Microbiological Quality of Peanuts: From Field to Consumption

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Abstract. Peanuts, including shelled peanuts, unshelled peanuts and roasted peanuts which are ready to eat foods, recently appeared as possible sources of infection with foodborne bacteria besides the high levels of aflatoxins. Peanuts which are taken from harvest, storage and snack shops were evaluated for the presence of *Listeria* spp. and *Salmonella* spp; also total aerobic mesophilic bacteria, coliforms, *E.coli*. and yeasts and moulds, were enumerated. A total of 92 samples were found to be clean in terms of *Salmonella* and *E-coli* O157: H7, but only in 1 of the samples of shelled peanut taken from harvest found *Listeria* spp. Shelled peanut samples collected from harvest and storage were found to be contaminated in terms of microorganisms, however, microbiological contamination was found to be less in the samples obtained when the shells of the same specimens were removed. In addition, in terms of microorganisms it was observed that the shell free samples taken from storage were dirtier than the samples which was being removed the shell taken from storage. Shell-free (unpackaged) roasted peanuts samples purchased from the snack shops was found clean in terms of the pathogens. When salt and heat treatment applied over 4%, existing microorganisms can be damaged. Besides, the water activity value of the product can reduce the level of that microorganisms growth. Therefore, processed peanuts are safer in the environment in which they are stored, unless the relative humidity increase and is not subject to cross-contamination.

Introduction

Peanuts (*Arachis hypogaea* L.) are one of the most important oilseed crops and snack foods in the world Agro-food trade market. The major producers/exporters of peanuts are the United States, China, Argentina, Sudan, Senegal, and Brazil. Peanuts are globally used as an economical food source as they contain high quality proteins, unsaturated fatty acid, and are rich in minerals. Peanuts are consumed whole roasted and boiled, and as ingredients in a variety forms of products include peanut brittle, peanut butter, and peanut oil, and so on [1].

In Turkey, the areas where peanut planting and production areas are most common are Adana and Osmaniye (80%). The plants are first excavated from the soil by special plows and the plants are drawn by the workers coming from behind and the fruits are laid on top of the soil. After the plants are kept in this way for up to three days in the field (when the ratio of peanuts to moisture falls to 20%), fruits are separated from their stalks by harvesting machines. Peanuts are often contaminated with different microorganisms from different environmental sources, such as soil, cattle or other animals and insects, reptiles, human resources when hygiene procedures are not performed at all or performed at little, in the post-harvest processes of conventional harvest and peanuts.

Peanuts can be consumed both raw or roasted in our country. Seeds are roasted after the outer shell is not broken or the shell is broken and it is presented for consumption. Peanuts can be sold as packaged and unpackaged in snack shops and grocery stores in room temperature. And it is sold also raw shelled peanuts on the street. These conditions make the peanut vulnerable to the microbiological risks. So far although there have not been studies on the microbiological characteristics of the peanut, which is thought to be clean from a microbiological point of view, since 1960, studies only on aflatoxin, a mycotoxin that the moulds have produced, have been

extensively carried out. However, almonds, cashews, coconut, hazelnuts, pine nuts, pecans, pistachios, sesame seeds, and peanuts, as well as several processed nut products, have been associated with foodborne outbreaks and/or recalls after isolation of foodborne pathogens. It was seen that, the majority of these outbreaks and recalls have been related with *Salmonella*, and many of the outbreaks have lasted for months and have included cases from multiple states in the United States and/or from other countries. Therefore, most published surveys have focused on indicator organisms and *Salmonella*. In the United Kingdom, the incidence of *Salmonella* on retail edible nuts and seeds was under 1%; samples included roasted nut kernels (25 g, n~727, 11 nut species, processed (heat or added preservatives) nuts (25 g, n~2,886, 12 nut species) and dried seeds (25 g, n ~ 3,735). The prevalence of *Salmonella* on raw almond kernels was about 1% over 8 years (100 g, n ~ 13,972) [2]. Outbreaks and recalls due to *Escherichia coli* O157:H7 and recalls due to *Listeria monocytogenes* contamination also are documented for some nuts and nut products [3].

