

Enumeration of Microorganisms and Detection of Some Pathogens in Commonly Used Spices Sold Openly from Retail Stores in Kars

Ibrahim BEKİ¹, Zeynep ULUKANLI^{1*}

¹ Kafkas University, Faculty of Science and Arts, Department of Biology, 36100, Kars, Turkey

Received: 03.05.2006 Revised: 22.05.2008 Accepted: 02.07.2008

ABSTRACT

A total of 75 spice samples from retail stores in Kars were analysed for the presence of microorganisms. Aerobic mesophilic counts of red pepper (14.3%) and cinnamon samples (14.3%) exceeded the Turkish Food Codex. The samples included high levels of enterobacteriaceae and coliforms. Red pepper (n=3), black pepper(n=3), cinnamon (n=2), cumin (n=2) and allspice (n=3), samples contained *E. coli*, but not *E. coli* O157:H7 nor *Salmonella* spp. Most samples contained staphylococci and micrococci but not *S. aureus*. Counts of Enterococci and lactobacilli were detected at low levels. Pseudomonads and Aeromonads were not detected in all samples. All samples included aerobic and anaerobic spore forming bacteria, yeast and moulds.

Key Words: Red pepper, Black pepper, Cumin, Cinnamon, Allspice, Microbiological quality.

1. INTRODUCTION

The use of spices in the preparation of many food products goes back to ancient times [1]. Today, there has also been an increasing interest in the consumption of spices in modern countries due to the health benefits. The modern food processor is also under increasing demands to produce natural, wholesome products in order to appease today's health conscious public and thus gain consumer acceptance [2]. Like in many countries in the world, in traditional Turkish industry and kitchens, the preparation of meat and meat-based products like salami, sausages, pastirma and soudjouk includes spices like red pepper, black pepper, cumin and allspice as major ingredients not only to give flavour but also to preserve the food [3, 4].

Kars is located in the northeastern part of the region and called the "Caucasian Gate" of Eastern Anatolia. The average altitude of Kars is 1750 meters. It has borders with Georgia, Armenia, Nakhichevan, Iran and Iraq. The province of Kars has a total population of 323,000. The main economic activities of the Kars region depend on agriculture and animal husbandry. The abundance of pastures in the region caused the number of animals to increase and priority was given to the production of

animal products. In fact, the production of animal products in the region is about one-fourth of the total production in Turkey [25].

Literature based on the microbiological quality of spices has been assessed in many countries as well as in some cities of Turkey [5- 19]. However, there has been no earlier microbiological study of spices sold in Kars (Turkey). In this work, our aim was to examine the microbial quality of red pepper, black pepper, cumin, cinnamon and allspice samples from Kars and to determine the possible health risk for consumers.

2. MATERIALS AND METHODS

A total of 75 spice samples were collected and examined from retail shops in the city of Kars. A 10 g of each sample was taken aseptically and homogenized with sterile 90 ml of 0.1% sterile peptone water. Decimal dilutions were carried out using the same diluent. An aliquot of 0.1 ml was plated directly. Colonies appearing on appropriate media were counted and expressed as the colony forming units per gram (CFU/g). The total aerobic mesophilic counts (TAM) were determined on Plate Count Agar (PCA) (30 °C/72 h). Coliform counts were performed using Violet Red Bile Agar (VRBA) (37 °C/24 h). Presumptive *E. coli*

*Corresponding author, e-mail: ulukanli@kafkas.edu.tr

colonies appearing on VRBA were then subcultured on Eosin Methylene Blue Agar (37 °C/24-48 h). Colonies with characteristic greenish metallic colour were subjected to IMVIC tests to identify *E.coli*. Enterobacteriaceae were isolated on Violet Red Bile Glucose Agar (37 °C/ 24-48 h).

Kanamycin Aesculin Azide agar was used for the isolation of Enterococci (37 °C/ 24-48 h). Staphylococci and micrococci were enumerated on Baird Parker Agar (35 °C/24-48 h). Starch Phenol Red Ampicillin agar was used for the enumeration of Pseudomonads and Aeromonas (37 °C/24-48 h). MRS agar (Lactobacillus agar acc. to De Man, Rogosa and Sharpe) was used for lactobacilli counts (37 °C/24-48 h). For the enumeration of aerobic spore forming bacteria (ASB) and anaerobic spore forming bacteria (AnSB), samples were initially treated with heat (80 °C) and then cooled immediately and subsequently plated on plate count agar for aerobes (30 °C/72 h) and on tryptose sulphite cycloserine agar for anaerobes (37 °C/ 24-48 h). For the total yeast and fungi, acidified Potato Dextrose Agar was used (25 °C/120 h).

