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Evaluating Contamination Level of Raw and Roasted Nuts Distributed in Commercial Markets in Mazandaran Province, Iran

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Abstract

BACKGROUND: Raw and roasted nuts are one of the most popular snacks consumed in Iran but mishandling and poor storage conditions and practices may lead to promote the growth of microorganisms and foodborne intoxications and infections.

OBJECTIVES: The purpose of this study was to investigate the microbial quality of raw and roasted (salted and unsalted) nuts, distributed in commercial markets in Mazandaran province, Iran.

METHODS: The moisture content, total colony counts, total coliform, fecal coliform, *Staphylococcus aureus*, and fungal contamination were evaluated according to procedures of Iran national standards.

RESULTS: The moisture content of raw and roasted hazelnut, pistachio, almond, and cashew nut samples were above the standard limits. The moisture content of the raw peanuts was within the standard approved limit (maximum 9%). But the moisture content of the roasted peanut was higher than the permissible limit of 5%. For yeasts and moulds, except for all almond samples and 56% of cashew nut samples, all products tended to be loaded with unsatisfactory limits. The total viable count was within the standard limits in hazelnut, almond, and cashew nut samples (less than 4log cfu/gr). Satisfactory limits of coliforms (lower than 1log cfu/gr) were observed in all almond samples, 91.6% of hazelnut, and 16.7% of pistachio samples. No fecal coliforms were detected in samples. *S.aureus* contamination was observed in 6.5% of hazelnut, 8% of almond, 25% of cashew nut, and 33% of peanut samples.

CONCLUSIONS: According to the findings, almond and hazelnut showed the least, and pistachio and peanut samples showed the highest microbial contamination.

KEYWORDS: Almond, Cashew nut, Hazelnut, Microbial load, Peanut, Pistachio

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Introduction

Nuts are generally consumed in many parts of the world directly as a snack with high nutritive value and desirable taste, accepted by almost everyone. Also, nuts are used in various parts of the food industry such as infant food, complementary food for athletes and kids, cooking, and extraction of oils is common for home and industrial uses. Types of nuts, hazelnut, pistachio, almonds, cashew nut, and peanuts, are rich in high-quality proteins, unsaturated good fats, minerals, and vitamins, and they have low water content; therefore, nuts are highly vulnerable to microbial spoilage especially fungal attack. Microorganisms may enter into the nuts ' shell while still on the trees and this usually occurs when the pods or hard shells of the nuts are split open and the seeds are attacked by insects or pests which make space for the microbial spores to access the developing seeds. Other possible ways of contamination of nuts by microorganisms include harvesting process, sorting practices, and washing of the nuts before storage. If the nuts are not properly treated during these stages, it could lead to bacterial and mould growth especially when nut seeds are not properly dried to the safe moisture content before storage or distribution (Adetunji et al., 2014). As mentioned above, both fungi and bacteria are responsible for microbial biodeterioration of food crops resulting in food and economic losses, hence reducing the consumer acceptance value of the food products. The fungal contamination occurs without necessarily showing any form of moldiness, moulds produce mycotoxins as secondary metabolites, which are consumed by humans and lead to different forms of diseases such as cancers, spontaneous abortion, cirrhosis, and other liver diseases, and also immune suppression interference with micronutrient metabolism and therefore stunted growth (Abbas et al., 2005; Luttfullah and Hussain, 2011; Ritter et al., 2011).

This research has focused on evaluating the microbiological quality and safety of different types of nuts and the effect of packaging type through analyzing the total colony counts, total coliform bacteria, fecal coliform bacteria, *Staphylococcus aureus* and fungal (yeasts and moulds) counts. The objective of the present study was to investigate the microbial quality of raw and roasted (salted and unsalted) nuts, distributed in commercial markets and consumed in Mazandaran province, Iran.