Low a_w foods have usually been considered safe regarding foodborne pathogens because the optimum a_w for growth of these pathogens is over 0.95. But, unfortunately, there have been several outbreaks were seen also due to foods which were low water activity contaminated with *E. coli* O157:H7, *S. Typhimurium*, and *L. monocytogenes* [4]. Dry foods like peanuts do not often support microbial growth, but they may still be able to allow survival of pathogens. Komitopoulou and Penaloza (2009) found that inoculated *Salmonella* could survive for 3-4 weeks on dry, raw materials including crushed cocoa and hazelnut shells, cocoa beans, and almond kernels at both room temperature and 5°C. *Salmonella* is typically inhibited by $a_w < 0.91$, but the a_w of these substances were likely much lower [5].

Pathogens can be transferred in several ways to food, such as through contaminated water and equipment, poor worker hygiene, and pests [5]. At harvest, if good agricultural and hygiene practices are followed, nuts are harvested with minimal damage and then dried to a water activity of less than 0.7. The Codex International Code of Hygienic Practice for tree nuts states that these products should be free from pathogenic microorganisms [6]. Although nut-associated outbreaks of infection are relatively uncommon, recent outbreaks of salmonellosis associated with the consumption of peanuts, peanut products, and almonds have raised awareness of nuts as a potential vehicle for foodborne illness [7]. For this reason, this study was designed to evaluate the microbiological quality of the peanuts produced in our country. This study was the first investigation the microbiological quality of peanuts in our country and peanut was evaluated for pathogens such as *Salmonella*, *Listeria* spp., *Coliform* and *E. coli*, as well as mold, yeast and total aerobic mesophilic bacteria.

Materials and Methods

Peanut samples supplied from the producers in Osmaniye during harvesting period and storage. Also, roasted peanuts were purchased from snack shops in Adana and Osmaniye. In the harvesting period, a total of 24 samples (12 shelled samples and 12 removed the shell samples) collected from different fields, a total of 36 samples (12 shelled samples and 12 removed the shell samples and 12 shell-free samples) taken from storage and 32 shell-free (unpackaged) roasted samples from snack shops, a total of 92 samples has been studied.

Total aerobic mesophilic bacteria were enumerated at Plate Count Agar (Merck). PCA plates were incubated for 48 h at 30°C. Potato Dextrose Agar (Merck) was used for isolate yeast and mould and incubated at 25°C for seven days [8].

Enumeration of coliform and *E. coli* were performed with Most Probable Number (MPN) technique and used Fluorocult Lauryl Sulfate Broth (Merck). Fluorocult Laurly Sulphate Broth tubes were incubated at 37 °C for 24-48 h incubation. After examining tubes of gas forming with UV light and applied Kovac's indole separator for indole test. Fluorescent positive and indole positive tubes as *Escherichia coli* type 1, fluorescent negative and indol positive tubes as *E. coli* O157: H7 were determined [9-10]. *E. coli* O157: H7 confirmation was also done using Fluorocult *E. coli* O157:H7 Agar.

Analyses of *Salmonella* spp. were carried out in four steps. Briefly, 25 grams of samples were weighed in 225 ml of Buffered Peptone Water (Merck) for non-selective pre enrichment step for 16-20 h at 37°C. Following the incubation, for selective enrichment step 0.1 mL and 10 mL were transferred to 10 mL of Rappaport Vassiliadis Broth (Merck) and 100 mL of Selenite Cystein Broth (Merck) and incubated at 42°C and 37°C for 24 h, respectively. After incubation a loopful were streaked on both Xylose Lysine Deoxycholate (XLD) Agar and Hektoen Enteric Agar (Merck), and incubated at 37°C for 24-48 h. Colonies isolated from each media were further analysed by biochemical reactions (lactose, gas from glucose, H₂S, urea, indole, citrate, lysine decarboxylase).

Listeria spp. isolation was performed according to FDA. The analyzed sample was weighed at 25 grams and incubated for 4 h at 30 °C in 225 ml Buffered Listeria Enrichment Broth (BLEB) medium for pre-enrichment. After 4 h, selective addition (10 mg / l acriflavine, 40 mg / l nalidixic acid) the incubation was completed in 48 hours. At the 24th and 48th hours from the beginning of the incubation, the samples was taken from BLEB medium to PALCAM Agar and the petri dishes were left incubation at 37°C for 48 hours. After incubation, on PALCAM agar gray-green or black colonies was suspected as *Listeria* and was taken and spiked to Tryptic Soy Agar-Yeast medium to purify. After incubation, colony and cell morphology of these cells were evaluated. Gram positive, catalase positive, oxidase negative, mobility positive and urea negative colonies were determined as *Listeria* spp. positive [11-12].

