The Research of Microbiological Quality in Some Edible Nut Kinds

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Abstract: The edible nuts samples (n: 217) were examined for total aerobic mesophilic bacteria, coliforms, *Escherichia coli, Staphylococcus-micrococcus* sp., mould, yeast, *Clostridium perfringens, Salmonella* sp. and *Bacillus cereus*. The highest range of bacterial count was noticed in water melon seed (3.9×10^6 cfu g^{-1}) and the lowest range in melon seeds (1.0×10^3 cfu g^{-1}). The existence of coliforms, *Bacillus cereus, Clostridium perfringens, Escherichia coli* and *Salmonella* sp., in the samples were found as 21.2, 16.13, 8.76, 2.77 and 2.77%, respectively.

Key words: Edible nut, microbiological quality, coliforms, bacteria

INTRODUCTION

Edible nuts are foodstuff which are rich in respect to protein and fiber, containing no cholesterol and which are rich in point of folic acid, niacin, vitamin E, vitamin B6, magnesium, copper, zinc, selenium, phosphorus and potassium. It was announced that these foodstuff, which had high unsaturated fat content (85%), included phytochemicals and antioxidants that were affective to prevent chronicle illnesses (Anon, 2006).

Extensive studies about the hygienic conditions of edible nuts that were often and for different reasons involved in daily diets by all the parts of the society are limited. It was informed that *E. coli* existed, like in other foodstuff, in edible nuts as fecal indicator bound to the kind of edible nuts and the conditions (Kokal, 1965; Hall, 1971; Beuchat, 1973). In a study about edible nuts, Douglas King *et al.* (1970) announced that they isolated bacteria like coliform, *Escherichia coli, Streptococcus, Bacillus, Xanthomonas, Achromobacter, Pseudomonas, Micrococcus, Staphylococcus* and *Brevibacterium*. Several studies about the existence of mould and aflatoxin in edible nuts were reported. Abdulkadar *et al.* (2000) found total aflatoxin in 19 of 81 edible nut samples (peanut, groundnut, hazelnut, almond, walnut and mixed) at the level of 0.53-289 µg kg^{-1} and in 48 of 101 peanut samples at the level of 1.2-275 µg kg^{-1}. Researchers, because of finding the existence of aflatoxin in the samples that were taken from different regions, announced that contacting these kinds of foodstuff by hand should be avoided and that the products which were going to be stored for a long time should be packed in the conditions of modified atmosphere or vacuum packed at low heat degrees. The aflatoxin contamination in edible nuts was also declared in several search results (Kershaw, 1985; Haydar *et al.*, 1990; Steiner *et al.*, 1992; Scholten and Spanjer, 1996).

The edible nuts which can be sold without packing and in unhygienic conditions, can encounter human or animal originate microorganisms' contamination at several steps like sale, storage, processing, transport and consumption. The direct consuming of these foods without having the processes like heat process is thought to cause risk for the health of the community.

In this study, it was aimed to examine the microbiological properties and probable public health risks of totally 217 edible nut samples that were sold markets, nut sellers and peddlers in Diyarbakir, Turkey.

MATERIALS AND METHODS

Collection of the samples: The edible nut samples (totally 217) which were sold without packing in several markets, nut sellers and peddlers in Diyarbakir, Turkey were taken as materials. Hazelnuts (n:15), Antep's pistachios (n:15), Dakota's sunflower seeds (n:15), almonds (n:15), peanut (n:18), roasted chickpeas (n:21), melon seeds (n:17), watermelon seeds (n:18), pumpkin seeds (n:16), sauced roasted chickpeas (n:16), mixed nuts (n:16), walnuts (n:15) and sauced roasted corn (n:15) samples (100 g) were put into sterile sample plastic bags and analyses were started immediately by bringing to 1he laboratories in cold protection conditions (4°C).