For *Salmonella*, 25 g of sample was aseptically weighted and added into 225 ml of sterile buffered peptone water and incubated at 37°C for 18-20 h. An inoculum of 0.1 ml was transferred into a tube containing 9 ml of Rappaport Vassiliadis Broth (42 °C/24 h) and subsequently plated onto Salmonella Shigella (SS) agar and Xylose Lysine Desoxycholate (XLD) agar plates (37°C/24-48 h) and then examined for typical colonies.

For *E. coli* O157:H7, 25 g of sample was pre-enriched in 225 ml modified Novobiocin EC Broth at 37 °C for 24 h. An inoculum of 0.1 ml enrichment broth was then spread onto selective CT-SMAC (Cefixime-Tellurite Supplement and Sorbitol MacConkey Agar) (42 °C/24-48 h) and then examined for typical colonies.

3. RESULTS AND DISCUSSION

The spice samples analyzed yielded a range of microbial contamination (Table 1). In our work, counts of TAM ranged from 10^4 to 10^7 CFU/g in red pepper samples. In other countries, levels as high as $>10^6$ CFU/g [5], 2×10^6 - 2×10^8 CFU/g [8], 10^4 - 10^9 CFU/g [11] and the levels as low as 8.1×10^5 CFU/g [9] and $\geq 10^4$ - $\geq 10^6$ CFU/g [10] were detected in red pepper samples. In Turkey, similar or high levels of 2.0×10^6 CFU/g, 63.0×10^4 - 1.0×10^7 CFU/g, 10^7 - 10^9 CFU/g, 3.1×10^6 CFU/g, 2.6×10^7 CFU/g, 8.1×10^6 CFU/g, 10^3 - 10^7 CFU/g were also reported in red pepper samples sold in the cities of Elazığ, Bursa, Ankara, İstanbul, İzmir, Erzurum and Iğdir, respectively [12, 14-19].

Counts of TAM varied from 10^4 to 10^6 CFU/g in black pepper samples. TAM counts in black pepper samples are similar to those reported by Garcia *et al.* [10], whereas our results are not in agreement with the findings of other studies, which reported TAM counts of black pepper as $>10^6$ CFU/g and $>10^7$ CFU/g, 12.1×10^7 - 81.9×10^8 CFU/g, 1.9×10^7 CFU/g, and 10^6 - 10^9 CFU/g [5-7, 9, 11]. Similar or higher levels of 10^7 - 10^9

CFU/g [15], 3.9×10^7 CFU/g [18], 4.6×10^6 CFU/g [12] and 6.8×10^6 CFU/g [16], 10^3 - 10^6 CFU/g [19] were also detected in black pepper by several workers in cities in Turkey.

Counts of TAM varied from 10^3 to 10^6 CFU/g in cumin samples. Levels of 1.0×10^4 - 1.0×10^8 CFU/g [8], 10^4 - 10^9 CFU/g, [11] and $\geq 10^4$ - $\geq 10^6$ CFU/g [10] were detected in spices examined from other countries. Levels of 3.9×10^5 CFU/g [17], 34×10^2 - 86×10^6 CFU/g [14], 1.2×10^4 CFU/g [12], 10^4 - 10^6 CFU/g [16] and 10^2 - 10^7 CFU/g [19] were also reported in cumin samples examined in Turkey. As compared to those stated in previous studies, the levels of TAM for cumin in the study reported here are more or less similar (Table 1).

Counts ranged from 10^2 to 10^7 CFU/g in cinnamon and 10^3 to 10^6 CFU/g in allspice samples. Various levels of 10^5 - 10^9 CFU/g [11] and 10^7 - 10^9 CFU/g [15] 1.1×10^5 CFU/g, 1.7×10^4 CFU/g, 5.2×10^3 - 1.2×10^5 CFU/g, 10^2 - 10^6 CFU/g [9, 17, 13, 19] in cinnamon samples were reported. High levels of $>10^6$ CFU/g and 10^7 - 10^8 CFU/g were reported for allspice samples in two earlier studies [5, 15].