Results and Discussion

A total of 92 samples taken from harvest, storage and snack shops were found to be clean in terms of *Salmonella* and *E-coli* O157: H7, but only in 1 of the samples of shelled peanut taken from harvest found *Listeria*. The analysis results are given in Tables 1-6 below.

Table 1. Microbiological analysis results of shelled peanut samples taken from harvested.

Samples	Coliform (MPN/g)	<i>E.coli</i> Tip 1	<i>E-coli</i> O157:H7	<i>Salmonella</i>	<i>Listeria</i> spp.	Yeast (log kob/g)	Mould (log kob/g)	Total aerobic mesophilic bacteria (log kob/g)
1	240	240	<3	N.D.	N.D.	4.7±0.1	N.D.	3.0±0.1
2	460	460	<3	N.D.	N.D.	3.9±0.2	3.0±0.2	4.5±0.1
3	9,1	9,1	<3	N.D.	N.D.	5.1±0.1	N.D.	5.0±0.2
4	15	<3	<3	N.D.	N.D.	3.7±0.3	3.6±0.1	4.3±0.3
5	23	<3	<3	N.D.	N.D.	4.0±0.2	2.7±0.3	4.3±0.2
6	460	<3	<3	N.D.	N.D.	3.6±0.3	N.D.	4.3±0.1
7	23	<3	<3	N.D.	N.D.	5.3±0.1	3.4±0.1	5.0±0.1
8	>1100	240	<3	N.D.	N.D.	4.7±0.3	5.3±0.1	5.6±0.2
9	290	<3	<3	N.D.	N.D.	3.6±0.2	5.6±0.2	6.9±0.3
10	>1100	>1100	<3	N.D.	Positive	8.7±0.2	N.D.	8.7±0.1
11	39	<3	<3	N.D.	N.D.	3.8±0.1	4.6±0.3	5.5±0.1
12	20	<3	<3	N.D.	N.D.	3.7±0.1	3.4±0.1	4.4±0.3

Table 2. Microbiological analysis results taken from harvested period samples that were being removed the shell.

Samples	Coliform (MPN/g)	<i>E.coli</i> Tip 1	<i>E-coli</i> O157: H7	<i>Salmonella</i>	<i>Listeria</i> spp.	Yeast (log kob/g)	Mould (log kob/g)	Total aerobic mesophilic bacteria (log kob/g)
1	<3	<3	<3	N.D.	N.D.	2.0±0.1	3.0±0.3	3.4±0.1
2	<3	<3	<3	N.D.	N.D.	2.7±0.1	N.D.	3.6±0.2
3	<3	<3	<3	N.D.	N.D.	3.6±0.3	3.0±0.2	4.5±0.3
4	<3	<3	<3	N.D.	N.D.	N.D.	3.9±0.1	4.3±0.1
5	<3	<3	<3	N.D.	N.D.	2.5±0.1	N.D.	3.8±0.1
6	<3	<3	<3	N.D.	N.D.	2.6±0.2	3.3±0.1	3.5 ±0.3
7	<3	<3	<3	N.D.	N.D.	3.6±0.1	2.7±0.2	3.8±0.2
8	<3	<3	<3	N.D.	N.D.	2.9±0.2	2.7±0.1	4.0±0.1
9	<3	<3	<3	N.D.	N.D.	5.2±0.1	N.D.	5.6±0.3
10	<3	<3	<3	N.D.	N.D.	2.4±0.1	3.4±0.2	3.7±0.2
11	<3	<3	<3	N.D.	N.D.	3.3±0.2	2.6±0.3	5.2±0.1
12	<3	<3	<3	N.D.	N.D.	2.7±0.3	3.0±0.1	3.4±0.1

Table 3. Microbiological analysis results of shelled peanut samples taken from storage.

