1} in all spices and were highest in the red chili pepper (4.09 log CFU g^{-1}). Ulukanli *et al.* (2005) reported that coliform levels were between 2.00-7.00 log CFU g^{-1} in all spices. Abou Donia (2008) reported *Coliform* and *E. coli* levels of 1.17-3.04 and 1.17-2.66 log CFU g^{-1}, respectively. Shamsuddeen (2009) reported the detection of *E. coli* in only one spice sample.

Bacillus cereus levels were 2.56, 2.77, 2.94, 2.52 and 3.04 log CFU g^{-1} in red sweet pepper, red chili pepper, red pepper flakes, cumin and black pepper, respectively. According to the Turkish Food Codec in spices (TFC., 2013), the maximum level of *Bacillus cereus* should be between 3-4 log CFU g^{-1}. In this study, 48 samples had levels between 3-4 log CFU g^{-1}, including 8 red sweet pepper, 9 red chili pepper, 11 red pepper flakes, 5 cumin and 15 black pepper. Previously reported levels of *B. cereus* were ≥5 log CFU g^{-1} (Little *et al.,* 2003), 2.08-2.85 log CFU g^{-1} (Erol *et al*., 1999) and 1.09-3.00 log CFU g^{-1} (Elmali and Yaman, 2005).

In this study, we detected *Salmonella* spp. in 2.8% and *L. monocytogenes* in 1.2% of the spice samples. *Salmonella* spp., was detected in red chili pepper, cumin and black pepper at 2, 6 and 6%, respectively. *Listeria monocytogenes* was detected in red chili pepper (2%) and cumin (4%) (Table 3). *Salmonella* spp., was detected in spices by Shamsuddeen (2009), Little *et al.* (2003) and Banerjee and Sarkar (2003), but was not detected by Ulukanli *et al.* (2005) and Abou Donia (2008). Contamination of spices with *Salmonella* spp. was identified as the cause of 95% of U.S. food recalls associated with spices between 1980-2000 (Vij *et al*., 2006). Contamination of retail spices is considered an indication of environmental or fecal contamination due to unhygienic practices during their production. Use of spices with high microbial content could increase the chance of food spoilage and transmission of food borne pathogens (Koohy-Kamaly-Dehkordy *et al*., 2013).

Seven *Salmonella* spp. and 3 *L. monocytogenes* isolates recovered from spice samples were tested for antimicrobial resistance against 12 antibiotics. Resistance to Gentamicin/Ampicillin/ Streptomycin, Nalidixic Acid and Tetracycline was found in 14, 42.86 and 57.14% of the *Salmonella* spp. isolates, respectively. All of the *Salmonella* spp. isolates were resistant to Vancomycin, Erythromycin and Penicillin G. On the other hand, *L. monocytogenes* isolates were resistant to Gentamicin/Amoxicillin and Ampicillin/Tetracycline/Penicillin in 33.33 and 66.67% of the samples, respectively. In addition, all of the *L. monocytogenes* isolates were resistant to Nalidixic Acid.

According to the results of various studies, spices may contain bacteria as a result of mistakes during harvest, inadequate storage conditions, lack of attention to staff hygiene and unrestrained sales without proper packaging.

CONCLUSION

Spices are traditionally used in the production and consumption of foods. In this study, we found that spices sold without proper packaging may contain indicator microorganisms and pathogens. Cross-contamination may occur, if contaminated spices are used for the production of food and this may pose a risk for public health. Additionally, in antibiograms, it was confirmed that obtained *Salmonella* spp. and *L. monocytogenes* strains are resistant to some antibiotics. Therefore, it is essential to apply all HACCP regulations during the harvest of spices and their processing and during all processes leading to consumption. Moreover, it is recommended that spices be packaged with suitable packaging material and regular inspections must be performed for bacteriological control, remnants and contaminants.

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