In spices, counts $>10^6$ CFU/g have not been at the acceptable level according to the Turkish Food Codex [26]. According to our results, it appears that some spices like red pepper and cinnamon have higher loads of TAM than other spices examined. Plate count of aerobic mesophiles in spices is regarded as an indicator of general hygiene and quality parameter [20]. High aerobic mesophilic counts found in our samples may reflect that samples had been exposed to poor handling, inappropriate storage or a general lack of hygiene [21, 22].

The number and the detection rate of Enterobacteriaceae and coliforms were presented in Table 1. Enterobacteriaceae count ranged from $<10^2$ to 10^6 CFU/g in red pepper and cinnamon, $<10^2$ to 10^5 CFU/g in black pepper and cumin, and $<10^2$ to 10^4 CFU/g in allspice samples. In other studies, spices showed enterobacterial counts of 10^3 - 10^5 CFU/g in 50% of red pepper, 10^1 - 10^8 CFU/g in 80% of black pepper, 10^1 - 10^5 CFU/g in 34% of cumin, 10^5 - 10^8 CFU/g in 20% of cinnamon [11], and $<10^1$ CFU/g in red pepper and cinnamon samples and 8.0×10^3 CFU/g in black pepper [9], and 10^3 CFU/g in 76% of black pepper, 96% of cumin, 100% of red pepper [16] and 10^2 - 10^6 CFU/g in 36.9% of red pepper and 42% of cinnamon samples, 10^6 CFU/g in 79% of cumin samples, $<10^4$ CFU/g in 52.6% of black pepper samples [19].

Table 1. The frequency distribution of microorganisms in spices.

| | <10 ² | 10 ² -<10 ³ | 10 ³ -<10 ⁴ | 10 ⁴ -<10 ⁵ | 10 ⁵ -<10 ⁶ | 10 ⁶ -<10 ⁷ | ≥ 10 ⁷ |
|-----------------------------|------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-------------------|
| TAM | | | | | | | |
| Red pepper (n=14) | - | - | - | 2 (14.3) | 4 (28.6) | 6(42.9) | 2 (14.3) |
| Black pepper (n=14) | - | - | - | 2 (14.3) | 5 (35.7) | 7 (50.0) | - |
| Cumin (n=14) | - | - | 5 (35.7) | 2 (14.3) | 5 (35.7) | 2 (14.3) | - |
| Cinnamon (n=14) | - | 3 (21.4) | 5 (35.7) | - | 2 (14.3) | 2 (14.3) | 2 (14.3) |
| Allspice (n=19) | - | - | 2 (10.5) | 4 (21.0) | 11 (57.9) | 2 (10.5) | - |
| Coliforms | | | | | | | |
| Red pepper (n=14) | 9 (64.3) | 3 (21.4) | 2 (14.3) | | | | |
| Black pepper (n=14) | 5 (35.7) | 5 (35.7) | 3 (21.4) | 1 (7.1) | | | |
| Cumin (n=14) | 6 (42.9) | 4 (28.6) | 4 (28.6) | | | | |
| Cinnamon (n=14) | 11(78.6) | 2 (14.3) | 1 (7.1) | | | | |
| Allspice (n=19) | 12 63.2) | 6 (31.6) | 1 (5.2) | | | | |
| Enterobacteriaceae | | | | | | | |
| Red pepper (n=14) | 5 (35.7) | 4 (28.6) | 2 (14.3) | - | 1 (7.1) | 2 (14.3) | |
| Black pepper (n=14) | 3 (21.4) | 2 (14.3) | 6 (42.9) | 1 (7.1) | 2 (14.3) | - | |
| Cumin (n=14) | 1 (7.1) | 5 (35.7) | 4 (28.6) | 3 (21.4) | 1 (7.1) | - | |
| Cinnamon (n=14) | 9 (64.3) | 1 (7.1) | 1 (7.1) | | 1 (7.1) | 2 (14.3) | |
| Allspice (n=19) | 7 (36.8) | 5(26.3) | 4 (21.0) | 3 (15.8) | | | |
| Enterococci | | | | | | | |
| Red pepper (n=14) | 11 (78.6) | 3 (21.4) | | | | | |
| Black pepper (n=14) | 5 (35.7) | 9 (64.3) | | | | | |
| Cumin (n=14) | 13 (92.8) | 1 (7.1) | | | | | |
| Cinnamon (n=14) | 11 (78.6) | 3 (21.4) | | | | | |
| Allspice (n=19) | 16 (84.2) | 3 (15.8) | | | | | |
| Staph/micrococci | | | | | | | |
| Red pepper (n=14) | 3 (21.4) | 3 (21.4) | - | 4 (28.6) | 3 (21.4) | 1 (7.1) | |
| Black pepper (n=14) | - | - | 1 (7.1) | 7 (50) | 5 (35.7) | 1 (7.1) | |
| Cumin (n=14) | 1 (7.1) | 3 (21.4) | 4 (28.6) | 4 (28.6) | 2 (14.3) | | |
| Cinnamon (n=14) | 5 (35.7) | 5 (35.7) | | 2 (14.3) | 2 (14.3) | | |
| Allspice (n=19) | 1 (5.2) | 3 (15.8) | 3 (15.8) | 6 (31.6) | 6 (31.6) | | |
| Pseudomonads/ Aeromonads | | | | | | | |
| Red pepper (n=14) | 14 (100) | | | | | | |
| Black pepper (n=14) | 14 (100) | | | | | | |
| Cumin (n=14) | 14 (100) | | | | | | |
| Cinnamon (n=14) | 14 (100) | | | | | | |
| Allspice (n=19) | 19 (100) | | | | | | |
| Lactobacilli | | | | | | | |
| Red pepper (n=14) | 10 (71.4) | 1 (7.1) | - | 1 (7.1) | 1 (7.1) | 1 (7.1) | - |
| Black pepper (n=14) | 9 (64.3) | 2 (14.3) | 2 (14.3) | - | 1 (7.1) | - | - |
| Cumin (n=14) | 13 (92.8) | - | - | - | 1 (7.1) | | |
| Cinnamon (n=14) | 11 (78.6) | - | - | - | 1 (7.1) | 2 (14.3) | |
| Allspice (n=19) | 13 (68.4) | 3 (15.8) | - | 2 (10.5) | 1 (5.2) | | |

