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Microbiological Quality and Safety of Some Dried Spices Obtained from Markets, Spice Shops and Homes

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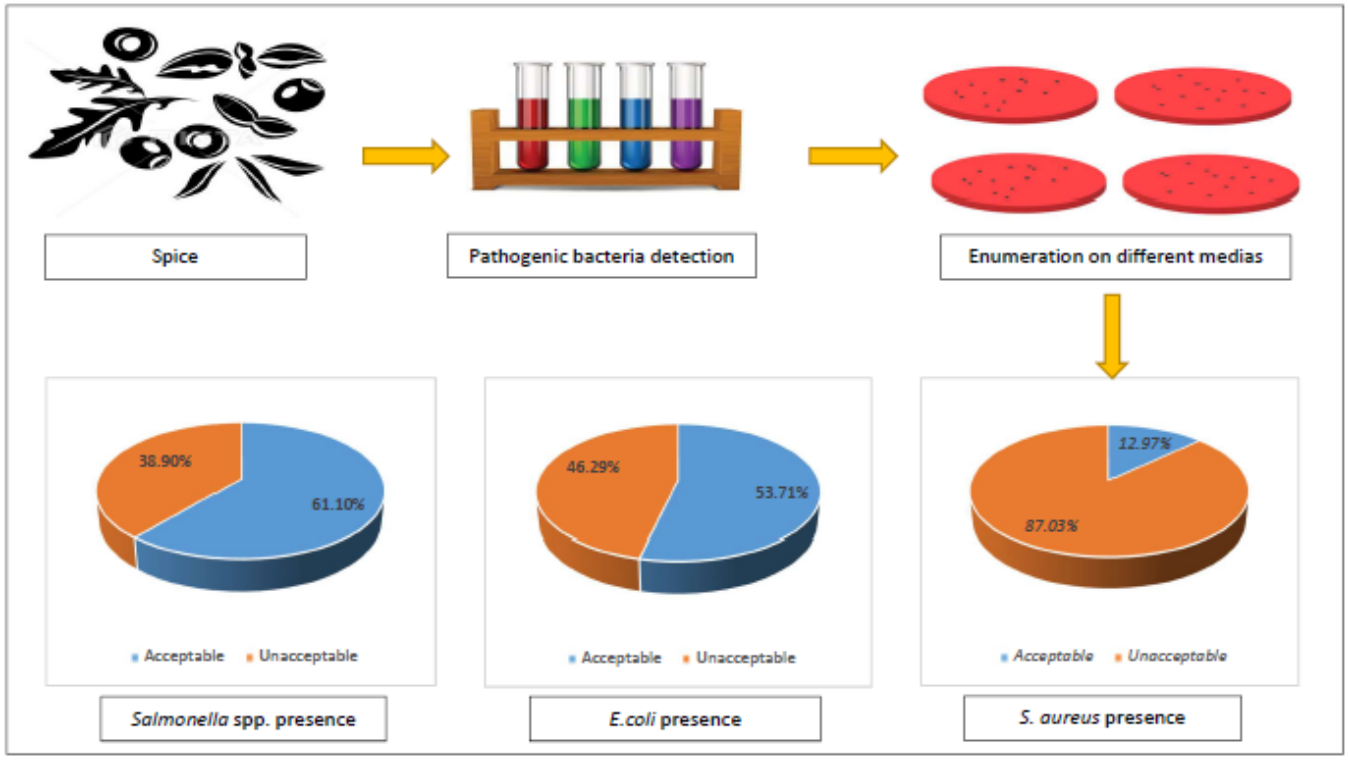
HIGHLIGHTS

- 46.29% of the samples were unacceptable according to the maximum limit of *E. coli*.
- 87.03% of the samples were unacceptable according to the maximum limit of *S. aureus*.
- 38.90% of the samples were unacceptable according to the presence of *Salmonella* spp.
- The kind of spice which had the best microbiological quality was thyme.

Abstract: This study was conducted to determine both the microbiological quality and safety of the spices as well as whether or not there are any pathogenic bacteria in fifty-four samples of six dried spices supplied from three markets, three spice shops, and three homes. Total mesophilic aerobic bacteria (TMAB), aerobic spore former bacteria (ASFB), total yeast-mold (TYM), coliform group bacteria (CGB), *Escherichia coli* (*E. coli*), and *Staphylococcus aureus* (*S. aureus*), and *Salmonella* spp. were found in red pepper flakes, red pepper powder, ground black pepper, dried mint, dried thyme, and ground cumin. The highest count of TMAB (11.20 ± 0.01 log cfu/g) was found in ground black pepper obtained from the second home ($P < 0.05$). 62.96% of the samples contained CGB ($P < 0.05$). 46.29% of the samples had unacceptable limits (2 log cfu/g) of *E. coli*, whereas 87.03% of the samples had unacceptable limits (2 log cfu/g) of *S. aureus* according to international microbiological standards. *Salmonella* spp. was isolated from 38.90% of the samples. The most common microorganisms found at the unacceptable limit included *S. aureus* (47/54), *E. coli* (25/54), and *Salmonella* spp. (21/54). The highest number of samples containing any microorganism at the unacceptable level was obtained from the home (18/18) and this rate was 14/18 in the samples obtained from both markets and spice shops. Dried thyme had the best microbiological quality. These results demonstrated that the pathogenic bacteria loads of the analyzed dried spices were high, and thus unsafe in terms of their microbiological quality.

Keywords: spice; microbiological quality; pathogenic bacteria; *Escherichia coli*; *Staphylococcus aureus*; *Salmonella* spp.

GRAPHICAL ABSTRACT



INTRODUCTION

The International Organization for Standardization located in Geneva defines spices as “vegetable products or mixtures thereof, free from extraneous matter, used for flavoring, seasoning, and imparting aroma to foods” [1]. Spices consist of leaves, flowers, seeds or stems of plants. They are food additives that have certain aromas and flavors, and are widely used for culinary purposes, medicinal purposes, and ease digestion [2, 3]. Since all the spices are derived from plants, they are generally considered as safe (GRAS) [4]. Spices are frequently used in food industry and found at homes. They are added to foods/meals in small amounts to alter or enhance the aroma and flavor of the base ingredient(s) [2, 5-7]. They are also used to preserve [8] and dye/color [9] foods. Through a secondary metabolism, plants synthesize many compounds with complex molecular structures. Such metabolites include alkaloids, flavonoids, isoflavonoids, tannins, cumarins, glycosides, terpenes, and phenolic compounds, all of which give spices their flavoring, antimicrobial [4], and antioxidant properties [10, 11].