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Table 1: The medias and incubation conditions used in microbiologic analysis

Microorganism	Media	Temperature (°C)	Time (h)	Aerob/Anaerobe
Aerobic mesophilic bacteria	Plate Count Agar (Oxoid CM 325)	35	48	Aerobe
Coliforms	Violet Red Bile Lactose Agar (Oxoid CM 107)	35	24	Anaerobe
Escherichia coli	TBX Agar (Oxoid CM 945)	30	4	Anaerobe
		44	18	
Staphylococcus- Micrococcus	Baird Parker Agar Base+ Egg yolk tellurite emülsion (Oxoid CM 275 +SR54)	35	48	Aerobe
Mould	Potato Dextrose Agar (Oxoid CM 139)	25	120	Aerobe
Yeast	Potato Dextrose Agar (Oxoid CM 139)	25	120	Aerobe
Clostridium perfringens	Perfringes Selective Agar Base (OPSP) + SupplementsAve B (Oxoid CM543	35	18-24	Anaerobe
Bacillus cereus	*Bacillus cereus* Selective Agar Base + Supplements (Oxoid CM617+ SR99+SR47)	30	18	Aerobe
Salmonella sp.	Buffered peptone water (Merck 1.07228)	35	24	Aerobe
	RVS broth (Merck 1.07700)	42	24	
	Selenite Cystine broth (Merck 1.07709)	35	24	
	BPLS agar (Merck 1.10747)	35	24	
	Salmonella Shigella agar (Merck 1.07667)	35	24	
	XLD agar (Merck 1.05287)	35	24	
E. coli O157:H7	mTSB (Merck 1.09205)	35	18-24	Anaerobe
	SMAC agar (Merck 1.09207)	35	24	

Microbiological analysis: When doing microbiological analysis of edible nut samples, 10 g sample that was taken in aseptic conditions was homogenized with 90 mL Peptone water (Merck, Darmstadt, Germany). Suitable medias were used with standard analysis methods by doing decimal dilutions Table 1 (Lancette and Harmon, 1980; AOAC, 1995; Anon, 1998; Downes; Ito, 2001; Anon, 2002).

Statistical analysis: SPSS packet program and variance analysis method were used for interpreting Analysis results (ANOVA). For determining the importance degree of the difference between the groups Duncan multiple analysis method was used. The importance degree between values was done in 95% reliance interspaces ($p<0.05$).

RESULTS AND DISCUSSION

The microorganism numbers that were determined in edible nut samples were shown in Table 2. The maximum and minimum numbers of TAMB, coliforms, *E. coli, Staphylococcus-Micrococcus* sp., mould, yeast, *Clostridium perfringens* and *Bacilus cereus* were found as 1.0×10^3-3.9×10^6 cfu g^{-1}, 1.0×10^1-1.2×10^5 cfu g^{-1}, 3.0×10^1-1.5×10^2 cfu g^{-1}, 1.0×10^1-4.2×10^4 cfu g^{-1}, 1.0×10^1-4.5×10^3 cfu g^{-1}, 2.0×10^1-8.6×10^3 cfu g^{-1}, 1.0×10^1-3.1×10^2 cfu g^{-1} and 1.0×10^2-4.5×10^3 cfu g^{-1}, respectively. The existence of coliforms, *Bacillus cereus, Clostridium perfringens, Escherichia coli* and *Salmonella* spp. were found as 21.2% (46/217), 16.13% (25/217), 8.76% (19/217), 2.77% (6/217) and 2.77% (6/217), respectively. *E. coli O157:H7* was not determined in any of the samples.

In this study, the highest numbers of TAMB, coliforms, *E. coli, Staphylococcus-Micrococcus* sp.,

mould, yeast, *Clostridium perfringens* and *Bacillus cereus* were found in watermelon seeds, hazelnut, walnut, watermelon seeds, almond, Dakota's sunflower seeds, sauced roasted chickpea and Dakota's sunflower seeds samples, respectively. The variations between samples are thought to source from the difference in kind of foodstuff, protection and selling conditions.

The importance degrees of the differences between the samples that were taken from markets, nut sellers and peddlers were evaluated statistically (Table 2). The difference between TAMB number were important in Antep's pistachios, Dakota's sunflower seeds, almond, peanut, roasted chickpea, watermelon seeds, pumpkin seeds, sauced roasted chickpea, mixed nuts, walnut and sauced roasted corn samples which were taken from markets, nut sellers and peddlers. The difference between TAMB number in hazelnut samples which were taken from nut sellers and the markets and it is melon seed samples which were taken from nut sellers and others were important. It is thought that this difference between the samples is related to the protection conditions (moisture and heat), shelf life, the practice of the sellers and the beginning microflora of the products.