Table 1 (continued). The frequency distribution of microorganisms in spices.

| | <10 ² | 10 ² -<10 ³ | 10 ³ -<10 ⁴ | 10 ⁴ -<10 ⁵ | 10 ⁵ -<10 ⁶ | 10 ⁶ -<10 ⁷ | ≥ 10 ⁷ |
|----------------------------------|------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-------------------|
| Aerobic Spore Forming Bacteria | | | | | | | |
| Red pepper (n=14) | - | 1 (7.1) | 1 (7.1) | 6 (42.9) | 4 (28.6) | 2 (14.3) | |
| Black pepper (n=14) | - | - | 1 (7.1) | 8 (57.1) | 3 (21.4) | 2 (14.3) | - |
| Cumin (n=14) | - | 1 (7.1) | 5 (35.7) | 6 (42.9) | 1 (7.1) | 1 (7.1) | - |
| Cinnamon (n=14) | - | 10 (71.4) | 3 (21.4) | 1 (7.1) | - | - | - |
| Allspice (n=19) | - | 4 (21) | - | 6 (31.6) | 9 (47.3) | - | - |
| Anaerobic Spore Forming Bacteria | | | | | | | - |
| Red pepper (n=14) | - | 1 (7.1) | 2 (14.3) | 6 (42.9) | 4 (28.6) | 1 (7.1) | |
| Black pepper (n=14) | - | - | 2 (14.3) | 9 (64.8) | 3 (21.4) | - | - |
| Cumin (n=14) | - | 4 (28.6) | 2 (14.3) | 6 (43) | 2 (14.3) | - | - |
| Cinnamon (n=14) | - | 10 (71.4) | 2 (14.3) | 2 (14.3) | - | - | |
| Allspice (n=19) | - | 4 (21) | 2 (10.5) | 5 (26.3) | 8 (42.1) | - | - |
| Yeast and moulds | | | | | | | |
| Red pepper (n=14) | - | 5 (35.7) | 5 (35.7) | 3 (21.4) | 1 (7.1) | - | - |
| Black pepper (n=14) | - | - | 1 (7.1) | 4 (28.6) | 5 (35.7) | 4 (28.6) | - |
| Cumin (n=14) | - | 4 (28.6) | 2 (14.3) | 4 (28.6) | 3 (21.4) | 1 (7.1) | - |
| Cinnamon (n=14) | - | 10 (71.4) | 1 (7.1) | 3 (21.4) | - | - | - |
| Allspice (n=19) | - | 3 (15.8) | 6 (31.6) | 8 (42.1) | 1 (5.2) | 1 (5.2) | - |