Spices generally go through processes such as washing, descaling, bleaching, drilling, curing, drying, cleaning, sorting, shredding, grinding, packaging, and storage, which can cause microbial contamination between the farm and the table [12, 13].

Spices host different microbial flora and load, depending on how they are produced as well as what part of the plant they hail from (e.g. roots or leaves). Many studies reveal that spices can contain high aerobic and anaerobic spore former bacteria, coliform group bacteria, xerophilic storage molds and bacteria, yeasts, and micrococci [14-21]. Their microflora can also contain various pathogenic bacteria that cause food poisoning – namely *Salmonella* spp., *Staphylococcus aureus* (*S. aureus*), *Bacillus cereus* (*B. cereus*), *Escherichia coli* (*E. coli*), and *Vibrio* spp. [16, 20-35]. Plants that grow close to the soil (e.g. black pepper, thyme, basil, and ginger) can become contaminated via fertilizer, soil, water, birds, insects, and rodents [17, 36]. Additionally, unpackaged spices sold in markets and bazaars can be contaminated through dust, waste water and animal/human feces [37, 38].

In Turkey, people either make their own spices at home or purchase them local producers, spice shops, or markets. As well as hygiene during production stage, how spices are used and stored is also important. Spices should not be stored in unsuitable packages (moisture- or air-permeable), unpackaged, or wherever there is heat or sunlight, or else since they are susceptible to developing microorganisms on them. Failing to store spices and herbs properly either at vendor locations or at home can pose many risks [22, 37, 38].

Since spices are bought ready-to-eat products, they are not subjected to heat treatment. Spices contaminated with pathogens can cause morbidity and mortality [39].

Spices containing pathogenic bacteria (*Salmonella* spp., *B. cereus*, *E. coli*, *Clostridium perfringens*, etc.) can cause food poisoning and food-borne infection in humans [15, 17, 36, 40-46]. Likewise, spices containing degrading agents can spoil canned food and meat products [16]. The use of contaminated spices in meat products can lead to early spoilage, food-borne infections, and poisoning [20]. Spoiled products and related illnesses can cost significant financial losses [16, 3].

The Turkish Food Codex Regulation on Microbiological Criteria [47] – which is currently in force in Turkey – sets the limit for how much coagulase (+) staphylococci, *B. cereus* and *Salmonella* commercial spices are allowed to contain. However, the microbiological hazards in spices are not limited to these three groups of bacteria.

This study aims to examine both the microbiological quality and safety of dried spices, as well as how much pathogenic bacteria exists in dried spices obtained from different places in Bitlis, Turkey. Ogur and Idikurt [48] found that the most common dried spices used by housewives in Bitlis were ground black pepper, red pepper flakes, dried mint, ground cumin, and dried thyme, respectively. These spices and red pepper powder were included in this study.

This study was conducted to examine both the microbiological quality and safety of dried spices as well as whether or not there are any pathogenic bacteria in fifty-four samples of six dried spices obtained from three markets, three spice shops, and three homes.

MATERIAL AND METHODS

Materials

Six dried spices obtained from three markets (18 samples), three spice shops (18 samples), and three homes (18 samples) in Bitlis, Turkey between February and March 2018 were used as the material in this study.

The spices from the markets were come in 100-250 g packages. They were kept in a dried food cabinet, and were not opened until they were analyzed. Six market samples were analyzed in a day.

The spice shop spices were sold unpackaged in large packages or boxes. They were weighed in appropriate quantities (100-200 g) and placed in small polyethylene bags. The home spices were kept in plastic bags/paper packages/jars/plastic boxes. Three or four tablespoon-sized (100-150 g) samples of them were also placed into small polyethylene bags. Six samples were obtained from each of sources every day and quickly sent to the lab for analysis in the same day.

In total, 54 samples were analyzed. They included red pepper flakes (RPF), red pepper powder (RPP), ground black pepper (GBP), dried mint (DM), dried thyme (DT), and ground cumin (GC). Table 1 shows the codes of the samples.

To find out whether or not the samples contained any pathogenic bacteria, counts were done for each of the total mesophilic aerobic bacteria (TMAB), aerobic spore former bacteria (ASFB), total yeast-mold (TYM), coliform group bacteria (CGB), *E. coli*, *S. aureus*, and *Salmonella* spp.. Each analysis was carried out twice. Colonies were counted using a colony counter.

Sample preparation

Ten g samples were taken from each spice and placed in sterile Stomacher bags (Seward Medical, London, UK). Ninety milliliters of 0.1% peptone water (Merck, 107228) was added onto each sample. The mixture was then homogenized using a 400 mL lab blender (Stomacher, IUL Instrument, Spain) at an appropriate speed for 120 seconds. This was the first dilution. Serial dilutions (1:10, diluents in 0.1% peptone water (Merck, 1.07228)) to 10^{-8} were then prepared from the first dilution [49] and later used to analyze TMAB, ASFB, TYM, CGB, *E. coli* and *S. aureus*.

Methods

Microbiological media and enumeration

To enumeration of TMAB, 0.1 mL of each serial dilution was spread onto plate count agar (PCA, Biomark B298), and incubated for 24-48 h at 37 °C. All colonies that developed afterwards were TMAB [50]. To enumeration of ASFB, dilutions were kept at 80 °C for 10 min and cooled to room temperature (20-24 °C). Zero point one milliliter of each serial dilution was spread onto PCA, and then incubated at 37 °C for 24-48 h. All colonies that developed afterwards were ASFB [51].

Table 1. The codes of the analyzed samples

Spice	Taken Place	No	Code	Spice	Taken Place	No	Code
Red Pepper Flakes (*Capsicum annuum*)	Market	1	RPFMA1	Dired Mint (*Mentha piperita*)	Market	1	DMMA1
		2	RPFMA2			2	DMMA2
		3	RPFMA3			3	DMMA3
	Spice Shop	1	RPFSH1		Spice Shop	1	DMSH1
		2	RPFSH2			2	DMSH2
		3	RPFSH3			3	DMSH3
	Home	1	RPFHO1		Home	1	DMHO1
		2	RPFHO2			2	DMHO2
		3	RPFHO3			3	DMHO3
Red Pepper Powder (*Capsicum annuum*)	Market	1	RPPMA1	Dried Thyme (*Thymus vıdgaris*)	Market	1	DTMA1
		2	RPPMA2			2	DTMA2
		3	RPPMA3			3	DTMA3
	Spice Shop	1	RPPSH1		Spice Shop	1	DTSH1
		2	RPPSH2			2	DTSH2
		3	RPPSH3			3	DTSH3
	Home	1	RPPHO1		Home	1	DTHO1
		2	RPPHO2			2	DTHO2
		3	RPPHO3			3	DTHO3
Ground Black Pepper (*Piper nigrum*)	Market	1	GBPMA1	Ground Cumin (*Cuminum cyminum*)	Market	1	GCMA1
		2	GBPMA2			2	GCMA2
		3	GBPMA3			3	GCMA3
	Spice Shop	1	GBPSH1		Spice Shop	1	GCSH1
		2	GBPSH2			2	GCSH2
		3	GBPSH3			3	GCSH3
	Home	1	GBPHO1		Home	1	GCHO1
		2	GBPHO2			2	GCHO2
		3	GBPHO3			3	GCHO3

