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Research Article

Bacteriological analysis of the red pepper spices marketed as packaged and unpackaged in Istanbul

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Abstract

The red pepper spice, which has been widely used in Turkey, must be examined in terms of possible pathogen content because it can be eaten raw. Therefore, in the current study, a total of 50 red pepper samples (40 unpackaged, 10 packaged) from retail shops in Istanbul were bacteriologically analysed for the presence of *Salmonella* spp. and bacteria of the *Aeromonas* genus. At the same time, unsuspicious colonies were tested for the determination of other enteric pathogens. The following results were recorded at the end of the analyses: although 10 % of the samples contained *Aeromonas* spp., none of the samples were positive for *Salmonella* spp. It was observed that the unpackaged red pepper samples were contaminated more than the packaged samples. The results obtained from the current study demonstrated that a better control in all aspects of production, processing and usage is required to prevent bacteriological contamination in the red pepper samples.

Keywords: Red pepper, Spices, *Salmonella* spp., *Aeromonas* spp., *Enterobacteriaceae*
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İstanbul'da paketlenmiş ve paketlenmemiş olarak pazarlanan kırmızıbiber baharatının bakteriyolojik analizi

Özet

Türkiye'de yaygın olarak kullanılan kırmızıbiber baharatı çiğ olarak kullanılabildiği için muhtemel patojen içeriği açısından incelenmelidir. Bu amaçla çalışmamızda, İstanbul'daki perakende marketlerden toplanan 50 farklı kırmızıbiber örneği (40 paketlenmemiş, 10 paketli) *Salmonella* spp. ve *Aeromonas* spp. varlığı açısından incelenmiştir. Aynı zamanda, şüpheli olmayan koloniler diğer enterik patojenler açısından test edilmiştir. Analiz sonuçları değerlendirildiğinde örneklerin %10'unda *Aeromonas* spp. bulunmasına rağmen hiçbir örnekte *Salmonella* spp.'ye rastlanmamıştır. Paketlenmemiş olarak satılan kırmızıbiber örneklerinin paketli örneklere oranla bakteriyolojik açıdan çok daha kirli olduğu tespit edilmiştir. Bu çalışmadan elde edilen sonuçlar, kırmızıbiber örneklerinde bakteriyolojik kontaminasyonun önlenmesi için üretim, işleme ve kullanım aşamaları sırasında daha iyi bir kontrolün gerekli olduğunu göstermektedir.

Anahtar Kelimeler: Kırmızıbiber, baharat, *Salmonella* spp., *Aeromonas* spp., *Enterobacteriaceae*

Introduction

Spices have been used to achieve a certain aroma and taste as flavoring agents in foods since ancient times (Ristori et al. 2007; Beki and Ulukanlı 2008). Although spices are known to have antimicrobial properties (Akgül and Kıvanç 1988; Liao and Fett 2001), different reports have indicated that spices may carry foodborne pathogens due to being in contact with water and soil that are contaminated with microorganisms (Üner et al. 2000; Küplülü et al. 2003). Previous studies have demonstrated that spices could be contaminated by total heterotrophs, *Bacillus cereus, Clostridium perfringens, Escherichia coli, Salmonella spp., Shigella spp., Pseudomonas aeruginosa, Aeromonas spp., Staphylococcus aureus* and toxigenic moulds (Manninen and Sjöberg 1991; Writanen et al. 1993; Aksu et al. 2000; Cadlish et al. 2001; Banarjee and Sarkar 2003; Kim et al. 2004; Elmalı and Yaman 2005). The production, the processing, the distribution and the storage of these products in non hygienic conditions increases the risk of contamination in spices (Banerjee and Sarkar 2003; Elmalı and Yaman 2005; Stankovich et al. 2006). Spices are also exposed to a wide range of environmental microbial contamination in the retail markets due to dust, waste water, and even animal and human excreta (Banerjee and Sarkar 2003). Spices generally do not undergo any processing, other than collection and preparation, such as washing, cutting and drying (Christensen et al. 1967; Schwab et al. 1982; McMahon and Wilson 2001). While some processing, for instance, ethylene oxide fumigation, cold sterilization, radiation ionizing etc., can be used to reduce a number of microorganisms. On the other hand these methods may be carcinogenic or ineffective after prolonged storage (Karapınar and Tuncel 1986; Agu et al. 2008).

Because spices have been associated with several outbreaks, their microbial quality should be investigated. The purpose of the present study is to determine the presence of bacteria such as Aeromonas spp. and Salmonella spp., which are the possible causative agents for infections in humans, in the red pepper spices currently available at retail shops in Istanbul.

Materials and methods

Sampling

A total of 50 different red pepper samples were purchased from retail outlets in Istanbul. The collected samples were packaged and unpackaged. Approximately 100 g spice samples were collected and transported to the laboratory, and analyzed as soon as possible (Banerjee and Sarkar 2003).