The counts of Enterobacteriaceae in food products indicate the hygienic quality of the samples rather than fecal contamination; therefore, hygienic quality of spices regarding this parameter seems to be low in this work. Poor quality in our samples may result from the use of low quality water and the unhygienic handling of the spices before and during storage [11, 23].

The coliform count ranged from <10² to 10³ CFU/g in red pepper, cumin, cinnamon, allspice and black pepper samples. On the other hand, higher than 10³ CFU/g of coliforms was present in 7.1% of black pepper sample. In other works, more or less similar counts of coliforms were reported between 42x10²-22x10³ CFU/g, ≥10²-≥10⁴ CFU/g, ≥10²->10⁴ CFU/g in black pepper samples [7, 10, 11] and 10²-10⁴ CFU/g in red pepper and cumin samples [10], and 10³ CFU/g in 100% of red pepper and 88% of black pepper, 10² CFU/g in 92% of cumin samples [16]. Variable coliform counts of 1.9x10⁵ CFU/g, 4.4x10⁴ CFU/g, 25.0-10³ CFU/g, 1.7x10² CFU/g, 10²-10⁴ CFU/g for red pepper [12, 17, 14, 18, 19], 9.3x10⁴ CFU/g, 3.3x10⁴ CFU/g, 4.0x10³ CFU/g, 4.9x10³ CFU/g, 10²-10⁴ CFU/g for black pepper [12,

17, 14, 18, 19] and 4.3x10³ CFU/g, 1.2x10⁴ CFU/g, 9.0x10³ CFU/g, 2.4x10⁴ CFU/g, 10²-10³ CFU/g for cumin [12, 17, 14, 18, 19]; 10² CFU/g for cinnamon [19] were also reported by other workers in cities in Turkey.

The presence of *E. coli* would seem to be of great value as an indicator of the presence of other enteric pathogens in food and food derived products. In this work, three red pepper (21.4%), three black pepper (21.4%), two cinnamon (14.3%), two cumin (14.3%) and three allspice (15.8%) samples contained *E. coli* (Table 2). It seems that the isolation of *E. coli* in the samples suggests that fecal contamination does occur. Our detection rate of *E. coli* is much lower than those obtained in other studies [7, 8, 10]. In those studies, *E. coli* was found in 100% of red pepper, 60% of black pepper, and 50% of red pepper and cumin [7, 8, 10]. Lower or higher incidence of *E. coli* was observed in the cities in Turkey. It was detected in 40% of red pepper and black pepper and in 77% of cumin samples [14], 50% of black pepper and cumin samples [18], 5% of red pepper and in 5% of black pepper samples [17],

11% of red pepper, 11% of black pepper, and 16% of cumin samples [19]. While *E. coli* was detected in some spices, as well as a considerable level of coliform and enterobacterial counts, none of the samples gave the presence of *E. coli* O157:H7 and *Salmonella* spp (Table 2). As far as we know, screening of *E. coli* O157:H7 in spices has been given little attention in comparison to other pathogenic bacteria. Absence of this pathogen was reported in only two previous studies [9, 19]. Rare occurrence of *Salmonella* spp in black pepper and red pepper was reported previously [9, 24]; nonetheless, the absence of *Salmonella* in our samples is in accordance with those reported by other authors [6, 7, 8, 10, 11, 19]. The apparent absence of *Salmonella* spp and *E. coli* O157:H7 may be due to the low or rare incidence of contamination with *Salmonella* spp. and *E. coli* O157:H7 or the intensive competition of the dominating flora.

