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Research Article
Effects of Storage Periods and Temperature on Mold Prevalence and Aflatoxin Contamination in Nuts

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Abstract

Background and Objective: Nuts are highly exposed to fungi contamination in the field during transport or storage and this can result in the production of mycotoxins. The aim of this study was to determine the prevalence and level of aflatoxins (AFB1, AFB2, AFG1 and AFG2), the mold and yeast counts in pistachios, cashews, walnuts, almonds and hazelnuts and the effect of different storage conditions (temperature and storage period) on aflatoxin formation and mold and yeast counts. **Materials and Methods:** One hundred and twenty-five samples of dehulled raw nuts (pistachio, cashew, walnut, almond and hazelnut) were purchased randomly from retail shops in the Kingdom of Saudi Arabia. The samples were first analyzed to obtain an estimation of the aflatoxin levels, mold and yeast counts prior to storage. The nut samples were then stored for 3 and 6 months at three temperatures: Room temperature (25°C), 45°C and under refrigeration (4°C). At the end of the storage periods, the samples were again analyzed for aflatoxin content (AFB1, AFB2, AFG1 and AFG2) and mold and yeast counts. **Results:** All of the nuts in the study were found to have safe aflatoxin levels before storage according to the European Union, Iranian and Australian/New Zealand food standard codes (<15 µg kg^{-1}). The aflatoxin levels rose above safe limits in all of the experimental nuts stored for 3 or 6 months at room temperature (25°C) and 45°C. However, storing the experimental nuts under refrigeration (4°C) allowed the pistachio, cashew and walnut aflatoxin levels to remain within the safe limit for up to 6 months. Both the almond and hazelnut aflatoxin levels exceeded the standards by 3 months of storage at 4°C and could not be considered safe. **Conclusion:** Storing nuts at a low temperature (refrigeration) reduces aflatoxin levels and mold and yeast counts for 3-6 months.

Key words: Aflatoxin, nuts, storage temperature, mold, yeast

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Aflatoxin contamination is an important problem for food safety and international trade. Aflatoxins, produced by *Aspergillus flavus*, *Aspergillus parasiticus* and other *Aspergillus* species, are secondary metabolites with carcinogenic, estrogenic, immunosuppressive and teratogenic effects in humans and farm animals[1-3]. Eighteen types of aflatoxins have been identified, but the naturally occurring and well-known forms are AFB1, AFB2, AFG1 and AFG2, which are closely associated with agricultural environments and crops, including nuts. Of these, AFB1 is the most frequent and most toxic metabolite found in contaminated nuts[4,5].

Nuts can be contaminated by fungi during growth, harvesting and storage under various climatic conditions and agricultural and storage practices, especially during storage in which adverse temperature and relative humidity conditions are present, which are conducive to fungal growth and toxin production[6,7]. Aflatoxin levels increase under storage conditions such as excessive heat, high humidity, lack of aeration and insect and rodent damage; those are common conditions in the tropics that aggravate toxin accumulation[8,9]. The most important factors influencing the development of fungi during nut storage are the storage temperature, moisture content and presence of oxygen[10].

The low aflatoxin threshold levels for major importing nations in the EU and Japan have significantly increased the probability of tree nut shipments being rejected. Therefore, to minimize food safety concerns and for purely economic reasons, the aflatoxin risk needs to be assessed and preventive measures implemented[11]. Limits vary according to the commodity but range from 2-12 $\mu g\ kg^{-1}$ for AFB1 and from 4-15 $\mu g\ kg^{-1}$ for total aflatoxins[12,13]. The Iranian and Australian/New Zealand food standard codes also set a maximum limit of 15 $\mu g\ kg^{-1}$ for aflatoxins in nuts[14]. The economic losses resulting from nuts contaminated with fungi and aflatoxin are difficult to estimate. These losses consist of direct nut losses, human illness and reduced productivity and livestock losses from deaths and slower growth rates. Additional economic losses include the indirect costs of implementing various systems to control mycotoxins in the nuts, the reduced value of the rejected nuts, the costs of detoxification to recover acceptable products and occasionally, the loss of export markets[15].