To enumeration of TYM, 0.1 mL of each serial dilution was spread onto Dichloran Rose Bengal Chloramphenicol Agar (Merck, 1.0046), and then incubated between 5 and 7 days at 25 °C in the dark. After incubation, bright pink yeast and spreading white/green mold TYM colonies developed, and were counted [52]. To enumeration of CGB, 0.1 mL of each serial dilution was spread onto Violet Red Bile Agar (Biomark, B350), and then incubated for 18 h at 37 °C. At the end of incubation, dark red colonies that were 1-2 mm in diameter were regarded as CGB. To enumeration of *E. coli*, 0.1 mL of each serial dilution was spread onto Violet Bile Agar Methylumbelliferyl-b-D-glucuronide (VRBA MUG, Biolife, 4021862) and then incubated for 18 h at 37 °C. After incubation, dark red colonies that were 1-2 mm in diameter were checked under a UV lamp and the fluorescent colonies from these colonies were evaluated as *E. coli* [53]. To enumeration of *S. aureus*, 0.1 mL of each serial dilution was spread onto Baird Parker Agar Base (Merck, 1.05406) containing Egg Yolk Tellurite Emulsion (Merck, 103785) and incubated for 48 h at 35 °C. Black glossy colonies with transparent zones that were 1-1.5 mm in diameter and developed after incubation, were regarded as *S. aureus* [54]. To enumeration of *Salmonella* spp., ISO 6579 was used to isolate *Salmonella* spp. First, a pre-enrichment culture was prepared. A twenty-five gram sample was homogenized in 225 mL of buffered peptone water (Merck 1.07228) and incubated for 16-20 hours at 35-37 °C for non-selective pre-enrichment purpose. After incubation, 0.1 mL and 10 mL of the pre-enrichment culture were transferred to 10 mL of Rappaport Vassiliadis Soy (RVS) Broth (Merck 1.07700) and 100 mL of Selenite Cystine (SC) Broth (Merck 1.07709), respectively, for selective enrichment. The RVS Broth was incubated at 42/43 °C for 24 hour, while the SC Broth was incubated at 37 °C for 24 hour. Later, the selective enrichment culture was streaked onto Brilliant Green Phenol Red Lactose Sucrose Agar (Merck 1.10747) as well as onto Xylose Lysine Tergitol-4 (XLT-4) Agar (Merck 1.13919) to which XLT-4 Supplement (Liofilchem, 80410) was added. Both were then left to incubate aerobically at 37 °C for 24 hours. To confirm pink-red colored suspicious colonies surrounded

by a bright red zone on Brilliant Green Phenol Red Agar and black suspicious colonies on XLT-4 Agar were indeed *Salmonella* ssp., the culture was then streaked onto Triple Sugar Iron Agar (Merck 1.03915) and Lysine Iron Agar (Merck 1.11640) and incubated at 37 °C for 24 hours. It was then inoculated into Urea Broth (Merck 1.08483), and incubated again at 37 °C for 48 hours. Last, a *Salmonella* Latex Test Kit (Oxoid FT0203A) was used for serological confirmation [55].

Counting TMAB, ASFB, TYM, CGB, E. coli, and S. aureus and indicating the presence / the absence of Salmonella spp.

The TMAB, ASFB, TYM, CGB, *E. coli*, and *S. aureus* counts were all indicated as a logarithm of colony-forming units per gram (log cfu/g) of the sample according to the number of colonies, dilution factor, and cultivation amount [56]. The results for *Salmonella* spp. were expressed as present (+) / absent (–) in 25 g [57].

Statistical analysis

The data was analyzed using Statistical Package for the Social Sciences (IBM SPSS Statistics, Version 25.0). The results were expressed in mean ± standard deviation. The one-way analysis of variance (ANOVA) was used to establish whether or not there was any difference between means according to microbial load. As the data were parametric and homogeneity of variance was provided, the Tukey test was conducted to find the sources of the differences between the groups. A P value of < 0.05 was accepted as a significant difference for each spice [58]. When the number of groups was greater than 50, they were divided into two because the Post-Hoc Test could not be performed.

RESULTS

Microbiological load of RPF, RPP, and GBP

Table 2 shows the microbiological loads of RPF, RPP, and GBP obtained from nine different places (three markets, three spice shops, three homes). The highest count of TMAB (11.20 ± 0.01 log cfu/g) was found in GBPHO2 ($P < 0.05$) (Table 2).

The difference between the dried spices in terms of TMAB count in Table 2 was significant ($P < 0.05$). The difference between the TMAB count in RPF samples obtained from the homes, the TMAB count in RPF samples obtained from the homes, and the TMAB count in GBP samples obtained from the spice shops was insignificant ($P > 0.05$) (Table 2).

The highest ASFB count (6.10 ± 0.02 log cfu/g) was found in GBPSH1 ($P < 0.05$) (Table 1). The difference between the dried spices in terms of ASFB count in Table 2 was significant ($P < 0.05$). The difference between RPP samples obtained from the homes in terms of ASFB count was insignificant ($P > 0.05$) (Table 2).

TYM was below the detection limit (1 log cfu/g) in twelve of the samples, including GBP obtained from the markets (Table 2). The highest count of TYM (3.82 ± 0.30 log cfu/g) was found in RPPSH3 ($P < 0.05$) (Table 1). The difference between the TYM count in the spices developing TYM in Table 2 was significant ($P < 0.05$). The difference between the TYM counts of GBPHE, GBPHO1, and GBPHO3 was insignificant ($P > 0.05$) (Table 2).

CGB and *E. coli* were below the detection limit (1 log cfu/g) in thirteen of the samples, including RPF samples obtained from the markets and the homes (Table 2). The highest CGB count (6.05 ± 0.16 log cfu/g) and the highest *E. coli* count (5.60 ± 0.16 log cfu/g) were found in RPPSH2 ($P < 0.05$) (Table 2). The difference between the spices developing CGB in terms of the count of CGB in Table 2 was significant ($P < 0.05$). The difference between the count of CGB in RPPSH1 and the count of CGB in RPPSH2 was insignificant ($P > 0.05$) (Table 2). The difference between the spices developing *E. coli* in terms of the count of *E. coli* was significant ($P < 0.05$). The difference between the counts of *E. coli* of GBPHO1 and GBPHO3 was insignificant ($P > 0.05$) (Table 2).