Table 2. The occurrence of *E. coli*, *E. coli* O157:H7 and *Salmonella* spp. in the samples examined.

| | <i>E. coli</i> | <i>E. coli</i> O157:H7 | <i>Salmonella</i> spp. |
|---------------------|----------------------------|---------------------------|---------------------------|
| samples | number of positive samples | | |
| Red pepper (n=14) | 3 | - | - |
| Black pepper (n=14) | 3 | - | - |
| Cumin (n=14) | 2 | - | - |
| Cinnamon (n=14) | 2 | - | - |
| Allspice (n=19) | 3 | - | - |

Enterococci were isolated in 64.3% of black pepper, 21.4% of red pepper, 21.4% of cinnamon, 15.8% of allspice and 7.1% of cumin samples at a level of $\geq 10^2$ CFU/g in all samples. Not only the level but also the presence of Enterococci found in this work is not in accordance with a previous report for red pepper and cinnamon samples collected from Austria [9]. But, the presence of enterococci in black pepper, red pepper, and cumin in our samples is significantly lower than reported in the previous two works carried out in Austria [9] and in Turkey [16]. More recently, similar counts were noted for black pepper and cumin samples in a recent study in Turkey [19], but up to the counts of 10^3 CFU/g in red pepper and cinnamon samples found in those studies appears to be higher than our results [19]. The presence of Enterococci in the spices examined may result from insects, soil or other sources associated with unsanitary conditions in the production and handling of foods.

The counts of staphylococci and micrococci as well as the incidence rate in spice samples were shown in Table 1. As can be seen from the table, the count ranged from $<10^2$ to 10^6 CFU/g in red pepper, 10^3 to 10^6 CFU/g in

black pepper, and $<10^2$ to 10^5 CFU/g in cumin, cinnamon and allspice samples. Our results showed that three different spices- black pepper, allspice and cumin- contained a higher contamination level than the other two spices. The findings of the present work are more or less similar to the results obtained in other surveys carried out in Turkey [12, 16, 18, 19]. In contrast to high counts of total staphylococci obtained in this work, *S. aureus* was not isolated from any of the spices examined. Our findings were in agreement with other works [5, 7, 11-13, 16, 18, 19]. The detection of *S. aureus* in spices has been reported in former studies [8, 9]. Rare recovery of this pathogen in spices may suggest that the numbers of this organism were present below the limit of detection.

Pseudomonads/Aeromonads were also not detected in any of the samples examined. This finding is consistent with that of Ulukanli *et al.*[19] who reported similar levels in retail spice samples. In contrast, Kneifel & Berger [9] reported values of 1.1×10^3 CFU/g in red pepper, 3.0×10^2 CFU/g in cinnamon, 1.6×10^5 CFU/g in black pepper [9]. Differences in the present and previous work might arise from level of contamination.

Lactobacilli was present in all samples analyzed. The counts occurred up to the level of 10^5 CFU/g in black pepper, cumin and allspice samples, and 10^6 CFU/g in red pepper and cinnamon. There have been reports implicating the occurrence of lactobacilli in spices. Lactobacilli counts were previously reported as being 2.4×10^3 CFU/g in black pepper and $<10^1$ CFU/g in red pepper and cinnamon samples [9], 10^6 CFU/g in red pepper, black pepper and cumin samples [18], and $<10^2$ CFU/g in red pepper, black pepper, cumin and cinnamon samples [19]. Differences in the present and previous works may be related to the quality of the spices assessed.

Total aerobic spore forming bacteria (ASB) were detected in all spices at various levels. ASB counts ranged from 10^2 - $\geq 10^6$ CFU/g in red pepper and cumin, 10^3 - $\geq 10^6$ CFU/g in black pepper, 10^2 - $\geq 10^5$ CFU/g in allspice and 10^2 - $\geq 10^4$ CFU/g in cinnamon. Lower levels than the present findings were reported in the other three studies [19]. In those studies, ASB counts were reported as 10^5 CFU/g in red and black pepper, and counts of 10^3 CFU/g and 10^4 CFU/g were found in cumin samples [12, 18]. Whereas, similar counts between 10^3 - 10^6 CFU/g for red pepper and black pepper and of 10^1 - 10^5 CFU/g for cumin was reported in another study [14]. Significantly higher levels than that the present data were reported as 10^5 - 10^7 CFU/g in red pepper and black pepper, 10^4 - 10^7 CFU/g in cumin, 10^5 - 10^8 CFU/g in cinnamon, 10^5 - 10^6 CFU/g in allspice [15] and $>10^5$ CFU/g in 48% of red pepper and 68% of black pepper; whereas, the ranges varied between 10^2 and 10^5 CFU/g in 100% of cumin samples [16].