Microbiological analysis
Salmonella spp. *isolation*

Conventional culture method procedures for isolation of *Salmonella* were performed according to the International Organization for Standardization (ISO 6579) (Cudjoe and Krona 1997; Vieira – Pinto et al. 2008). Firstly, each of the red pepper samples (25 g) were aseptically weighed and placed in 225 mL sterile Buffered Peptone Water (BPW) (Oxoid, Basingstoke, Hampshire, UK) and mixed for one minute. The homogenized samples were incubated at 37ºC for 24 hours. An aliquot of 0.1 mL BPW pre-enriched samples was inoculated to 9.9 mL Rappaport-Vasiliadis (RV) broth (Merck, Darmstadt, Germany) and incubated at 42°C for 24 h (Mansfield and Forsythe 2001). After 24 h incubation, 0.1 mL aliquots were spread on the Brilliant-green Phenol-red Lactose Sucrose Agar (BPLS-A), (Merck, Darmstadt, Germany) and incubated at 37 °C for 24 h. Following 24 h incubation, plates were examined for typical colonies, picking at least one colony of each typical colonial type from each of the plates for identification and suspicious colonies were carried out onto Nutrient Agar (NA) slants, (Oxoid, Basingstoke, Hampshire, UK) (Anğ-Küçüker et al. 1993). At the same time, unsuspicious colonies were tested for the determination of other enteric pathogens.

Aeromonas spp. isolation

Each of the red pepper samples (25g) were inoculated into Alkaline Peptone Water (Oxoid, Basingstoke, Hampshire, UK). After the incubation at 22ºC for 24-48 hours, samples were subcultured on the BPLS-A overnight at

35 ºC and then suspicious colonies were inoculated onto NA slants (Meçgel 1992).

Identification of bacteria

The general key which was used for the identification was Bergey's Manual of Determinative Bacteriology (Holt et al. 1994). All isolates were immediately examined for Gram stain reaction and cell morphology. Isolates determined as Gram-negative rod bacteria were selected to determine oxidase activity (Kovacs' test) and identification. Oxidase negative and oxidase positive rods were accepted as suspicious strains for Salmonella and Aeromonas, respectively. Gram-negative bacteria were identified using API 20E strips (BioMérieux, Marcy-l'Etoile, France) and API 20NE strips for oxidase negative and positive strains, respectively.

The isolated bacterial strains were stored at -20°C in tryptic soy broth containing 10% of glycerol.

Results

The distribution of isolated bacteria in packaged and unpackaged red pepper is given in Tables 1 and 2. The unpackaged and the packaged spices had high rates of bacterial contamination with 70% and 60%, respectively. In this study, the 6 different species were found in 5% of unpackaged spices and 10% of the packaged spices. Moreover, 5 species in 5%, 4 species in 10%, 3 species in 5%, 2 species in 15% and 1 species in 30% of unpackaged spices were detected. In packaged spices, 4, 3, 2 and 1 species were isolated with the range of 10%, 10%, 10% and 20%, respectively. It is shown that in 16 of 50 red pepper samples tested, any suspicious bacterial colonies were not found in terms of Salmonella spp. and Aeromonas spp. (Table 1 and 2).

Table 1. Distribution of isolated bacteria in unpackaged red pepper samples

No. of red pepper	*Isolated bacteria*
1	*Enterobacter sakazakii** *Enterobacter sakazakii**
2	*Enterobacter agglomerans* *Enterobacter sakazakii* *Pseudomonas luteola* *Pseudomonas oryzihabitans*
3	*Cedecea lapagei*
4	*Enterobacter agglomerans*
5	*Serratia odorifera*
6	*Aeromonas salmonicida** *Aeromonas salmonicida**
7	*Enterobacter sakazakii* *Klebsiella pneumoniae* spp *pneumoniae* *Serratia odorifera*
8	*Enterobacter agglomerans*
9	*Enterobacter sakazakii*
10	*Aeromonas salmonicida** *Aeromonas salmonicida**
11	*Enterobacter sakazakii*
12	Any suspicious colony could not be found.
13	*Enterobacter sakazakii** *Enterobacter sakazakii** *Pantoea* spp2 *Serratia rubidaea*
14	Any suspicious colony could not be found.
15	Any suspicious colony could not be found.
16	Any suspicious colony could not be found.
17	Any suspicious colony could not be found.
18	*Aeromonas sobria*
19	Any suspicious colony could not be found.
20	*Aeromonas salmonicida** *Aeromonas salmonicida** *Enterobacter intermedium* *Enterobacter agglomerans*
21	Any suspicious colony could not be found.
22	Any suspicious colony could not be found.
23	Any suspicious colony could not be found.
24	Any suspicious colony could not be found.