Table 2. Microbiological load of red pepper flakes, red pepper powder, and ground black pepper

Sample Code	Total Mesophilic Aerobic Bacteria (log cfu/g)	Aerobic Spore Former Bacteria (log cfu/g)	Total Yeast-Mold (log cfu/g)	Coliform Group Bacteria (log cfu/g)	*E. coli* (log cfu/g)	*S. aureus* (log cfu/g)	*Salmonella* spp.
RPFMA1	4,96±0,01^{a*}	4,69±0,02^{a}	1,70±0,01^{b}	-	-	3,56±0,46^{a}	–
RPFMA2	2,20±0,55^{b}	1,70±0,01^{b}	-**	-	-	-	–
RPFMA3	4,25±0,02^{c}	4,18±0,08^{c}	-	-	-	3,73±0,03^{ac}	–
RPFSH1	3,30±0,02^{d}	3,07±0,01^{d}	-	-	-	2,77±0,22^{b}	–
RPFSH2	4,92±0,03^{a}	3,99±0,13^{c}	2,12±0,46^{bc}	3,84±0,06^{a}	3,69±0,04^{a}	4,03±0,03^{cd}	+
RPFSH3	4,00±0,33^{c}	3,46±0,11^{e}	-	-	-	2,78±0,20^{b}	–
RPFHO1	5,70±0,01^{e}	4,83±0,01^{af}	2,24±0,26^{bcf}	-	-	4,86±0,01^{ehk}	–
RPFHO2	5,78±0,05^{ef}	4,70±0,01^{a}	-	-	-	5,17±0,02^{efgij}	–
RPFHO3	5,74±0,02^{e}	5,16±0,02^{fg}	-	-	-	5,03±0,03^{efgj}	–
RPPMA1	5,95±0,01^{efg}	5,18±0,08^{go}	3,74±0,49^{d}	4,17±0,02^{b}	4,15±0,04^{b}	4,94±0,33^{ekj}	–
RPPMA2	5,70±0,56^{e}	5,33±0,06^{gijko}	2,95±0,38^{eghj}	4,33±0,01^{bc}	3,99±0,02^{c}	5,14±0,13^{efijl}	–
RPPMA3	3,86±0,13^{c}	3,00±0,01^{d}	-	-	-	3,31±0,56^{a}	–
RPPSH1	4,91±0,01^{a}	4,64±0,02^{a}	2,98±0,41^{eghj}	3,35±0,07^{d}	3,18±0,07^{d}	4,48±0,01^{dkm}	–
RPPSH2	6,33±0,47^{g}	5,86±0,49^{hlk}	3,61±0,53^{de}	6,05±0,16^{e}	5,60±0,16^{e}	5,48±0,25^{gijlno}	+
RPPSH3	6,19±0,65^{fg}	5,57±0,56^{him}	3,82±0,30^{d}	5,18±0,36^{f}	4,86±0,02^{f}	5,42±0,26^{gijlnp}	–
RPPHO1	6,09±0,07^{eg}	5,52±0,05^{ij}	2,79±0,54^{fgi}	4,63±0,07^{g}	4,55±0,01^{g}	4,80±0,30^{ekm}	+
RPPHO2	5,98±0,03^{eg}	5,64±0,07^{jhk}	3,03±0,27^{ei}	4,48±0,07^{cg}	4,33±0,01^{h}	4,85±0,16^{ekm}	–
RPPHO3	5,95±0,09^{eg}	5,46±0,50^{gji}	-	2,91±0,22^{h}	2,45±0,16^{i}	4,42±0,01^{dhm}	+
GBPMA1	5,22±0,10^{a}	4,70±0,08^{a}	-	2,70±0,01^{h}	2,70±0,01^{j}	4,79±0,31^{ekm}	–
GBPMA2	3,24±0,26^{d}	3,09±0,10^{d}	-	-	-	2,84±0,30^{b}	–
GBPMA3	6,36±0,01^{g}	5,89±0,02^{hlmn}	-	3,63±0,03^{a}	3,30±0,01^{d}	5,69±0,25^{gijno}	–
GBPSH1	8,88±0,01^{h}	6,10±0,02^{nl}	3,36±0,20^{dehi}	-	-	5,58±0,01^{ino}	+
GBPSH2	9,31±0,01^{h}	5,13±0,01^{fg}	3,48±0,01^{deh}	2,70±0,01^{h}	-	4,98±0,02^{ep}	+
GBPSH3	9,18±0,01^{h}	5,99±0,01^{l}	2,70±0,01^{cij}	-	-	5,38±0,13^{jipn}	+
GBPHO1	10,97±0,01^{i}	5,63±0,01^{kmj}	2,70±0,01^{cij}	2,70±0,01^{h}	2,70±0,01^{j}	5,82±0,25^{no}	+
GBPHO2	11,20±0,01^{i}	5,29±0,03^{gio}	-	-	-	5,91±0,22^{o}	+
GBPHO3	10,45±0,01^{j}	5,06±0,02^{fo}	2,70±0,01^{cij}	2,70±0,01^{h}	2,70±0,01^{j}	5,73±0,35^{no}	–

* Lower case letters indicate the difference between lines in the same column ($P < 0.05$).
The difference between the mean values indicated by the same letter is insignificant ($P > 0.05$).
** Microorganism load was below the detection limit (1 log cfu/g).
+It indicate the presence of the microorganism in 25 g sample.
–It indicate the absence of the microorganism in 25 g sample.

The highest count of *S. aureus* (5.92 ± 0.22 log cfu/g) was found in GBPHO2 ($P < 0.05$) (Table 2). The difference between the spices in terms of the count of *S. aureus* was significant ($P < 0.05$). The difference between RPF and GBP samples obtained from the homes in terms of the count of *S. aureus* was insignificant ($P > 0.05$) (Table 2).

Salmonella spp. was isolated from nine of the spices (RPFSH2, RPPSH2, RPPHO1, RPPHO3, GBPSH1, GBPSH2, GBPSH3, GBPHO1, and GBPHO2) (Table 2).

Microbiological load of DM, DT, and GC

Table 3 shows microbiological load of DM, DT, and GC obtained from nine different places (three markets, three spice shops, and three homes). The highest count of TMAB (11.08 ± 0.01 log cfu/g) was found in DMHO1 ($P < 0.05$) (Table 3).