The aim of this study was to determine the prevalence and level of aflatoxins (AFB1, AFB2, AFG1 and AFG2) and the mold and yeast counts in pistachios, cashews, walnuts, almonds and hazelnuts, which are the most common consumed nuts in Saudi Arabia. The effects of different storage conditions (temperature and storage period) on aflatoxin formation and mold and yeast counts were determined. In addition, the total aflatoxin levels were compared with the maximum limits in EU, Iranian and Australian/New Zealand food standards (15 $\mu g\ kg^{-1}$) to determine their safety profile.

MATERIALS AND METHODS

Experimental protocol: One hundred and twenty-five samples of dehulled raw nuts (pistachios, cashews, walnuts, almonds and hazelnuts) were purchased randomly from retail shops in the Kingdom of Saudi Arabia. The 200 g samples were placed in Zip-Pak containers and stored at -20°C until analysis. The samples were first analyzed prior to placement in the experimental storage conditions to obtain a baseline estimate of the initial aflatoxin levels and mold and yeast counts. The nut samples were then stored for 3 or 6 months at three different temperatures: 25°C (room temperature), 45°C and 4°C (refrigeration). At the end of each storage period, the samples were analyzed for their total and individual aflatoxin levels (AFB1, AFB2, AFG1 and AFG2) and their mold and yeast counts.

The microbiological examinations to determine the mold and yeast counts were performed according to the International Commission on Microbiological Specifications for Foods[16]. The individual aflatoxin levels were estimated with the RIDASCREEN® aflatoxin total (R4701) and RIDASCREEN® Aflatoxin B1 30/15 (R1211) test kits in combination with a RIDA® aflatoxin column (R5001/5002) following the manufacturer instructions (R-Biopharma, Darmstadt, Germany) and according to the procedures described by Anonymous[17-19] and Senyuva and Gilbert[20]. All nut samples were analyzed three times.

Precautions while in contact with aflatoxins: Precautions were taken by wearing suitable protective clothing (laboratory coat, mask and gloves) during all procedures. Aflatoxins were deactivated by autoclaving in the presence of ammonium and by treatment with hypochlorite[13,21]. Therefore, all laboratory instruments were pre-washed with 10% sodium hypochlorite before cleaning or discarding after use and laboratory surfaces were cleaned with 1% sodium hypochlorite.

Statistical analysis: Statistical analysis was performed with a one-way analysis of variance (ANOVA) with a significance level of $p<0.05$. Average values were expressed as the Mean±SD according to Snedecor and Cochran[22].

RESULTS AND DISCUSSION

The baseline aflatoxin contamination levels in the experimental nuts are shown in Table 1 and Fig. 1. The aflatoxin AFB1 levels ranged from 1.11±0.45 to 2.18±0.24 μg kg^{-1}, with cashews having the lowest level and walnuts the highest. The AFB2 levels ranged from 1.04±0.23 to 1.76±0.40 μg kg^{-1}, with walnuts having the lowest level and pistachios the highest. This was also true of the AFG1 levels, which ranged from 1.17±0.23 μg kg^{-1} (walnuts) to 1.85±0.35 μg kg^{-1} (pistachios). There were no significant differences in the AFB1, AFB2 and AFG1 levels among the nuts studied. The lowest AFG2 level was found in the cashews (1.20±0.32 μg kg^{-1}), with the highest levels found in the hazelnuts and almonds (2.53±0.42 and 2.06±0.40 μg kg^{-1}, respectively). The mold and yeast counts ranged from 3.0×10^3 CFU g^{-1} in the pistachios and cashews to 4.0×10^3 CFU g^{-1} in the walnuts, almonds and hazelnuts. Figure 1 shows that the total aflatoxin levels in the nuts prior to storage were considered safe under the EU, Iranian and Australian/New Zealand food standard codes, which set limits of 15 μg kg^{-1} for the total aflatoxin (AFB1, AFB2, AFG1 and AFG2) levels permitted in nuts.