Samples	Coliform (MPN/g)	<i>E.coli</i> Tip 1	<i>E-coli</i> O157 :H7	<i>Salmonella</i>	<i>Listeria</i> spp.	Yeast (log kob/g)	Mould (log kob/g)	Total aerobic mesophilic bacteria (log kob/g)
1	>1100	>1100	<3	N.D.	N.D.	7.3±0.1	3.3±0.2	7.4±0.1
2	<3	<3	<3	N.D.	N.D.	3.9±0.2	3.4±0.1	4.8±0.2
3	23	9,1	<3	N.D.	N.D.	5.7±0.1	5.0±0.3	6.1±0.3
4	23	<3	<3	N.D.	N.D.	N.D.	N.D.	3.1±0.2
5	>1100	35	<3	N.D.	N.D.	N.D.	N.D.	N.D.
6	39	3,6	<3	N.D.	N.D.	N.D.	N.D.	3.3±0.1
7	>1100	>1100	<3	N.D.	N.D.	4.7±0.1	3.4±0.2	4.9±0.2
8	>1100	15	<3	N.D.	N.D.	5.2±0.1	4.6±0.1	5.5±0.1
9	7,2	<3	<3	N.D.	N.D.	N.D.	N.D.	N.D.
10	<3	<3	<3	N.D.	N.D.	3.6±0.1	3.0±0.2	4.6±0.3
11	<3	<3	<3	N.D.	N.D.	3.4±0.2	3.0±0.1	3.8±0.1
12	15	6	<3	N.D.	N.D.	4.7±0.1	3.3±0.2	5.0±0.2

Table 4. Microbiological analysis results of samples that were being removed the shell taken from storage.

Samples	Coliform (MPN/g)	<i>E.coli</i> Tip 1	<i>E-coli</i> O157:H7	<i>Salmonella</i>	<i>Listeria</i> spp.	Yeast (log kob/g)	Mould (log kob/g)	Total aerobic mesophilic bacteria (log kob/g)
1	<3	<3	<3	N.D.	N.D.	3.1±0.2	3.7±0.1	4.1±0.3
2	<3	<3	<3	N.D.	N.D.	N.D.	N.D.	4.9±0.2
3	<3	<3	<3	N.D.	N.D.	3.5±0.2	3.2±0.2	3.7±0.2
4	<3	<3	<3	N.D.	N.D.	2.7±0.3	2.3±0.3	3.0±0.1
5	<3	<3	<3	N.D.	N.D.	2.6±0.1	1.6±0.1	4.2±0.3
6	<3	<3	<3	N.D.	N.D.	3.2±0.1	N.D.	4.4±0.2
7	<3	<3	<3	N.D.	N.D.	3.6±0.2	2.0±0.3	3.7±0.1
8	150	<3	<3	N.D.	N.D.	4.8±0.2	3.6±0.2	3.5±0.1
9	<3	<3	<3	N.D.	N.D.	3.6±0.3	N.D.	3.7±0.3
10	<3	<3	<3	N.D.	N.D.	N.D.	N.D.	3.0±0.2
11	<3	<3	<3	N.D.	N.D.	N.D.	N.D.	2.0±0.2
12	<3	<3	<3	N.D.	N.D.	2.3±0.1	N.D.	2.8±0.3

Table 5. Microbiological analysis results of shell-free peanuts samples taken from storage.

Samples	Coliform (MPN/g)	<i>E.coli</i> Tip 1	<i>E-coli</i> O157:H7	<i>Salmonella</i>	<i>Listeria</i> spp.	Yeast (log kob/g)	Mould (log kob/g)	Total aerobic mesophilic bacteria (log kob/g)
1	93	<3	<3	N.D.	N.D.	N.D.	N.D.	N.D.
2	9,1	9,1	<3	N.D.	N.D.	N.D.	N.D.	N.D.
3	<3	<3	<3	N.D.	N.D.	N.D.	N.D.	N.D.
4	1100	<3	<3	N.D.	N.D.	5.3±0.2	3.1±0.2	5.7±0.2
5	21	21	<3	N.D.	N.D.	4.7±0.1	2.1±0.3	4.8±0.1
6	<3	<3	<3	N.D.	N.D.	4.4±0.1	N.D.	5.1±0.3
7	>1100	36	<3	N.D.	N.D.	4.7±0.2	N.D.	5.3±0.2
8	15	3	<3	N.D.	N.D.	3.4±0.3	3.1±0.1	2.9±0.1
9	<3	<3	<3	N.D.	N.D.	4.9±0.2	N.D.	5.3±0.1
10	36	<3	<3	N.D.	N.D.	N.D.	N.D.	N.D.
11	>1100	3	<3	N.D.	N.D.	5.0±0.3	N.D.	5.2±0.1
12	<3	<3	<3	N.D.	N.D.	5.2±0.2	N.D.	5.5±0.2