The difference between the spice samples in terms of the count of TMAB in Table 3 was significant ($P < 0.05$). The difference between the counts of TMAB of DMSH1 and DMSH2 was insignificant ($P > 0.05$) (Table 3).

Table 3. Microbiological load of dried mint, dried thyme, and ground cumin.

Sample Code	Total Mesophilic Aerobic Bacteria (log cfu/g)	Aerobic Spore Former Bacteria (log cfu/g)	Total Yeast-Mold (log cfu/g)	Coliform Group Bacteria (log cfu/g)	*E. coli* (log cfu/g)	*S. aureus* (log cfu/g)	*Salmonella* spp.
DMMA1	5,66±0,03^{a*}	3,95±0,06^{a}	5,01±0,03^{a}	5,61±0,07^{a}	5,50±0,07^{a}	4,04±0,02^{a}	+
DMMA2	5,15±0,04^{b}	4,11±0,08^{a}	4,32±0,04^{bdh}	5,07±0,06^{b}	4,93±0,01^{b}	3,67±0,28^{ab}	+
DMMA3	4,82±0,07^{c}	3,69±0,10^{bci}	3,48±0,52^{cf}	3,59±0,12^{c}	3,30±0,33^{c}	2,87±0,10^{cj}	+
DMSH1	10,45±0,01^{d}	10,38±0,01^{d}	-	3,95±0,06^{dg}	3,87±0,19^{dhi}	7,78±0,01^{e}	–
DMSH2	10,51±0,01^{d}	9,48±0,01^{e}	3,76±0,31^{cej}	4,79±0,02^{e}	4,68±0,08^{eg}	8,04±0,06^{e}	–
DMSH3	9,48±0,01^{e}	9,40±0,01^{e}	3,39±0,10^{cfg}	4,58±0,05^{f}	4,43±0,10^{fj}	7,94±0,13^{e}	+
DMHO1	11,08±0,01^{f}	9,78±0,01^{f}	-	-	-	8,79±0,01^{f}	–
DMHO2	10,90±0,01^{g}	10,60±0,01^{d}	4,21±0,06^{deh}	4,98±0,08^{be}	4,90±0,11^{be}	7,85±0,26^{e}	+
DMHO3	10,18±0,01^{h}	10,08±0,01^{g}	3,54±0,59^{cf}	3,84±0,07^{d}	3,65±0,06^{d}	7,62±0,10^{e}	+
DTMA1	3,48±0,01^{i}	3,00±0,01^{h}	3,30±0,01^{fg}	-	-	2,31±0,38^{dim}	–
DTMA2	4,00±0,01^{j}	-	3,00±0,01^{g}	-	-	1,66±0,01^{g}	–
DTMA3	3,45±0,16^{i}	-	-	-	-	2,31±0,71^{dm}	–
DTSH1	-**	-	3,24±0,26^{cg}	-	-	-	–
DTSH2	4,00±0,01^{j}	-	3,00±0,01^{g}	-	-	1,66±0,01^{g}	–
DTSH3	-	-	-	-	-	-	–
DTHO1	4,56±0,05^{k}	3,09±0,10^{hj}	3,55±0,16^{cf}	4,51±0,09^{fi}	4,51±0,01^{gf}	1,96±0,01^{dgi}	–
DTHO2	4,33±0,04^{l}	3,48±0,01^{bk}	4,05±0,07^{hj}	3,86±0,08^{d}	3,71±0,12^{di}	3,49±0,35^{bkln}	–
DTHO3	4,21±0,06^{m}	3,71±0,19^{ic}	3,18±0,01^{cg}	3,57±0,19^{c}	3,15±0,16^{ck}	3,59±0,17^{ab}	–
GCMA1	4,45±0,09^{n}	3,90±0,09^{ac}	3,28±0,04^{fg}	4,08±0,03^{g}	3,95±0,04^{hi}	3,21±0,27^{bc}	–
GCMA2	3,78±0,01^{o}	3,54±0,26^{bik}	-	2,00±0,01^{h}	-	1,96±0,01^{dgi}	–
GCMA3	4,13±0,03^{m}	3,30±0,01^{jk}	3,51±0,07^{cf}	3,95±0,06^{dg}	3,93±0,04^{i}	3,07±0,21^{ck}	–
GCSH1	4,83±0,01^{c}	3,33±0,16^{k}	3,28±0,22^{fg}	4,36±0,02^{i}	3,93±0,08^{i}	2,26±0,01^{dim}	+
GCSH2	5,89±0,02^{p}	4,67±0,04^{l}	4,08±0,01^{hj}	4,84±0,17^{e}	4,59±0,05^{gf}	5,28±0,23^{h}	+
GCSH3	6,01±0,01^{r}	5,26±0,01^{m}	4,12±0,10^{hj}	4,89±0,18^{be}	4,21±0,06^{j}	5,21±0,21^{h}	+
GCHO1	2,96±0,01^{s}	2,70±0,01^{n}	-	1,70±0,01^{j}	-	2,45±0,01^{jm}	+
GCHO2	3,65±0,06^{t}	2,94±0,26^{h}	-	3,00±0,01^{k}	2,93±0,01^{k}	3,04±0,01^{cl}	+
GCHO3	4,98±0,01^{u}	4,58±0,07^{l}	1,70±0,01^{i}	2,53±0,14^{l}	2,42±0,13^{l}	3,79±0,19^{an}	+

* Lower case letters indicate the difference between lines in the same column ($P < 0.05$).
The difference between the mean values indicated by the same letter is insignificant ($P > 0.05$).
** Microorganism load was below the detection limit (1 log cfu/g).
+It indicate the presence of the microorganism in 25 g sample.
–It indicate the absence of the microorganism in 25 g sample.

The highest count of ASFB (10.60 ± 0.01 log cfu/g) was found in DMHO2 ($P < 0.05$) (Table 3). The difference between the spices in terms of the count of ASFB was significant ($P < 0.05$). The difference between the ASFB counts of DMMA1 and DMMA2 was insignificant ($P > 0.05$) (Table 3).

TYM was below the detection limit (1 log cfu/g) in seven of the samples, including GCHO1 and GCHO2 (Table 3). The highest count of TYM (4.32 ± 0.04 log cfu/g) was found in DMMA2 ($P < 0.05$) (Table 3). The difference between the spices in terms of the count of TYM in Table 3 was significant ($P < 0.05$). The difference between the TYM counts of DTMA1, DTMA2, DTSH1, and DTSH2 was insignificant ($P > 0.05$) (Table 3).