Total anaerobic spore forming bacteria (AnSB) were present in the spices examined. Counts of anaerobes exceeded the acceptable limits ($>10^4$ CFU/g) in 37% of red pepper, 21.4% of black pepper, 14.3% of cumin, and 42.1% of allspice. A similar detection rate was also reported in 100% of red pepper and black pepper, and

75% of cumin [18]. Besides, high levels of 10^5 - 10^8 CFU/g in black pepper, 10^4 - 10^7 CFU/g for cumin and cinnamon, 10^5 - 10^6 CFU/g for allspice samples [15], and 10^3 CFU/g in red pepper and cumin, 10^5 CFU/g in black pepper [12], and 10^2 - 10^5 CFU/g in red pepper and cumin samples, 10^3 - 10^5 CFU/g in black pepper, and 10^2 - 10^4 CFU/g in cinnamon samples [19] were also reported.

All the samples included yeast and moulds. However, 7.1% of red pepper, 64.3% of black pepper, 28.5% of cumin, and 10.4% of allspice in this work had levels above 10^4 CFU/g, which does not comply with Turkish Food Codex [26]. Lower or higher yeast/fungal counts were previously reported in spices [7, 8, 11]. As compared to previous studies in Turkey, levels of yeast and fungi in black pepper, cumin, cinnamon, and allspice in this work are similar to the previous findings [15]. In that study, yeast and mould counts was found between 10^4 - 10^6 CFU/g in black pepper, and 10^3 - 10^5 CFU/g in cumin, cinnamon and allspice samples. In another study [19], counts of 10^2 - 10^6 CFU/g found in black pepper appear to be similar to our data (Table 1). On the other hand, results obtained from other spices examined are not similar to the findings of other surveys [12, 13, 16-19]. Yeast and fungi are widely found in nature and they have been known as the quality criteria for products exposed to open air. Storage type and temperature are also important factors affecting the number of microorganisms. Additionally, the cultivation of spices in hot and/or wet regions and the harvesting of these products in non hygienic conditions may increase the contamination risks in spices [17]. The high load of fungi and yeast counts in the samples examined is presumably due to these factors.

The occurrence of significant numbers of spore bearing microorganisms in the samples may indicate that improper pre- or post-harvesting and inadequate temperature storage conditions exist. The reasons for the high occurrence and levels are not known at the moment. The presence of aerobic and anaerobic spore formers and moulds are important since their survival or the presence of their toxins after the cooking process may result in food poisoning or deterioration of the product in which spices have been used. This is of vital importance for the microbiological quality and safety of the experimental shelf stable products [5]. Further study is necessary to identify Bacilli and Clostridia as well as mould and yeast contaminants at species level.

In conclusion, considering the health risks, spices in retail markets should not be sold in open containers and appropriate sterilization and/or reduction of load of microorganisms appears to be required via proper methods. More studies are also needed to confirm the findings of the present study, such as identification of risk factors for the presence of high load of microorganisms and assessment of the impact of spice contamination on public health.