The studies about the microbiological quality of the edible nuts are very limited. According to Microbiological Criteria Announcement of Turkish Food Codex (Anon, 2001), although the acceptable maximum values for aerobic mesophilic bacteria, *E. coli* and mould in edible nut are 1×10^5 cfu g^{-1}, 9 MPN g^{-1} and 1×10^4 cfu g^{-},[1] respectively and it is not wanted to find *Salmonella* sp. in 25 g. In this study, the existence of *E. coli* and *Salmonella* sp. was found at a rate of 2.77. 46.54% (101/217) of the samples contained microorganisms above the legal limits in regard to mean value TAMB numbers. Mould numbers in the samples were lower than legal limits.

Table 2: The microbiological properties of some edible nuts (mean value cfu g^{-1})

	n	TAMB*	Coliforms	E. coli	Staphyloccus -Micrococcus	Mould	Yeast	C. p erfr ingens	B. cereus
Hazelnut									
Market	5	.4×10^{5b}	4.4×10^{2a}	ND**	2.7×10^{2a}	2.0×10^{1a}	ND	ND	ND
Nut seller	5	7.7×10^{3a}	ND	ND	ND	3.0×10^{2b}	1.0×10^{2a}	ND	ND
Peddler	5	2.0×10^{5ab}	1.2×10^{5b}	ND	6.5×10^{2b}	6.0×10^{2c}	1.8×10^{2b}	3.0×10^{1a}	4.0×10^{1a}
Antep's pistachio									
Market	5	3.0×10^{4a}	3.0×10^{1b}	ND	ND	ND	ND	1.0×10^{1a}	ND
Nut seller	5	1.6×10^{6b}	9.0×10^{1c}	ND	1.0×10^{1a}	2.0×10^{1a}	ND	ND	ND
Peddler	5	2.0×10^{6c}	1.0×10^{1a}	ND	3.0×10^{1b}	3.0×10^{1a}	4.0×10^{1a}	ND	ND
Dakota's sunflower seeds									
Market	5	9.2×10^{5c}	3.5×10^{3b}	ND	1.5×10^{4b}	ND	8.6×10^{3b}	ND	4.5×10^{3a}
Nut seller	5	3.1×10^{5b}	7.0×10^{3a}	ND	1.5×10^{4b}	1.0×10^{2a}	4.4×10^{3a}	ND	4.5×10^{3a}
Peddler	5	3.0×10^{3a}	ND	ND	8.0×10^{2a}	ND	ND	ND	ND
Almond									
Market	5	1.1×10^{3a}	ND	ND	6.0×10^{2b}	2.0×10^{1a}	1.0×10^{2a}	ND	ND
Nut seller	5	2.5×10^{4b}	ND	ND	2.0×10^{1a}	4.5×10^{3c}	5.8×10^{3c}	ND	ND
Peddler	5	1.4×10^{5c}	ND	ND	1.0×10^{3c}	4.0×10^{3b}	1.0×10^{3b}	ND	ND
Peanut									
Market	5	3.0×10^{3a}	ND	ND	ND	ND	ND	ND	ND
Nut seller	6	7.7×10^{4c}	1.0×10^{1a}	ND	6.0×10^{2b}	1.3×10^{2a}	8.0×10^{1a}	ND	ND
Peddler	7	9.0×10^{3b}	1.0×10^{1a}	ND	1.8×10^{2a}	2.7×10^{2b}	ND	ND	4.1×10^{2a}
Roasted chickpeas									
Market	5	7.6×10^{5c}	4.1×10^{1b}	ND	1.0×10^{1a}	ND	ND	ND	2.0×10^{2a}
Nut seller	11	1.3×10^{5b}	1.0×10^{1a}	ND	3.3×10^{3b}	1.3×10^{2a}	ND	2.0×10^{1a}	ND
Peddler	5	2.0×10^{3a}	ND	ND	1.0×10^{1a}	ND	ND	ND	ND
Melon seeds									
Market	5	3.0×10^{3a}	ND	ND	ND	1.0×10^{2b}	2.6×10^{2c}	ND	ND
Nut seller	7	3.0×10^{5b}	5.0×10^{3a}	ND	1.3×10^{4a}	3.4×10^{2c}	1.7×10^{2b}	3.0×10^{1a}	ND
Peddler	5	1.0×10^{3a}	ND	ND	ND	4.0×10^{1a}	2.0×10^{1a}	NDND	
Watermelon seeds									
Market	5	1.2×10^{5a}	ND	ND	7.1×10^{3a}	1.0×10^{1a}	ND	ND	ND
Nut seller	5	3.9×10^{6c}	8.2×10^{2a}	ND	4.2×10^{4b}	5.3×10^{2c}	3.1×10^{3a}	3.0×10^{1a}	5.0×10^{2b}
Peddler	8	2.9×10^{5b}	1.6×10^{3b}	ND	9.8×10^{3a}	1.2×10^{2b}	4.4×10^{3b}	ND	2.2×10^{2a}
Pumpkin seeds									