CGB and *E. coli* were below the detection limit (1 log cfu/g) in seven of the samples including DT obtained from the markets and the spice shops (Table 3). The highest count of CGB (5.61 ± 0.07 log cfu/g) and the highest count of *E. coli* (5.50 ± 0.07 log cfu/g) were found in DMMA1 ($P < 0.05$) (Table 3). The difference between the spices in terms of the count of CGB was significant ($P < 0.05$). The difference between the counts of CGB of GCSH2 and GCSH3 was insignificant ($P > 0.05$) (Table 3). The difference between the samples developing *E. coli* in terms of the count of *E. coli* was significant ($P < 0.05$). The difference between the counts of *E. coli* of GCMA1, GCMA3 and GCSH1 was insignificant ($P > 0.05$) (Table 3).

The highest count of *S. aureus* (8.04 ± 0.06 log cfu/g) was found in DMSH2 ($P < 0.05$) (Table 3). The difference between the spices in terms of the count of *S. aureus* count was significant ($P < 0.05$). The difference between the count of *S. aureus* in DM obtained from the spice shops, the count of *S. aureus* in DMHO2, and the count of *S. aureus* in DMHO3 was insignificant ($P > 0.05$) (Table 3).

Salmonella spp. was isolated from twelve of the spices (DMMA1, DMMA2, DMMA3, DMSH3, DMHO2, DMHO3, GCSH1, GCSH2, GCSH3, GCHO1, GCHO2, and GCHO3) (Table 3).

DISCUSSION

According to the herbal production statistics of Turkey Statistical Institute [59] in 2018, Turkey produced 227,380 tons of pepper (dried, unprocessed), 24,195 tons of cumin, and 15,895 tons of thyme. Since Turks tend to pick and consume mint fresh from nature, data about Turkey's mint output as the dried spice is not very reliable. No data could be found in the Turkey Statistical Institute [59] about the country's 2018 black pepper output either.

TMAB count is the most important indicator of overall microbiological quality in any product. No limit was found in the Turkish Food Codex Regulation on Microbiological Criteria [47] for TMAB count in spice. Both the International Commission on Microbiological Specifications for Foods [60] and Food Safety Authority of Ireland [61] set the maximum TMAB limit in dried spice to 10^6 cfu/g (6 log cfu/g). As ICMSF standard [60], spices containing a TMAB count of $<10^4$ cfu/g (4 log cfu/g) are of acceptable quality, whereas those containing 10^4–10^6 cfu/g (4-6 log cfu/g) are of marginal quality. The samples of all RPF and RPP obtained from the markets, RPPSH1, RPPHO2, and RPPHO3, GBPMA2, DM obtained from the markets, DTMA1 and DTMA3, DTSH2, and DT obtained from the homes, and the samples of GC (expect for GCSH3) did not exceed the maximum limit of TMAB. RPFMA2, RPFSH1, RPPMA3, GBPMA2, DTMA1 and DTMA3, GCMA2, GCHO1 and GCHO2 were of acceptable quality (Tables 2 and 3).

The count of TMAB was deemed unacceptable in 28% (35/125) of the samples in an Iranian study conducted on 25 different spices and herbs collected from the city of Tabriz [62]. Almost 50% of the herbs did not exceed the aerobic mesophilic level of 10^4 cfu/g (4 log cfu/g) in a study conducted on 99 samples of aromatic herbs collected from retail shops across Algarve, Southern Portugal [63]. Nur and coauthors [64] found that 13 out of 33 samples (dried herb and spice) from Dhaka, Bangladesh exceeded the permissible limit of bacterial count (>10^5 cfu/g/5 log cfu/g). However, 20 samples showed bacterial count ranging between 3.1×10^2 and 2×10^3 cfu/g (2.49-3.30 log cfu/g) [64]. The aerobic mesophilic microorganisms did not exceed 10^5 cfu/g in most of 46 samples of the aromatic herbs from Algarve, Portugal [65].

Gulati and Das [66] found that 80% of spice samples (n=100) on sale in Uttarakhand, India – including 10 samples each of cumin and black pepper – were of marginal quality according to the count of TMAB. Total bacteria contamination accounted for 45% in 80 samples of medicinal herbs (including 40 traditional and 40 industrial herbs) from Ahvaz, Iran [67]. Dghaim and coauthors [68] found that 50% of herb samples exceeded the World Health Organization's [69] limit for the total aerobic bacteria count in a study examining 20 herb samples from Dubai, United Arab Emirates. This rate was 31.48% (17/54) in the current study (Tables 2 and 3).

The total aerobic heterotrophic bacterial load of six spices (including cumin and black pepper) collected from different local markets across Dhaka city, Bangladesh ranged between 6.6×10^3 and 2.1×10^5 cfu/g (3.82-5.32 log cfu/g) [70]. The total aerobic bacterial count was 6.2 log cfu/g in HACCP certificated spices and 5.4 log cfu/g in non-certificated HACCP spices in a study examining 119 commercial spices from Suncheon, South Korea [71]. The total number of bacteria in the spices was between 5×10^5 and 95×10^5 cfu/g (5.70-6.98 log cfu/g) in a study including 11 spices hailing from local markets across Tikrit, Iran [72]. The total aerobic bacteria number in red pepper in Korea fell within the range of 2.97 and 8.13 log cfu/g [73]. The highest total bacterial count (8×10^8 cfu/g/8.90 log cfu/g) was detected in black pepper samples in a study conducted on 5 spices randomly collected from different catering enterprises in Tehran, Iran [74]. Abd El-Rahman [75] found that the count of TMAB was 10.80×10^3 cfu/g (4.03 log cfu/g) in cumin whole seeds, 45.04×10^4 cfu/g (5.65 log cfu/g) in ground cumin, 68.87×10^4 cfu/g (5.84 log cfu/g) in black pepper whole seeds, 63.66×10^6 cfu/g (7.80 log cfu/g) in ground black pepper, and 16.49×10^4 cfu/g (5.22 log cfu/g) in thyme powder, respectively. But, TMAB was not detected in thyme whole seeds [75].

No limit value was found in the Turkish Food Codex Regulation on Microbiological Criteria [47] for ASFB in dried spices. Expect for five samples (DTMA2, DTMA3, and DT obtained from the spice shops), all the dried spices contained ASFB. GBPSH1, DM obtained from the spice shops and the homes, the samples of all DT, and the samples of all GC did not exceeded the limit of 6 log cfu/g (Tables 2 and 3).