No. of red pepper	*Isolated bacteria*
25	*Enterobacter sakazakii*
26	*Enterobacter agglomerans*
27	Any suspicious colony could not be found.
28	*Enterobacter agglomerans* *Enterobacter cloacae* *Enterobacter sakazakii* *Pseudomonas aeruginosa* *Serratia rubidaea** *Serratia rubidaea**
29	*Enterobacter agglomerans* *Enterobacter cloacae* *Klebsiella oxytoca* *Serratia ficaria* *Serratia rubidae*
30	*Enterobacter agglomerans** *Enterobacter agglomerans** *Enterobacter cloacae* *Enterobacter spp.* *Yersinia frederiksenii* *Serratia odorifera*
31	*Serratia odorifera* *Pantoea* spp4
32	*Enterobacter agglomerans** *Enterobacter agglomerans** *Enterobacter cloacae* *Enterobacter sakazakii** *Enterobacter sakazakii**
33	*Enterobacter agglomerans* *Pseudomonas luteola*
34	*Citrobacter freundii*
35	*Serratia odorifera* *Enterobacter agglomerans* *Enterobacter sakazakii** *Enterobacter sakazakii**
36	Any suspicious colony could not be found.
37	*Enterobacter cloacae*
38	*Yersinia frederiksenii* *Enterobacter sakazakii*
39	*Enterobacter agglomerans* *Enterobacter cloacae* *Enterobacter sakazakii*
40	*Yersinia frederiksenii*

*: species obtained from different colonies

Table 2. Distribution of isolated bacteria in packaged red pepper samples

No. of red pepper	Isolated bacteria
1	*Pseudomonas luteola** *Pseudomonas luteola**
2	*Aeromonas salmonicida* *Enterobacter agglomerans* *Pseudomonas aeruginosa* *Pseudomonas luteola*
3	*Enterobacter agglomerans* *Enterobacter sakazakii* *Klebsiella pneumoniae spp pneumoniae* *Serratia odorifera** *Serratia odorifera** *Serratia rubidaea*
4	*Enterobacter agglomerans*
5	Any suspicious colony could not be found.
6	Any suspicious colony could not be found.
7	Any suspicious colony could not be found.
8	Any suspicious colony could not be found.
9	*Serratia odorifera* *Enterobacter agglomerans* *Raoultella ornithinolytica*
10	*Enterobacter agglomerans*

*: species obtained from different colonies

The number of positive samples for bacteria which is isolated from the red pepper is summarized in Table 3. As seen in Table 3, a total of 85 bacterial isolates were obtained from the 50 red paper samples. The results of the present study show that *Enterobacter agglomerans* is the most frequently isolated bacterium in the examined samples with the range of 22% (Table 3). The remaining strains were identified as *Enterobacter sakazakii* (20%), *Serratia odorifera* (9%), *Aeromonas salmonicida* (8%), *Enterobacter cloacae* (7%), *Pseudomonas luteola* (6%), *Serratia rubidaea* (6%), *Yersinia frederiksenii* (4%), *Pseudomonas aeruginosa* (2%), *Klebsiella pneumoniae spp pneumoniae* (2%), *Enterobacter spp.* (1%), *Enterobacter intermedium* (1%), *Pseudomonas oryzihabitans* (1%), *Cedecea lapagei* (1%), *Serratia ficaria* (1%), *Aeromonas sobria* (1%), *Klebsiella oxytoca* (1%), *Pantoea spp2* (1%), *Pantoea spp4* (1%), *Citrobacter freundii* (1%), *Raoultella ornithinolytica* (1%). *Salmonella* spp. could not be detected in any of the analyzed samples both packaged and unpackaged (Tables 1 and 2).

Table 3. Incidence of bacteria in the red pepper samples

Isolated bacteria	Number of positive samples	Total no. of isolates
Enterobacter spp.	1	1
Enterobacter agglomerans	17	19
Enterobacter cloacae	6	6
Enterobacter intermedium	1	1
Enterobacter sakazakii	13	17
Pseudomonas oryzihabitans	1	1
Pseudomonas luteola	4	5
Pseudomonas aeruginosa	2	2
Cedecea lapagei	1	1
Serratia odorifera	7	8
Serratia ficaria	1	1
Serratia rubidaea	4	5
Aeromonas salmonicida	4	7
Aeromonas sobria	1	1
Klebsiella pneumoniae spp *pneumoniae*	2	2
Klebsiella oxytoca	1	1
Pantoea spp2	1	1
Pantoea spp4	1	1
Citrobacter freundii	1	1
Yersinia frederiksenii	3	3
Raoultella ornithinolytica	1	1
	Total	85

Discussion

Spices are made from different parts of the plants, such as dried seeds, roots, fruits and the barks of trees and are used to flavor foods in preparation and processing throughout the

world (Stankovich et al. 2006). Spices may contain many microorganisms which are possible causes of foodborne infections (Erdoğrul 2000; Vural et al. 2004). Although spices and herbs are not major contributors to foodborne diseases, a potential hazard exists, particularly if the spices are added to foods at the end of cooking or to foods that do not go under further heat treatment (Banerjee and Sarkar 2003; Stankovich et al. 2006; Vij et al. 2006; Sagoo et al. 2009).