Market	5	9.6×10^{5c}	1.0×10^{2a}	ND	ND	ND	ND	ND	ND
Nut seller	6	9.4×10^{4b}	ND	ND	6.0×10^{1a}	1.0×10^{1a}	ND	ND	ND
Peddler	5	1.0×10^{4a}	ND	ND	1.5×10^{2b}	ND	ND	2.5×10^{2a}	ND
Sauced roasted chickpeas									
Market	5	2.5×10^{6c}	3.4×10^{2b}	7.0×10^{1b}	2.3×10^{2a}	ND	ND	ND	7.5×10^{2b}
Nut seller	5	2.2×10^{5b}	ND	ND	3.0×10^{2b}	6.0×10^{1a}	ND	ND	ND
Peddler	6	1.8×10^{4a}	3.0×10^{1a}	3.0×10^{1a}	3.1×10^{2b}	2.7×10^{2b}	3.0×10^{1a}	3.1×10^{2a}	1.1×10^{2a}
Mi×ed nuts									
Market	5	3.0×10^{5c}	ND	ND	1.0×10^{1a}	ND	ND	ND	4.0×10^{2b}
Nut seller	5	1.5×10^{4a}	ND	ND	6.4×10^{2c}	6.7×10^{2a}	5.0×10^{1a}	ND	1.0×10^{2a}
Peddler	6	4.6×10^{4b}	ND	ND	5.0×10^{2b}	1.0×10^{3b}	1.8×10^{2b}	ND	4.6×10^{2c}
Walnut									
Market	5	8.3×10^{4b}	4.7×10^{2b}	1.5×10^{2a}	1.0×10^{3a}	1.0×10^{1a}	ND	ND	ND
Nut seller	5	2.1×10^{4a}	1.6×10^{2a}	ND	7.5×10^{2a}	1.2×10^{3b}	4.0×10^{1b}	2.0×10^{1a}	ND
Peddler	5	3.2×10^{5c}	1.0×10^{3c}	ND	1.0×10^{4b}	4.0×10^{1a}	2.0×10^{1a}	ND	ND
Sauced roasted corn									
Market	5	2.1×10^{4b}	ND	ND	1.0×10^{2a}	3.7×10^{3c}	4.8×10^{2b}	ND	ND
Nut seller	7	3.7×10^{4c}	1.0×10^{1a}	ND	2.7×10^{2b}	7.1×10^{2b}	8.8×10^{2c}	1.0×10^{1a}	1.5×10^{2a}
Peddler	8	3.9×10^{3a}	2.0×10^{1b}	ND	5.0×10^{2c}	2.0×10^{1a}	2.0×10^{1a}	ND	2.8×10^{2b}
Total	217								

*TAMB: total aerobic mesophilic bacteria, ** ND: none detected, [a, b, c] Means within the same column with the different superscript letter are significantly different (p<0.05)

Evaluation of results of this study suggests that edible nuts might significant risk for public health. It was found that pathogen bacteria existed and it could be possible to find other pathogens, in edible nut samples. The applications that change according to people and sale places cause the selling of products which are not appropriate for the standards. The risk of the public health is important because of the protection of edible nut without packing and in inappropriate (heat and moist) conditions, touching by hand and unhygienic application by the sellers and the customers and the probability of microbial and physical contaminations from other resources.

In all the steps, starting from the harvest to transportation, processing, protection, sale and consuming, practicing Good Agriculture Practices (GAP),

Good Manufacturing Practices (GMP) and HACCP system are going to be effective to prevent the existence of probable dangers. Vacuum-packed products or the products that were packed in modified atmosphere as suitable for their origin of the product are thought to be prevented from microbial, physical and chemical contaminations and that they extend the shelf life of the products. Acquainting of the society the risks of these products and the conscious consumption will be benefit.

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