Lesanifar and Hanifan [76] stated that the count of ASFB was 4.15 ± 0.56-7.02 ± 0.60 log cfu/g in 25 spices and aromatic herbs in Tabriz, Iran. Sedaghat and coauthors [77] reported that mean count of total

aerobic mesophilic spores was 1.6×10^7 cfu/g (7.20 log cfu/g) for red pepper (at highest rate), 6.6×10^6 cfu/g (5.82 log cfu/g) for black pepper, when they found that a total of 80 samples of packed and unpacked spices were collected randomly from the food supplying centers and local retail stores in Zanjan city, Iran. Average total aerobic mesophilic spore counts for packed and unpacked spices was 3.0×10^6 cfu/g (6.48 log cfu/g) and 8.4×10^6 cfu/g (6.92 log cfu/g), respectively [77].

Yeast and mold is a group (fungus) of microorganisms commonly found in the microbial flora of dried spices [78], some of which pose a risk for food safety and public health because they produce mycotoxins [79]. No limit value was found in the Turkish Food Codex Regulation on Microbiological Criteria [47] for TYM in dried spice. But, International Commission on Microbiological Specifications for Foods [60] set the maximum limit to 10^4 cfu/g (4 log cfu/g) of TYM in dried spice. DMMA1 and DMMA2, DMHO2, DTHO2, GCSH2 and GCSH3 exceeded this critical limit (Table 3).

Yeast and mold contamination accounted for 77% in 80 samples of medicinal herbs (40 traditional herbs and 40 industrial herbs) in Ahvaz, Iran [67]. The fungi count regarded as unacceptable (10^6 cfu/g/6 log cfu/g) was not found in any of the tested herbs, while 84% of the samples ranged from ≤10^2 to 10^4 cfu/g (≤2-4 log cfu/g) in a study conducted on 99 samples of aromatic herbs collected from retails shops in the region of Algarve, Southern Portugal [63]. Fungi did not exceed 10^5 cfu/g in most of 46 samples of the aromatic herbs in Algarve, Portugal [65]. Dghaim and coauthors [68] observed that 75% exceeded the permissible limit for total molds and yeast count in a study examining 20 herb samples in Dubai, United Arab Emirates. The rate of unacceptable samples was 11.11% in the current study according to the limit of TYM (Table 2 and 3).

Abd El-Rahman [75] found that the count of TYM was 19.26 x 10^4 cfu/g (5.28 log cfu/g) in whole cumin seeds, 37.00 x 10 cfu/g (2.57 log cfu/g) in ground cumin, 68.00 cfu/g (1.83 log cfu/g) in whole black pepper seeds, 17.30 x 10 cfu/g (2.24 log cfu/g) in ground black pepper, and 42.26 x 10^3 cfu/g (4.63 log cfu/g) in ground thyme. But TYM was not detected in whole thyme seeds [74]. Mold and yeast above the acceptable limit was observed in 5 (3%), and 7 (4.3%) of 162 samples of 25 spices collected from retail and production sites around different regions of Ethiopia, respectively [39].

The prevalence of molds in spices and herbs was 45/60 (75%) samples and 34/45 (76%) samples of spices and 11/45 (24%) samples of herbs in a study investigating a total of 60 samples composed of 5 herbs (n=14) and 15 spices (n=46) collected from the local market and supermarket chain in Riga, Latvia. Molds were found in four (9%) cumin samples with 3.13-4.48 log cfu/g. Cumin contained the highest mold concentration level (3.6 log cfu/g) [80]. Mold-yeast content in packaged samples (n=15) collected from markets and stores across Istanbul, Turkey was 1.07×10^4 cfu/g (4.03 log cfu/g) in red pepper and 1.75×10^4 cfu/g (4.24 log cfu/g) in black pepper. In unpackaged samples (n = 15), it was 7.82×10^3 cfu/g (3.89 log cfu/g) in red pepper, 7.62×10^3 cfu/g (3.88 log cfu/g) in black pepper [81].

CGB and *E. coli* are indicators of hygiene. They show whether products have been contaminated with feces; furthermore, they are an agent of many dangerous foodborne diseases [79]. No limit value was found in the Turkish Food Codex Regulation on Microbiological Criteria [47] for CGB and *E. coli* in dried spices. The International Commission on Microbiological Specifications for Foods [60] set the maximum limit to 10^4 cfu/g (4 log cfu/g) of CGB. RPPMA1, RPPMA2, RPPSH2, RPPSH3, RPPHO1, RPPHO2, DMMA1, DMMA2, DMSH2, DMSH3, DMHO2, DTHO1, GCMA1, and GC obtained from the spice shops exceeded the limit of CGB (Tables 2 and 3). The standard recommended by the International Commission on Microbiological Specifications for Foods [60] is <10^3 cfu/g (3 log cfu/g) for *E. coli* in dried spices. Accordingly, this study's findings revealed that all of the samples except for six (RPPHO3, GBPMA1, GBPHO1, GBPHO3, GCHO2, and GCHO3) contained unacceptable levels of *E. coli* (Tables 2 and 3).

The count of CGB was unacceptable in 40% (50/125) of the samples in an Iranian study conducted on 25 spices and herbs from Tabriz [62]. Coliform and *E. coli* contamination accounted for 55% and 31.2% in 80 samples of medicinal herbs (40 traditional and 40 industrial herbs), respectively from Ahvaz, Iran [67]. Dghaim and coauthors. [68] found that 75% of herb samples were contaminated with *E. coli* count in a study examining 20 herb samples from Dubai, United Arab Emirates. The rate of unacceptable sample was 29.62% according to the limit of CGB and 46.29% according to the limit of *E. coli* in the current study (Tables 2 and 3).

Nur and coauthors [64] found that about 48.5% of dried herb and spice samples from Dhaka, Bangladesh contained *E. coli*, therefore indicating the presence of coliform bacteria. Likewise, 21.2% of the samples contained other enteric bacteria (unidentified) [64]. Coliform bacteria were detected in 13 (35.1%) out of 37 HACCP certificated spices and 27 (32.9%) out of 82 non-certificated HACCP spices in a study examining 119 commercial spices from Suncheon, South Korea [71].

No sample was positive for the presence of *E. coli* in a study conducted on 99 samples of aromatic herbs collected from retail shops across Algarve, Southern Portugal [63]. *E. coli* was not detected in any sample of

aromatic herbs from Algarve, Portugal [65]. The coliforms in red pepper in Korea fell within the range of 1.87 to 6.71 log cfu/g. However, *E. coli* was not detected [73]. There was no *E. coli* contamination in any spice samples in a study conducted on 5 spices randomly collected from different catering enterprises from Tehran, Iran [74].