Table 6. Microbiological analysis results of shell-free roasted peanuts samples taken from snack shops.

Samples	Coliform (MPN/g)	<i>E.coli</i> Tip 1	<i>E.coli</i> O157:H7	<i>Salmonella</i>	<i>Listeria</i> spp.	Yeast (log kob/g)	Mould (log kob/g)	Total aerobic mesophilic bacteria (log kob/g)
1	<3	<3	<3	N.D.	N.D.	N.D.	N.D.	2.0±0.2
2	<3	<3	<3	N.D.	N.D.	N.D.	N.D.	3.6±0.1
3	<3	<3	<3	N.D.	N.D.	N.D.	N.D.	3.6±0.3
4	<3	<3	<3	N.D.	N.D.	N.D.	N.D.	2.6±0.1
5	<3	<3	<3	N.D.	N.D.	N.D.	N.D.	2.1±0.2
6	<3	<3	<3	N.D.	N.D.	N.D.	N.D.	2.3±0.2
7	<3	<3	<3	N.D.	N.D.	N.D.	N.D.	3.6±0.3
8	<3	<3	<3	N.D.	N.D.	N.D.	N.D.	N.D.
9	<3	<3	<3	N.D.	N.D.	N.D.	N.D.	N.D.
10	<3	<3	<3	N.D.	N.D.	N.D.	N.D.	N.D.
11	<3	<3	<3	N.D.	N.D.	N.D.	N.D.	N.D.
12	<3	<3	<3	N.D.	N.D.	N.D.	N.D.	N.D.
13	<3	<3	<3	N.D.	N.D.	2.7±0.1	N.D.	4.4±0.2
14	<3	<3	<3	N.D.	N.D.	N.D.	N.D.	4.4±0.1
15	<3	<3	<3	N.D.	N.D.	2.9±0.1	2.4±0.1	3.5±0.3
16	23	<3	<3	N.D.	N.D.	3.0±0.1	N.D.	4.1±0.2
17	<3	<3	<3	N.D.	N.D.	4.9±0.2	2.6±0.2	5.0±0.1
18	<3	<3	<3	N.D.	N.D.	3.2±0.3	N.D.	3.7±0.2
19	<3	<3	<3	N.D.	N.D.	3.6±0.2	1.6±0.2	3.7±0.1
20	<3	<3	<3	N.D.	N.D.	3.5±0.1	N.D.	3.6±0.1
21	<3	<3	<3	N.D.	N.D.	3.3±0.1	N.D.	4.3±0.2
22	<3	<3	<3	N.D.	N.D.	3.6±0.2	N.D.	4.2±0.2
23	<3	<3	<3	N.D.	N.D.	3.3±0.2	N.D.	3.5±0.1
24	<3	<3	<3	N.D.	N.D.	3.8±0.1	N.D.	4.0±0.3
25	<3	<3	<3	N.D.	N.D.	3.5±0.1	N.D.	4.2±0.1
26	<3	<3	<3	N.D.	N.D.	3.9±0.2	N.D.	4.7±0.2
27	<3	<3	<3	N.D.	N.D.	3.7±0.1	N.D.	3.8±0.1
28	<3	<3	<3	N.D.	N.D.	3.9±0.2	N.D.	4.3±0.2
29	<3	<3	<3	N.D.	N.D.	4.5±0.1	N.D.	4.7±0.1
30	<3	<3	<3	N.D.	N.D.	3.7±0.3	3.3±0.1	3.9±0.3
31	3	<3	<3	N.D.	N.D.	3.9±0.2	2.6±0.2	4.2±0.1
32	<3	<3	<3	N.D.	N.D.	3.8±0.1	N.D.	4.3±0.2