Table 1: Mean aflatoxin levels±SD and mold/yeast counts for the nuts prior to storage

	Variables				
	Nuts before storage (zero time)				
	Aflatoxins (μg kg^{-1})				
Samples	AFB1	AFB2	AFG1	AFG2	Mold/yeast count (CFU g^{-1})
Pistachios	2.10±0.23^a	1.76±0.40^a	1.85±0.35^a	1.44±0.33^b	3×10^3
Cashews	1.11±0.45^b	1.27±0.31^a	1.45±0.35^a	1.20±0.32^{bc}	3×10^3
Walnuts	2.18±0.24^a	1.04±0.23^a	1.17±0.23^a	1.63±0.34^{ab}	4×10^3
Almonds	2.05±0.31^a	1.23±0.33^a	1.33±0.24^a	2.06±0.40^a	4×10^3
Hazelnuts	2.04±0.42^a	1.35±0.21^a	1.19±0.31^a	2.53±0.42^a	4×10^3

Mean values in each column with different superscripts (a, b, c, d) differ significantly

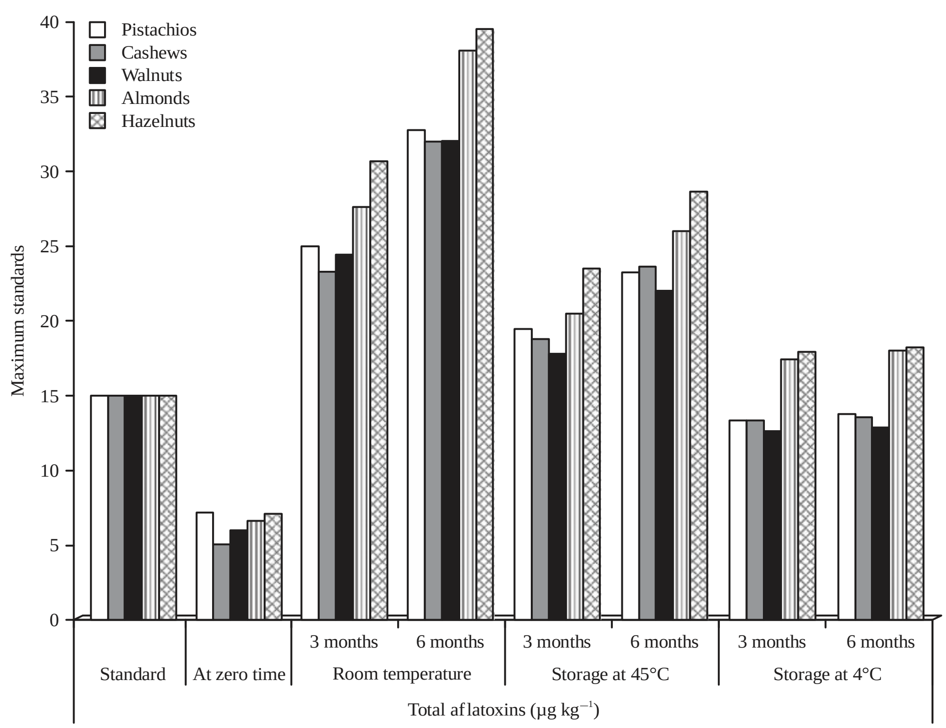

Fig. 1: Total aflatoxins in the pistachios, cashews, walnuts, almonds and hazelnuts after different storage periods at different temperatures in comparison with the initial levels and the maximum standards

Nut contamination and toxin production are particularly likely to occur in subsistence farming communities in tropical and sub-tropical regions with high temperatures and humidity, as well as in certain crop genotypes, soil types, minimum and maximum daily temperatures and daily net evaporation. Moreover, aflatoxin contamination can be promoted by stress or damage to the crop due to events such as drought before harvest, insect activity, poor harvest timing, heavy rains during and after harvest and inadequate drying of the crop before storage[5]. The moisture content of the plants is an important factor in determining the growth of *Aspergillus* species and their toxin production. The optimum conditions for aflatoxin growth are temperatures between 25 and 32°C, moisture contents between 12 and 16% and a relative humidity of 85%. Aflatoxins are known carcinogens with toxic metabolites, with AFB1 being the most frequently occurring and most toxic metabolite found in contaminated nuts and nut product samples[5,23].

The results of the current study agree with an investigation in Turkey that reported detecting AFB1 contamination in 43 samples (84.32%) of dehulled hazelnut samples, with levels ranging from <1-10 ppb[24]. In contrast, an investigation in Malaysia found the highest level of AFB1 incidence was in raw groundnut without the shell, having a total concentration of 711 ppb, which is higher than the results found in this study. It was also reported that walnuts had the lowest AFB1 contamination levels, while in current study, cashews had the lowest levels[13].