REFERENCES

- [1] Nasar, A.S., Halkman, K., "Baharat Mikroflorasi Uzerine Isinlamanin Etkisi", *Orlab-Online Mikrobiyol. Derg.*, 1:43-65 (2003).
- [2] Moore, J.E., "Gastrointestinal outbreaks associated with fermented meats", *Meat Sci.*, 67: 565-568 (2004).
- [3] Basoglu, F., "Gıdalar da kullanılan bazı baharatların mikroorganizmalar üzerine etkileri ve kontaminasyondaki rolleri", *Gıda Derg.*, 7: 19-24 (1982).
- [4] Akgul, A., "Baharatlar: lezzet, koku ve renk dunyasi", *Gıda San.*, 48: 27-34 (1997).
- [5] Baxter, R., Holzapfel, W.H., "A microbial investigation of selected spices, herbs, and additives in South Africa", *J. Food Sci.*, 47: 570-578 (1982).
- [6] de Boer, E., Spiegelenberg, W.M. and Janssen, F.W., "Microbiology of spices and herbs", *Ant. van Leeuwenhoek.*, 51:435-438 (1983).
- [7] Geeta, H., Kulkarni, P.R., "Survey of the microbiological quality of whole, black pepper and turmeric powder sold in retail shops in Bombay", *J. Food Prot.*, 50: 401-403 (1987).
- [8] Bhat, R., Geeta, H., Kulkarni, P.R., "Microbial profile of cumin seeds and chili powder sold in retail shops in the city of Bombay", *J. Food Prot.*, 50: 418-419 (1987).
- [9] Kneifel, W., Berger, E., "Microbiological criteria of random samples of spices and herbs retailed on the Austrian Market", *J. Food Prot.*, 57: 839-901 (1994).
- [10] Garcia, S., Iracheta, F., Galvan, F., Heredia, N., "Microbiological quality of retail herbs and spices from Mexican markets", *J. Food Prot.*, 64: 99-103 (2001).
- [11] Banarjee, M., Sarkar, P.K., "Microbiological quality of some retail spices in India", *Food Res. Int.*, 36: 469-474 (2003).
- [12] Tekinsen, O.C., Sarigol, C., "Elazığ yöresinde tüketime sunulan bazı öğütülmüş baharatın mikrobiyal florası", *F. U. Vet. Fak. Derg.*, 1: 151-162 (1982).
- [13] Karapinar, M., Aktug, S.E., "Microbiological quality of ground cinnamon: incidence of *Bacillus cereus*", *Mitt Gebiete Lebensm. Hyg.*, 77: 520-527 (1986).

- [14] Karapinar, M., Tuncel, G., "Parekende satılan bazı toz baharatların mikrobiyolojik kaliteleri", *E. U. Muh Fak. Derg.*, 4: 27-36 (1986).
- [15] Kivanc, M., Sert, S., "Erzurum'da perakende satış mağazalarında ki bazı öğütulmuş baharatların mikrobiyel kalitesi", *Doga*, TUBITAK, 13: 316-325 (1989).
- [16] Erol, I., Kuplulu, O., Karagoz, S., "Ankara'da tüketime sunulan bazı baharatın mikrobiyolojik kalitesi", *A. U. Vet Fak Derg.*, 46: 116-125 (1999).
- [17] Uner, Y., Ergun, O., "Piyasada satışa sunulan çeşitli baharatın bazı patojenler ve genel mikrobiyolojik kriterler yönünden incelenmesi", *I.U. Vet Fak Derg.*, 25: 245-251 (1999).
- [18] Filiz, N., "Bursa'da tüketime sunulan bazı baharatların mikrobiyal florası", *U.U. Vet Fak Derg.*, 20: 103-107 (2001).
- [19] Ulukanli, Z., Karadag, E., Elmali, M., Gurbuz, A., "İgdir'da acıkta satışa sunulan bazı öğütulmuş baharatların mikrobiyolojik kalitesi", *S.U. Vet Fak Derg.*, 21: 31-37 (2005).
- [20] Kneifel, W., Czech, E., Kopp, B., "Microbial contamination of medicinal plants: A Review", *Planta Medica*, 68: 5-15 (2002).
- [21] Gillespie, I., Little, C., Mitchell, R., "Microbiological examination of cold ready-to-eat sliced meats from catering establishments in the United Kingdom", *J. App. Microbiol.*, 88: 467-474 (2000).
- [22] Richardson, I.R., Stevens, A.M., "Microbiological examination of ready-to-eat stuffing from retail premises in the north-east of England. The 'Get Stuffed' survey", *J Appl. Microbiol.*, 94: 733-737 (2003).
- [23] Jay, J.M., "Modern Food Microbiology", *Chapman & Hall*, 5th ed., New York (1996).
- [24] Pafumi, J., "Assessment of the microbiological quality of spices and herbs", *J. Food Prot.*, 49: 958-963 (1986).
- [25] BTC (The Baku-Tbilisi-Ceyhan Crude Oil Pipeline Community Investment Programme Turkey), <http://www.btcinvestment.com>.
- [26] Anonymous., "Turk Gıda Kodeksi. Baharat Tebliği", Tebliğ No:2000/16, Resmi Gazete: Tarih ve Sayı:31.07.2000, 24126 (2000).