Total coliform ranged between 0 and 1.7 log cfu/ml, whereas *E. coli* ranged between 0 and 3.14 log cfu/ml in four spices collected from five popular stores in Temale, Ghana [82]. The total coliform above the acceptable limit was observed in 20 (12.3%) of 162 samples of 25 spices collected from retail and production sites in different regions of Ethiopia; this rate was 9 (5.6%) for *E. coli* [39]. The presence of *E.coli* ranging between 35×10^5 and 93×10^5 cfu/g (6.54-6.97 log cfu/g) was found in all of the samples in an Iran study that looked at 11 spices from local markets in Tikrit [67].

S. aureus causes forms toxins that make people ill [83]. The enterotoxin produced by S. aureus bacteria causes Staphylococcal poisoning (a gastrointestinal disorder). The cooking process does not destroy enterotoxin, leading the person consuming the food to develop food poisoning [84]. The vegetative cells of *S. aureus* can be deactivated with heat treatment done for 2-50 min at 60 °C [85]. However, the enterotoxin produced by *S. aureus* does not disintegrate during subsequent cooking because it is heat resistant. A heat treatment at higher temperatures is required to inactive staphylococcal enterotoxin. The norms of heat treatment vary between several hours at 80-100 °C and 5-10 min at 121 °C depending on the suspending medium and toxin type [86].

It was stated that the number of pathogens should reach 10^5 cells/g for *S. aureus* to form enterotoxins [87]. Therefore, one can deduce that nineteen of the samples in this study (RPFHO2, RPFHO3, RPPMA2, RPPSH2, RPPSH3, GBPMA3, GBPSH1, GBPSH3, GBPHO1, GBPHO2, GBPHO3, DMSH1, DMSH2, DMSH3, DMHO1, DMHO2, DMHO3, GCSH2, and GCSH3) were risky in terms of enterotoxins – meaning that *S. aureus* could form (Tables 2 and 3).

The Turkish Food Codex Regulation on Microbiological Criteria [47] specified the limit value for coagulase (+) staphylococci as 10^3 cfu/g (3 log cfu/g) in dried spices. Since there are also coagulase-producing species other than *S. aureus* among staphylococci, no evaluation has been made according to this standard. The standards recommended by the Food Safety Authority of Ireland [61] and the Food Standards Australia New Zealand [88] is $<10^2$ cfu/g (2 log cfu/g) for *S. aureus* in dried spices. In this study, all of samples (100%) with the exception of four samples (DTMA2, DTSH2, DTHO1, GCMA2) contained unacceptable levels of S. aureus (Tables 2 and 3).

Gulati and Das [66] observed that 50% of spice samples (n=100), including 10 samples of cumin and 10 samples of black pepper from Uttarakhand, India had *S. aureus* contamination. No sample was tested positive for the presence of staphylococci in a study conducted on 99 samples of aromatic herbs collected from retail shops in Algarve, Southern Portugal [63]. Dghaim and coauthors [68] reported that all herb samples were within the World Health Organization's [69] acceptable limit for *S. aureus* in a study examining 20 herb samples from Dubai, United Arab Emirates.

S. aureus was above the acceptable limit in 19 (11.7%) of 162 samples of 25 spices collected from retail and production sites from different regions of Ethiopia [39]. *S. aureus* was the most isolated among pathogenic bacteria in six spices (including cumin and black pepper) collected from different local markets of Dhaka city, Bangladesh [70]. Coagulase positive staphylococci was not detected in any of the samples of the aromatic herbs in Algarve, Portugal [65]. The count of *S. aureus* was 1×10^5 - 17×10^5 cfu/g (5.00-6.23 log cfu/g) in an Iranian study that tested 11 spices hailing from local markets in the city of Tikrit [72].

As *Salmonella* spp. causes foodborne gastrointestinal disease worldwide, it therefore is an important microorganism when it comes to food safety [89]. *Salmonella* spp. should be not found in 25 g of dried spice [47, 60]. However, it was isolated in 38.88% (21/54) of the samples in the current study (Table 2 and 3). Since *Salmonella* spp. was also found in GC shop and home samples; people instead should buy from spice shops. Alas, unpackaged GC can pose a risk for *Salmonella* spp.

Salmonella species was not detected in any of 162 samples of 25 spices collected from retail and production sites in different regions of Ethiopia [39]. No *Salmonella* positive samples (based on analysis of 125 g) were found among commercial samples of cumin seed (whole or ground) in an American study featuring 7,250 retail samples of 11 spices. In that study, *Salmonella* prevalence estimates (confidence intervals) for the other spice types were 0.24% (0.049 to 0.69%) for black pepper (whole, ground, or cracked) and 0.64% (0.17 to 1.6%) for red pepper (hot red pepper, e.g., chili, cayenne; ground, cracked, crushed, or flakes). But, *Salmonella* prevalence estimates (based on analysis of two 375 g composite samples) for shipments of imported spices were 1.7-18% [90].

No sample tested positive for the presence of *Salmonella* spp. in a study conducted on 99 samples of aromatic herbs collected from retail shops around Algarve, Southern Portugal [63]. Dghaim and coauthors

[68] found that 75% of 20 herb samples were contaminated with *Salmonella* spp. (Dubai, United Arab Emirates). *Salmonella* was not detected in any of the samples of the aromatic herbs in Algarve, Portugal [65]. *Salmonella* spp. was not isolated in red pepper in South Korea [73]. *Salmonella* contamination was negative in all spice samples in a study conducted on 5 spices randomly collected from different catering enterprises in Tehran, Iran [74]. *Salmonella* spp. was not found in any of the samples in a study conducted on packaged (n=15) and unpackaged (n=15) spices collected from markets and stores across Istanbul, Turkey [81].

CONCLUSION

Different microorganism groups were found at unacceptable levels in the majority of dried spices examined in this study – regardless of whether they were sold in packages in markets, unpackaged in spice shops, or kept in bags or spice jars at home. Microorganisms found at the unacceptable level included *S. aureus* (47/54), *E. coli* (25/54) and *Salmonella* spp. (21/54), respectively. Samples taken from the home contained the highest number of microorganisms at an unacceptable level (18/18). This rate was 14/18 in the samples taken from both markets and spice shops. The dried spices that had the highest microbiological load were DM, GBP, and GC, respectively. The dried spice that had the best microbiological quality was DT – this indicates that mint may have antimicrobial properties. These results also demonstrate that the pathogenic bacteria loads of the dried spices were high, not to mention microbiologically unsafe.

The results of the current study suggest that dried spices can contain microorganisms at different levels. These microorganisms can in turn contaminate meals and infect humans. Therefore, where and how they are collected, grown, produced, stored, purchased, and kept (at home) are all important. In order to reduce the damage caused by microbial hazards in dried spices, they should not be left to sit on dining tables or used to spice up already cooked meals/foods. Instead, they should only be used when cooking meals.

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