N.D: not determined

Table 1 shows that microbiological analysis results of shelled peanut samples taken from harvested. A total of 12 shelled peanut samples which were taken from harvest, as the range of in 12 samples total aerobic mesophilic bacteria 3.0±0.1-8.7±0.1 log CFU/g, in 12 samples coliform group bacteria 9.1->1100 MPN/g, in 12 samples *E.coli* Type 1 <3->1100 MPN/g, in 12 samples yeast 3.6±0.2-8.7±0.2 log CFU/g, in 8 samples mold 2.7±0.3-5.6±0.2 log CFU/g were found. In 1 of the samples of shelled peanut taken from harvest found *Listeria* spp. When removing the shell of 12 of peanut samples from harvested period (Table 2), total aerobic mesophilic bacteria 3.4±0.1-5.6±0.3 log CFU/g in 12 samples, in 11 samples yeast 2.0±0.1-5.2±0.1 log CFU/g, and in 9 samples mold 2.6±0.3-3.9±0.1 log CFU/g in the range of were found.

Listeria monocytogenes is a microorganism of significant public concern that can cause severe illness and death in susceptible populations. It was estimated in 1999 that *L.monocytogenes* causes 500 deaths per year in the United States. Pregnant women, neonates, elderly persons, and immunocompromised patients are most susceptible to listeriosis [13]. *Escherichia coli* O157:H7 is an increasingly common cause of illness, including bloody diarrhea and hemolytic uremic syndrome [4].

In order to keep pathogens at a minimum level in the peanuts, it is necessary to follow up with the requirements of good agriculture and hygiene practices such as harvesting the peanuts without damaging and drying them to the desired moisture level.

Microbiological analysis results of shelled peanut samples taken from storage are shown in Table 3. In 12 shelled peanut samples which were taken from storage, in 10 of them total aerobic mesophilic bacteria 3.1 ± 0.2 - 7.4 ± 0.1 log CFU/g, in 12 samples coliform group bacteria $<3->1100$ MPN/g, *E.coli* Type 1 $<3->1100$ MPN/g, in 8 samples yeast 3.4 ± 0.2 - 7.3 ± 0.1 log CFU/g, mold 3.0 ± 0.1 - 5.0 ± 0.3 log CFU/g in the range of were found. When removing the shell of 12 of peanut samples (Table 4), were found coliform group bacteria (150 MPN/g) only in one sample, and *E.coli* was not found in any of the samples. Besides, total aerobic mesophilic bacteria was found in the range of 2.0 ± 0.2 - 4.9 ± 0.2 log CFU/g in 12 samples. Also, yeast 2.3 ± 0.1 - 4.8 ± 0.2 log CFU/g in 9 samples, and mold 1.6 ± 0.1 - 3.7 ± 0.1 log CFU/g in 6 samples were found.

Of the 12 shell-free peanut samples taken from storage, total aerobic mesophilic bacteria 2.9 ± 0.1 - 5.7 ± 0.2 log CFU/g in 8 samples, coliform group bacteria $<3->1100$ MPN/g in 12 samples as was found (Table 5). Moreover, *E.coli* Tip1 as $<3-36$ MPN/g in 12 samples, yeast as 3.4 ± 0.3 – 5.3 ± 0.2 log CFU/g in 8 samples, and mold 2.1 ± 0.3 – 3.1 ± 0.2 log CFU/g in 3 samples, were found.

Table 6 shows that microbiological analysis results of shell-free roasted peanut samples. 27 of the 32 peanut samples which purchased from the snack shops, the total aerobic mesophilic bacteria were found to be between 2.0 ± 0.2 - 5.0 ± 0.1 log CFU/g. While in 2 samples coliform group bacteria were found to be 3-23 MPN /g and *E.coli* was not found. In 19 samples, yeast were found to be between 2.7 ± 0.1 - 4.9 ± 0.2 log CFU/g and mold were found to be between 1.6 ± 0.2 - 3.3 ± 0.1 log CFU/g in 5 samples. In study by Silverio et al. (2010), eleven samples were contaminated by molds, with average fungal content of 3.7×10^3 CFU/g. No contamination was observed in salted shell-free peanuts. Despite the lack of official microbiological criteria on peanuts, there is evidence of fungal contamination during peanut production/shipping procedures that may not be eliminated by roasting [14].