Materials and Methods

Sampling

The analyses of the bulk nut samples were conducted in four different sampling times (interval between successive samples, also called the sampling interval), for a total period of four months (April-July 2018). The sampling procedure was conducted according to sampling guidelines of the European Commission for nuts (in a shell and shelled or otherwise prepared). So, the random selection was done from different parts of the consignment, at least five conventional points (one at the top, three at different depths in the middle, and one at the bottom). The recommended minimum quantity for each final sample was 100-500 gr. In this study, the sample size was equivalent to 200 gr. For sample collection, five different types of bulk nuts (raw, roasted and salted, roasted and unsalted) including shelled hazelnut, unshelled pistachio, shelled almonds, cashew nut and shelled peanuts were obtained from local markets of different cities in Mazanda-

ran province (Iran). Samples were collected from four different markets in each city. The mixed samples were blended aseptically using a mixer grinder and 100 g portions kept in zip lock envelopes. Samples that were not used immediately were kept at −20 °C before analysis.

Chemicals

All were purchased from Sigma Chemical Co. (St. Louis, USA).

Moisture content

Moisture content was determined according to Iran national standard (2015) for dry fruits. Briefly, 5 gr of samples were oven-dried in a pre-weighed dish at 105 °C for 4 h and cooled in a desiccator until a constant weight was obtained. The moisture content was calculated from the reduction in weight and expressed as a percentage of the original weight.

Total viable count

Ten grams of each nut sample (shelled) were weighted in a sterilized stomacher bag and homogenized using a sterile physiological normal saline solution (0.85% NaCl), then serial dilutions were prepared. Pour plate method was applied for aerobic mesophilic bacteria count, using PCA (Plate Count Agar). PCA plates were incubated at 30 ºC for 48 h (Iran national standard, 2015).

Total coliform bacteria and fecal coliform bacteria

For total coliforms counts, the method described by Iran national standard (2007) was used. Growth of coliforms was detected using VRBA (Violet Red Bile Agar) incubated at 37 ºC for 48 h, and confirmation step was performed through subculturing of suspected colonies into BGLB (Brilliant Green Lactose Bile Broth) and evaluating gas production at 37 ºC for 48 h, and at 44 ºC for 24 h for fecal coliforms.

Staphylococcus aureus

To detect the presence of *S.aureus*, 0.2 ml of dilution 1 (first dilution) was spread onto an of Baird Parker Agar plate complemented with 5% of Egg Yolk Tellurite Emulsion. The plates were incubated at 37 ºC for 48 h, and then they were evaluated for the typical morphology of *S. aureus* colonies (Iran national standard, 2005).

Fungal contamination

One gram of each blended and well-mixed nut was added to 9 ml of sterile peptone water in a sterile test tube and the solution was diluted decimally. 20 ml of sterilized molten Potato Dextrose Agar (PDA) plates supplemented with 0.01% chloramphenicol were cooled to 45 °C and poured into plates (in triplicate) and 0.1 ml aliquots of each sample were added. The plates were gently swirled and allowed to solidify. The PDA plates were incubated at 25 °C for 5 days for the determination of fungal counts (Spangenbergi and Ingham, 2000).

Statistical analysis

Means and standard divisions of the microbial counts and moisture content were analyzed using SPSS 22.0 by the One-Sample t-test. Means were compared by the least significant difference (LSD) test when the P-value was <0.05.

Results

Different samples of nuts, including hazelnut, pistachio, almond, cashew nut, and peanut, collected from the local markets of Mazandaran province (Iran) during four months were analyzed. The samples included raw and roasted-salted and roasted-unsalted nuts. The moisture content of all the samples was measured and presented in Table 1. The moisture content of raw and roasted hazelnut, pistachio, almond, and cashew nut samples

was above the standard limits (5%, 5 and 3%, 5 and 4%, 4%, respectively) according to the Iran national standards. The moisture contents of the raw peanuts were within the permitted limits (max 9%) but that of roasted peanut samples was above the permissible recommended moisture limit of 5% (Iran national standard, 2015).