Table 2 shows the effects of storing the experimental nuts at room temperature (25°C) for 3 months. The AFB1 levels ranged from 5.65±0.66 (cashews) to 8.21±0.71 μg kg^{-1} (hazelnuts). The lowest AFB2 levels were found in the almonds (5.55±0.54 μg kg^{-1}) and cashews (5.59±0.57 μg kg^{-1}), while the highest levels were found in the walnuts (6.68±0.42 μg kg^{-1}). Conversely, the lowest AFG1 levels were in the walnuts (5.57±0.003 μg kg^{-1}) and cashews (5.75±0.44 μg kg^{-1}), while it was the hazelnuts that had the highest levels (7.86±0.54 μg kg^{-1}). Finally, the lowest AFG2 levels were in the cashews (6.33±0.33 μg kg^{-1}) and walnuts (6.44±0.005 μg kg^{-1}), while the highest levels were found in the hazelnuts (8.27±0.81 μg kg^{-1}).

The mold and yeast counts in the nuts ranged from 4.0×10^4 to 7.0×10^4 CFU g^{-1} after storage for 3 months at room temperature (25°C). The total aflatoxin levels in the nuts were no longer considered safe after 3 months of storage at room temperature according to the EU, Iranian and Australian/New Zealand food standard codes for nuts (Fig. 1).

The major factors impacting fungal growth during nut storage are high water content, temperature and air humidity higher than 70%[25]. These data are in line with Pacheco and Scussel's[26] results. The slight increase in the total fungi count after storing the nuts at room temperature may be due to a slight increase in the moisture content of the dried nuts. Rosso and Robinson[27] reported that the optimum conditions for growth and toxin production in aflatoxin-producing mold species such as *Aspergillus nomius* and *Aspergillus flavus* were a temperature of 30°C and a relative humidity of 97%.

Table 3 presents the aflatoxin contamination levels in the experimental nuts after 6 months of storage at room temperature (25°C). The raw walnuts and cashews had the lowest AFB1 levels (7.46±0.55 and 7.76±0.43 μg kg^{-1}, respectively), while the hazelnuts had the highest (10.35±0.61 μg kg^{-1}). The pistachios and cashews had the lowest AFB2 levels, 7.68±0.44 and 7.78±0.45 μg kg^{-1}, respectively. The lower AFG1 and AFG2 levels were in the pistachios, cashews and walnuts, while the almonds (9.83±0.56 and 9.95±0.63, respectively) and hazelnuts (9.99±0.66 and 10.58±0.82, respectively) had higher levels. The mold and yeast counts ranged from 6×10^4 to 8×10^4 CFU g^{-1}. As seen in Fig. 1, the total aflatoxin levels in the nuts after 6 months of storage at room temperature no longer met the safety standards set by the EU, Iranian and Australian/New Zealand food standard codes.

Table 2: Mean aflatoxin levels±SD and mold/yeast counts in the nuts after 3 months of storage at room temperature (25°C)

	Variables				
	Nuts stored for 3 months at 25°C				
	Aflatoxins (μg kg^{-1})				
Samples	AFB1	AFB2	AFG1	AFG2	Mold/yeast count (CFU g^{-1})
Pistachios	6.52±0.62^{c}	5.93±0.68^{ab}	5.94±0.73^{b}	6.66±0.63^{b}	6×10^4
Cashews	5.65±0.66^{cd}	5.59±0.57^{b}	5.75±0.44^{bc}	6.33±0.33^{b}	4×10^4
Walnuts	5.80±0.32^{d}	6.68±0.42^{a}	5.57±0.003^{c}	6.44±0.005^{b}	5×10^4
Almonds	7.26±0.64^{ab}	5.55±0.54^{b}	6.95±0.55^{ab}	7.93±0.89^{ab}	6×10^4
Hazelnuts	8.21±0.71^{a}	6.38±0.61^{a}	7.86±0.54^{a}	8.27±0.81^{a}	7×10^4

Mean values in each row with different superscripts (a, b, c, d) differ significantly

Table 3: Mean aflatoxin levels±SD and mold/yeast counts in the nuts after 6 months of storage at room temperature (25°C)