A total of 32 samples purchased from snack shops were found to be clean in terms of *Salmonella*, *E.coli* and *Listeria* spp. Ready-to-eat foods contaminated with *Salmonella* spp. are considered to be harmful to health and/or unsuitable for human consumption and they contravene the food safety requirements [15]. Researchers evaluated microbiologically to detect the levels of total aerobic mesophilic bacteria, coliforms, *Staphylococcus aureus* and *Salmonella* spp. and yeasts and moulds in several types of pistachio and pistachio containing products. They reported that unsatisfactory limits of aerobic mesophilic bacteria, coliforms and yeasts and moulds were obtained in 55% (15/27), 18.5% (5/27) and 22% (6/27) of the samples respectively. Besides, no *Staphylococcus aureus* were detected [15].

The use of *E. coli* as a faecal indicator organism is based on the concept that its detection in food or water samples indirectly provides evidence that the sample has been contaminated with faecal material and that pathogenic organisms may also be present [7]. Researchers found that in only one of 148 samples of peanut taken from snack shops in Brazil, *E. coli* was at an undesirable level (≥ 100 / g) [4].

In this study, shell-free roasted peanuts samples purchased from the snack shops was found clean in terms of the pathogens. When salt and heat treatment applied over 4%, existing microorganisms can be damaged. Besides, the water activity value of the product can be reduced to the level that microorganisms can not develop. For this reason, the environment in which they are stored in processed peanuts is safer as long as the relative humidity does not increase and they are not exposed to cross-contamination.

Evaluation in terms of mold and mycotoxin in peanuts consumed as a snack is less common than in raw peanuts. The evaluation of mold and aflatoxin in snack peanuts was first done in 1987 by Var [16] and the samples were found clean in terms of aflatoxin. Var and Evliya [17] reported that as a result of molding, aflatoxin formation is inevitable, however molds and aflatoxins are mostly damaged as a consequence of thermal processing with dry salt. Hence, the small amount of aflatoxin left in the samples after thermal treatment could not be in detection limits, consequently the spots were difficult to identify.

Shelled peanut samples collected from harvest and storage were found to be contaminated in terms of microorganisms, however microbiological contamination of shell free same samples were found to be less. In addition, in terms of microorganism it was observed that the shell free samples taken from storage were dirtier than the samples which was being removed the shell taken from storage. According to this, peanut shells are natural protective structures and internal seeds can be preserved as long as mechanical and insect damages do not occur. However, the excess of microbial load on the shells can be transmitted to the product if it is not observed after shell removal and processing. For this reason, the hygienic quality of equipment and the processing environment is becoming important. Besides, the storage conditions are important in terms of the internally stored peanuts, and the appropriate temperature, relative humidity and hygienic conditions must be observed. Peanuts can be stored under conditions that maintain moisture below 10%. Temperature should be maintained between 0 and 10 °C (32-50 °F).

Conclusions

Although this is the first large-scale research study on the microbiological assessment of peanuts in Turkey, there is a study where the microbiological quality of other nuts including the roasted peanuts was done. Var et al. [18] were studied a total of 50 nut samples including walnut kernel, peanut, hazelnut, almond, and shelled pistachios. Of the 50 samples, 40 were found to be contaminated with the microorganisms investigated except *Salmonella* spp. and yeast. Both coliform bacteria and *E.coli* were found at the range of 3.6 to 1100 MPN/g. *Staphylococcus aureus* was found as 5×10^1 - 2×10^2 CFU/g. Molds and total aerobic bacterial count were at the range of 5×10^1 - 1×10^2 CFU/g and 5×10^1 - 36×10^2 CFU/g, respectively. The presence of suspicious *Listeria* colonies were seen in 3 of the samples including walnut kernels and peanuts.

These studies are preliminary studies and have been studied by the government to encourage routine inspections of these products and to start auditing in state laboratories. Although there was no found *Salmonella* spp. causing microbiological outbreaks in peanuts in this study, presence of *E.coli* and *Listeria* spp. in shelled peanuts is important. The absence of such a consumption in another country does not create problems but in our country the crusts of nuts in the mouth are eaten by breaking. Therefore, this situation makes routine evaluation of this product in our country important.