Table 1. Moisture content in different nut products

	Moisture content (%)				
	T_1*	T_2	T_3	T_4	Standard limit
Hazelnut					
Raw	**6.25±0.114[a]	6.38±0.713[a]	5.85±0.320[a]	6.41±0.328[a]	Max 5%
Roasted-salted	5.45±0.371[a]	4.02±0.836[b]	5.47±0.513[a]	6.25±0.914[a]	-
Roasted-unsalted	5.11±0.230[a]	5.5±0.937[a]	5.03±0.184[a]	5.95±0.320[a]	-
Pistachio					
Raw	7.23±0.922[a]	7.5±0.691[a]	6.92±0.515[a]	7.54±0.216[a]	Max 5%
Roasted-salted	6.45±0.560[a]	7.11±0.573[a]	7.05±0.384[a]	6.90±0.738[a]	Max 3%
Roasted-unsalted	6.70±0.732[a]	6.83±0.447[a]	6.55±0.821[a]	7.04±0.580[a]	Max 3%
Almonds					
Raw	8.50±0.673[a]	7.33±0.838[a]	7.15±0377[a]	8.60±0.511[a]	Max 5%
Roasted-salted	7.73±0.315[a]	6.15±0190[a]	5.90±0.834[a]	6.74±0.809[a]	Max 4%
Roasted-unsalted	7.50±0.528[a]	6.55±0.411[a]	5.17±0.610[a]	6.35±0.314[a]	Max 4%
Cashew nut					
Raw	8.25±0.451[a]	7.92±0.174[a]	8.5±0.116[a]	8.47±0.219[a]	-
Roasted-salted	7.10±0.603[a]	6.75±0.900[a]	6.68±0.450[b]	5.81±0.564[b]	Max 4%
Roasted-unsalted	5.05±0.380[b]	6.44±0.815[a]	7.28±0.315[a]	6.5±0.259[b]	Max 4%
Peanut					
Raw	7.65±0.825[a]	8.5±0.794[a]	8.13±0.620[a]	7.75±0.310[a]	Max 9%
Roasted-salted	6.80±0.254[a]	7.90±0.433[a]	7.15±0.340[a]	6.55±0.241[a]	Max 5%
Roasted-unsalted	6.47±0.318[a]	7.63±0.651[a]	7.84±0.895[a]	7.50±0.414[a]	Max 5%

* Time of sampling

** Data are presented as mean ± standard deviation

a Mean values with the same superscript along the same column are not significantly different ($P< 0.05$).

The whole samples were evaluated microbiologically to detect the levels of a total viable count, coliforms, fecal coliforms, *S. aureus*, yeasts, and moulds (Tables 2 and 3). For yeasts and moulds (Table 2), except all almond samples and 56% of cashew nut samples, all kinds of pistachio products ended up being loaded with unsatisfactory limits (>2.69logcfu/gr) (Iran national standard, 2014). There is still no microbiological permissible limit for hazelnut and peanut in Iran national standards but it is obvious that yeast and mould count in peanut samples is high.