In Turkey, the red pepper is one of the most commonly used spices and is generally used in cooked food (Vural et al. 2004). Therefore, the contaminated red pepper may have an important role in increasing the microbial load of prepared foods (Temelli and Anar 2002; Banerjee and Sarkar 2003). There are a number of studies related to the isolation of different pathogens in the red pepper (Boer et al. 1985; Aksu et al. 2000; Garcia et al. 2001; Temelli and Anar 2002; Banarjee and Sarkar 2003; Küplülü et al. 2003; Ulukanlı et al. 2005; Beki and Ulukanlı 2008). Studies on the microbiology of spices have demonstrated that spices such as red pepper, in generally contain different pathogens such as *Salmonella*, *Aeromonas*, *Bacillus cereus* (Temelli and Anar 2002; Banerjee and Sarkar 2003; Küplülü et al. 2003; Elmali and Yaman 2005; Beki and Ulukanlı 2008).

In the current study, although bacteria of the *Aeromonas* genus were found in the red pepper samples, none of the samples had *Salmonella*. The absence of *Salmonella* in all samples is in agreement with results reported by other authors (Boer et al. 1985; Banarjee and Sarkar 2003; Ulukanlı et al. 2005; Beki and Ulukanlı 2008). It is thought that the absence of *Salmonella* spp. could originate from the low or incidental occurence of contamination with *Salmonella* spp. Although the absence of *Salmonella* spp. in 25 g of spice samples is accepted according to the Turkish Food Codex (2000), it should be considered that it is due to the competition effect of the dominant flora. On the other hand, the rare occurrence of *Salmonella* spp. in red pepper is reported by Kneifel and Berger (1994).

In the current study, it was found that 9% of isolates were *Aeromonas* genus bacteria. *Aeromonas salmonicida* was frequently isolated as the predominant species among *Aeromonas* bacteria in unpackaged red pepper samples that were surveyed. Of the examined samples, 4 and 1 were detected as *Aeromonas salmonicida* and *Aeromonas sobria*, respectively. This finding is inconsistent with those of Ulukanlı et al. (2005) and Beki and Ulukanlı (2008) who reported that *Aeromonas* bacteria were not isolated in retail red pepper samples. In contrast, in this study, *Aeromonas* bacteria were isolated in ways similar to that of Kneifel and Berger (1994).

In the present study, species belonging to the family *Enterobacteriaceae* such as *Enterobacter agglomerans, Enterobacter sakazakii, Enterobacter cloacae, Enterobacter intermedium, Enterobacter* spp*, Serratia rubidaea, Serratia odorifera, Serratia ficaria, Yersinia frederiksenii, Serratia marcescens, Cedecea lapagei, Pantoea* spp2*, Klebsiella pneumoniae* spp *pneumoniae, Klebsiella oxytoca, Citrobacter freundii, Raoultella ornithinolyticai* have been isolated from red pepper samples. Although these bacteria are opportunistic pathogens for humans (except *Enterobacter intermedium*), it is usually not considered as important for food hygiene (Abadias et al. 2006). Because they are often isolated from water, soil and vegetation, it is not suprising that these species were isolated from red pepper in the present study. When the relevant literature were examined, although the levels of contamination of spices have been reported by several researchers (Christensen et al. 1967; Banerjee and Sarkar 2003; Beki and Ulukanlı 2008), no study was found about isolation and identification of enterobacteriaceae. There are many different reports about *E. coli* isolation in the red pepper in Turkey (Karapınar and Tuncel 1986; Ulukanlı et al. 2005; Beki and Ulukanlı 2008). None of the samples were positive for *E. coli* in this study.

When results are evaluated by taking into account the packaging properties of samples, it was seen that unpackaged red pepper samples were contaminated more than packaged samples. The results obtained from the current study revealed that the sterilization stage of

production and air proof packaging in spices is important with regard to the microbial quality of the product, as packaging materials may be in contact with contaminated surfaces, thus passing the contamination on to the product.

Food safety is an increasing concern worldwide. All spices are susceptible to microbial contamination. To prevent bacterial contamination in dried herbs and spices, control measures must be taken at all stages of the food production: growing, harvesting and processing, manufacturing, transporting, storing, and preparation of foods.

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