Control measures to prevent bacterial cross-contamination of raw and processed nuts require procedures for maintaining the hygienic quality of the processing environment and equipment [6]. Application of good agricultural, manufacturing and storage practices together with the hazard analysis and critical control points (HACCP) system that encompass all stages of production, processing and distribution will help ensure the microbial safety of edible nut kernels.

Conflict of Interest

The authors declare that there is no conflict of interest.

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References

- [1] H.W. Huang, B.B. Yang, C.Y. Wang, Effects of high pressure processing on immunoreactivity and microbiological safety of crushed peanuts, *Food Control*. 42 (2014) 290-295.
- [2] T. Blessington, E.J. Mitcham, L.J. Harris, Survival of *Salmonella enterica*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* on inoculated walnut kernels during storage, *Journal of Food Protection*, 75(2) (2012) 245–254.
- [3] P.K. Brar et al., Survival of *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* on raw peanut and pecan kernels stored at -24, 4, and 22°C, *Journal of Food Protection*. 78(2) (2015) 323–332.
- [4] W.J. Song, D.H. Kang, Influence of water activity on inactivation of *Escherichia coli* O157:H7, *Salmonella Typhimurium* and *Listeria monocytogenes* in peanut butter by microwave heating, *Food Microbiology*. 60 (2016) 104-111.
- [5] A.S. Chang, A. Sreedharan, K.R. Schneider, Peanut and peanut products: A food safety perspective, *Food Control*. 32 (2013) 296-303.
- [6] Codex Alimentarius Commission (CAC), Recommended international code of hygienic practice for tree nuts. CAC/RCP 6–1972, (1972). Available: www.codexalimentarius.net/download/standards/266/CXP_006e.pdf.
- [7] C.L. Little et al., Survey of *Salmonella* contamination of edible nut kernels on retail sale in the UK, *Food Microbiology*. 27 (2010) 171-174.
- [8] Anonymous, Merck Gıda Mikrobiyolojisi, Ankara, Turkey, 2007.
- [9] K. Halkman, H. Doğan, M. Rahati Noveir, Gıda maddelerinde *Salmonella* ve *E.coli* aranma ve sayılma yöntemlerinin karşılaştırılması. Gıda Teknolojisi Derneği Yayın no; 21. Ankara. (1994).
- [10] Anonymous, Merck Gıda Mikrobiyolojisi, Ankara, Turkey, 1998.
- [11] I. Var et al., Adana’da Satışa Sunulan Tavuk Etlerinde *Listeria* spp. Varlığının Araştırılması. 7.Gıda Mühendisliği Kongresi, Konya. s:46 (2012).
- [12] FDA, Detection and Enumeration of *Listeria monocytogenes* in Foods. Bacteriological Analytical Manual, 8th Edition, Revision A. (Erişim tarihi: 12.07.2014). (1998).
- [13] S.J. Kenney, L.R. Beuchat, Survival, growth, and thermal resistance of *Listeria monocytogenes* in products containing peanut and chocolate, *Journal of Food Protection*. 67(10) (2004) 2205–2211.
- [14] A. Silverio, M.M. Lopes, G. de Freitas, Determination of fungal contamination, aflatoxin B1 and ochratoxin A content in peanuts available in Portuguese market, *Journal of Biotechnology*. 150S (2010) S1–S576.
- [15] M. Al-Moghazy, S. Boveri, A. Pulvirenti, Microbiological safety in pistachios and pistachio containing products, *Food Control*. 36 (2014) 88-93.
- [16] I. Var, Çerezlik yerfıstıklarında aflatoksin saptanması üzerine bir araştırma, Çukurova Üniversitesi, Fen Bilimleri Enstitüsü YL tezi. Adana, 1987.
- [17] I. Var, B. Evliya, Çerezlik yerfıstıklarında aflatoksin saptanması üzerine bir araştırma, *Gıda dergisi*. 13(3) (1988) 217-222.
- [18] I. Var et al., The microbiological quality of edible nuts, International Conference on Raw Materials to Processed Foods, Antalya, s:53, 2018.