Table 2. Fungal contamination of different nut products

	Yeast and Moulds (log cfu/g)				
	T_1*	T_2	T_3	T_4	Standard limit
Hazelnut					
Raw	2.30±0.518a**	2.51±0.445a	1.98±0.095a	2.07±0.359a	
Roasted-salted	1.81±0.655a	1.97±0.314a	2.05±0.141a	1.84±0.319a	-
Roasted-unsalted	2.14±0.430a	2.55±0.909a	2.23±0.550a	2.37±0.881a	
Pistachio					
Raw	4.85±0.672a	3.73±0.811a	3.72±0.850a	4.61±0.372a	
Roasted-salted	3.70±0.201a	4.05±0.935a	4.15±0.618a	3.80±0.195a	2.69
Roasted-unsalted	3.31±0.622a	3.68±0.412a	3.28±0.475a	4.51±0.262a	
Almonds					
Raw	1.33±0.217a	1.21±0.150a	1.55±0.091a	1.38±0.101a	
Roasted-salted	0.57±0.010a	0.90±0.013a	1.06±0.085a	1.05±0.067a	2
Roasted-unsalted	1.08±0.105a	1.14±0.208a	1.28±0.119a	0.95±0.035a	
Cashew nut					
Raw	1.05±0.118a	1.41±0.315a	2.36±0.175a	2.10±0.560a	
Roasted-salted	2.60±0.214a	1.58±0.220a	2.15±0.105a	1.21±0.137a	2
Roasted-unsalted	1.90±0.272a	2.11±0.508a	1.30±0.097a	2.4±0.715a	
Peanut					
Raw	4.50±0.335a	3.91±0.182a	4.58±0.350a	3.76±0.613a	
Roasted-salted	3.11±0.525a	2.85±0.166a	3.25±0.284a	3.55±0.117a	-
Roasted-unsalted	3.47±0.680a	3.05±0.485a	3.18±0.505a	3.45±0.282a	

* Time of sampling

** Data are presented as mean ± standard deviation

a Mean values with the same superscript along the same column are not significantly different ($P< 0.05$).

The total viable count was within the standard limits in hazelnut, almond, and cashew nut samples (less than 4log cfu/gr). Satisfactory limits of coliforms (lower than 1log cfu/gr) were observed in all almond samples, 91.6% of hazelnut and16.7% of pistachio samples (Table 3). No fecal coliforms were detected in samples (results are not shown). *S.aureus* contamination was found in 6.5% of hazelnut, 8% of almond, 25% of cashew nut, and 33% of peanut samples.

Table 3. Results of bacteriological contamination of different nut products

	Total viable count (log cfu/g)				Total coliform (log cfu/g)			
	T_1*	T_2	T_3	T_4	T_1	T_2	T_3	T_4
Hazelnut								
Raw	3.05±0.185a**	3.41±0.117a	3.38±0.179a	3.25±0.275a	1.65±0.095a	0.91±0.83a	1.86±۰,۱۷۲a	1.60±0.119a
Roasted-salted	3.89±0.446a	2.20±0.238a	3.50±0.233a	3.40±0.165a	1.13±0.092a	1.08±0.066a	1.75±0.105a	2.11±0.145a
Roasted-unsalted	3.55±0.475a	2.15±0.289a	3.85±0.171a	3.23±0.155a	1.40±0.085a	1.61±0.048a	0.80±0.055a	1.90±0.121a
Pistachio								
Raw	4.58±0.713a	5.27±0.671a	5.13±0.91a	4.66±0.715a	1.01±0.040a	2.16±0.151a	0.70±0.032a	2.11±0.156a
Roasted-salted	3.95±0.483b	3.81±0.115b	3.70±0.238a	3.98±0.585a	1.82±0.150a	1.58±0.075a	2.63±0.115a	1.91±0.083a
Roasted-unsalted	4.35±0.550a	4.05±0.205a	4.39±0.871a	5.13±0.820b	1.50±0.085a	1.44±0.056a	1.87±0.095a	0.26±0.009a
Almonds								
Raw	3.51±0.225a	3.90±0.426a	2.85±0.220a	3.08±0.172a	0.38±0.416a	0.90±0.075a	0.85±0.054a	0.49±0.010a
Roasted-salted	2.07±0.135a	1.72±0.085a	2.35±0.175a	2.75±0.215a	0.67±0.045a	0.81±0.060a	0.52±0.019a	0.19±0.015a
Roasted-unsalted	2.66±0.190a	2.81±0.115a	3.05±0.471a	3.40±0.385a	0.58±0.022a	0.74±0.038a	0.48±0.011a	0.97±0.082a
Cashew nut								
Raw	3.67±0.275a	3.11±0.455a	3.55±0.128a	4.41±0.317a	2.71±0.303a	2.18±0.145a	2.27±0.183a	2.66±0.131a
Roasted-salted	2.14±0.129a	2.51±0.167a	3.50±0.262a	3.49±0.232a	2.59±0.110a	2.50±0.265a	2.03±0.116a	2.05±0.090a
Roasted-unsalted	2.80±0.320a	3.07±0.285a	2.95±0.150a	3.16±0.155a	2.38±0.439a	2.15±0.106a	2.20±0.195a	2.75±0.118a
Peanut								
Raw	4.09±0.239a	4.57±0.360a	4.68±0.310a	4.91±0.412a	2.76±0.113a	3.25±0.140a	3.16±0.301a	2.81±0.115a
Roasted-salted	4.31±0.181a	4.89±0.172a	4.75±0.241a	4.06±0.163a	3.13±0.220a	3.28±0.211a	3.35±0.295a	2.57±0.200a
Roasted-unsalted	3.22±0.290a	4.07±0.158a	4.67±0.150a	4.15±0.201a	2.55±0.337a	2.92±0.127a	3.87±0.194a	3.80±0.146a
Standard limit	Max 4				Max 1			

* Time of sampling ** Data are presented as mean ± standard deviation a Mean values with the same superscript along the same column are not significantly different ($P< 0.05$).

Discussion

The moisture contents of all the raw and roasted nuts, except for raw peanut, were above the permissible recommended moisture limit. In comparison to our study, lower moisture content, 5.10-7.2%, and 6.48-7.05% were reported for peanuts from different states of Nigeria by Adetunji et al. (2018) and Oyedele et al. (2017), respectively. Also, the moisture content of our raw and roasted cashew nuts was higher than the results of Adebajo and Diyaolu (2003) who reported a range of moisture content of 4.1–6.8% and higher than the findings of Oluwafemi et al. (2009) who reported a low moisture content range of 3.7–4.3 and 3.2–5.4% for cashew nuts during the dry and raining seasons. Similar results to our work were found by Adetunji et al. (2018) who obtained 5.2-8.6% of moisture content for cashew nuts. There is no report on the moisture content of hazelnut, pistachio or almond. As nuts are hygroscopic materials, they absorb moisture from the surrounding atmosphere. The higher moisture content of the raw samples could be as a result of their raw nature as they have not yet been treated by any form of the drying process. The high moisture contents of the samples may also be as a result of inappropriate packaging, use of improper packaging compositions, and harvesting methods in the farms (Oladapo et al., 2014).

Nuts are vulnerable to fungal attacks at different stages of cultivation, harvesting, sorting, processing, and storage. If the nuts are not properly handled at these stages, it could result in mould development especially when they are not dried enough to be considered as the safe moisture level and stored under conditions suitable for mould growth such as high humidity and temperature (Abdulla, 2013; Adetunjiet et al., 2014). However, the total bacteria, total coliforms, and fungi load of the almond samples were less than the limits recommended in nuts by the Iran National Standard Commission on Specification for almond as 10^4cfu/gr, 10^1cfu/gr and10^2cfu/gr, respectively. As reported by Adetunji et al. (2018), total viable count of groundnut and cashew nut in Nigeria was 4.85 and 4.47 log cfu/gr, respectively, which is almost the same as our findings of peanut samples and a little higher than our results from cashew nut microbiological analysis. The lower counts of viable organisms in the almond, hazelnut and cashew nut samples possibly is due to the presence of amygdalin in almond and phenolic compounds in all these three nuts which exert inhibitory effects against bacteria, yeast, and fungi. Although the cashew nut is known to be a rich substrate for microbial growth, an outbreak of intoxication and infections through its consumption had rarely been reported. Also, Torquato et al. (2004) observed that the cashew nut contains the anacardic acid which is an inhibitor for microbial growth. Anacardic acid has lateral chains that are effective against the growth of *S. aureus*. Contaminated food handlers, surfaces, and types of equipment may result in staphylococcal infections that is one of the most important pathogens as a cause of foodborne intoxication. The results show *S.aureus* contamination was observed in all groups of nuts samples except the pistachio. It seems that the shell of pistachio decreases the probability of staphylococcal cross-contamination through handlers or surfaces. Adetunji et al. (2018) reported the presence of *S.aureus* in groundnuts and roasted cashew nuts in Nigeria (2 and 2.1 log cfu/gr). Also, Oluwafemi et al. (2009) announced high contamination of *S.*

aureus (> 4 logs cfu/gr) in Nigerian roasted cashew nuts.

As it is observed in the findings of the present work, almond and hazelnut showed the least and pistachio peanut samples the highest microbial contamination. Higher microbial counts of the pistachio and peanut samples than the recommended limit show poor handling practices of the products by the farmers, producers, and sellers. It is recommended that nut processors and handlers meet all the sanitary principles such as personal hygiene, avoid handling nuts in conditions such as sneezing, coughing and getting cold, flu, diarrhea, wounds and lesions, and following proper cleaning standard operation procedure (SOP) for the surfaces and equipment.

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Conflict of Interest

The authors declared that there is no conflict of interest.

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ارزیابی آلودگی در آجیل خام و بو داده توزیع شده در بازار مصرف استان مازندران (ایران)

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چکیده

زمینه مطالعه: آجیل های خام و بو داده یکی از محبوب ترین میان وعده های غذایی مورد مصرف در ایران هستند. اما شرایط و روش نگهداری نامناسب می تواند منجر به رشد میکروارگانیسم ها در آجیل و در نتیجه بروز مسمومیت و عفونت های غذایی در مصرف کننده شود.

هدف: هدف از مطالعه حاضر، بررسی کیفیت میکروبی آجیل خام و برشته شده (شور و بدون نمک)، توزیع شده در بازارهای تجاری استان مازندران (ایران) بود.

روش کار: میزان رطوبت، تعداد کل باکتری، کلی فرم کل، کلی فرم مدفوعی، استافیلوکوکوس اورئوس و آلودگی قارچی مطابق با روش های استاندارد ملی ایران مورد ارزیابی قرار گرفت.

نتایـــج: مقدار رطوبت محصولات فندق، پسته، بادام درختی و بادام هندی خام و برشته شده بالاتر از حد استاندارد بود. میزان رطوبت بادام زمینی خام در محدوده مجاز (حداکثر ۹٪) قرار داشت اما نمونه های بادام زمینی برشته شده دارای رطوبت بالاتر از حد توصیه شده (۵٪) بودند. در مورد مخمرها و کپک ها، به جز همه نمونه های بادام درختی و ۵۶٪ نمونه های بادام هندی، سایر نمونه‌ها دارای بار قارچی بیش از حد مجاز بودند. تعداد کل باکتری ها در نمونه‌های فندق، بادام درختی و بادام هندی در محدوده استاندارد (کمتر از ۴ log cfu/gr) بود. تعداد کلی فرم در همه نمونه های بادام، ۹۱/۶٪ و نمونه‌های فندق و ۱۶/۷٪ از نمونه های پسته در محدوده قابل قبول (کمتر از ۱ log cfu/gr) قرار داشت. هیچ کلی فرم مدفوعی در نمونه‌ها مشاهده نشد. آلودگی به استافیلوکوکوس اورئوس در ۶/۵٪ از نمونه های فندق، ۸٪ بادام درختی، ۲۵٪ بادام هندی و ۳۳٪ بادام زمینی شناسایی شد.

نتیجه گیری نهایی: مطابق یافته‌ها، بادام درختی و فندق کمترین و نمونه‌های بادام زمینی و پسته بیشترین میزان آلودگی میکروبی را نشان دادند.

واژه‌های کلیدی:

بادام درختی، بادام زمینی، بادام هندی، پسته، فندق، بار میکروبی.

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