	Variables				
	Nuts stored for 6 months at 25°C				
	Aflatoxins (µg kg^{-1})				
Samples	AFB1	AFB2	AFG1	AFG2	Mold/yeast count (CFU g^{-1})
Pistachios	8.65 ± 0.38^{b}	7.68 ± 0.44^{b}	7.72 ± 0.63^{b}	8.71 ± 0.66^{c}	7×10^{4}
Cashews	7.76 ± 0.43^{bc}	7.78 ± 0.45^{b}	7.81 ± 0.55^{b}	8.65 ± 0.67^{c}	6×10^{4}
Walnuts	7.46 ± 0.55^{bc}	8.35 ± 0.44^{ab}	7.85 ± 0.54^{b}	8.40 ± 0.71^{bc}	8×10^{4}
Almonds	9.38 ± 0.53^{ab}	8.88 ± 0.47^{a}	9.83 ± 0.56^{a}	9.95 ± 0.63^{ab}	7×10^{4}
Hazelnuts	10.35 ± 0.61^{a}	8.56 ± 0.51^{a}	9.99 ± 0.66^{a}	10.58 ± 0.82^{a}	8×10^{4}

Mean values in each column with different superscripts (a, b, c, d) differ significantly

Table 4: Mean aflatoxin levels±SD and mold/yeast counts in the nuts after 3 months of storage at 45°C

	Variables				
	Nuts stored for 3 months at 45°C				
	Aflatoxins (µg kg^{-1})				
Samples	AFB1	AFB2	AFG1	AFG2	Mold/yeast count (CFU g^{-1})
Pistachios	4.49 ± 0.58^{c}	4.92 ± 0.41^{b}	4.68 ± 0.44^{b}	5.45 ± 0.52^{a}	7×10^{4}
Cashews	4.73 ± 0.32^{c}	4.88 ± 0.64^{b}	4.46 ± 0.32^{b}	4.76 ± 0.51^{b}	5×10^{4}
Walnuts	3.96 ± 0.14^{cd}	4.14 ± 0.61^{b}	4.86 ± 0.50^{b}	4.88 ± 0.70^{b}	7×10^{4}
Almonds	5.75 ± 0.60^{ab}	4.41 ± 0.31^{b}	4.90 ± 0.62^{b}	5.47 ± 0.60^{a}	6×10^{4}
Hazelnuts	6.58 ± 0.43^{a}	5.76 ± 0.55^{a}	5.61 ± 0.90^{a}	5.59 ± 0.52^{a}	7×10^{4}

Mean values in each column with different superscripts (a, b, c, d) differ significantly

These results are in line with those of Liu *et al.*[28], who reported that the *Aspergillus* fungus is essentially a part of the grain storage flora. This fungus grows optimally at 25°C with a minimum water activity of 0.75. While it begins producing secondary metabolites at 10-12°C, the most toxic ones are produced at 25°C. The storage of crops in hot and humid conditions, particularly prolonged storage, promotes the growth of aflatoxin-producing fungi and an accumulation of toxins. The AFB1, is a toxin most frequently found in contaminated human foods, is the most potent aflatoxin and has the highest hepatocarcinogenic potential[29]. The results of the current study were also in agreement with a previous study conducted by Lee *et al.*[30]. Although consumers store nuts at different temperatures, ranging from -18°C to over 38°C depending on the storage period, the most common practice is to store nuts at room temperature for 2-4 weeks.

Table 4 shows the effect on aflatoxin levels and mold and yeast counts after 3 months of storage at 45°C. The AFB1 levels ranged from 3.96 ± 0.141 µg kg^{-1} in the walnuts to 6.58 ± 0.43 µg kg^{-1} in the hazelnuts. The pistachios, cashews, walnuts and almonds had the lower AFB2 levels, with walnuts having the least (4.14 ± 0.6 µg kg^{-1}), while the highest levels were found in the hazelnuts (5.76 ± 0.55 µg kg^{-1}). Similarly, the lowest AFG1 levels were found in the pistachios, cashews, walnuts and almonds, with cashews having the least (4.46 ± 0.32 µg kg^{-1}), while the hazelnuts again had the highest levels (5.61 ± 0.90 µg kg^{-1}).

The lowest AFG2 levels were found in the cashews (4.76 ± 0.51 µg kg^{-1}) and walnuts (4.88 ± 0.70 µg kg^{-1}), with pistachios, almonds and hazelnut having the higher levels. The mold and yeast counts ranged from 5.0×10^{4} to 7.0×10^{4} CFU g^{-1}. After 3 months of storage at 45°C, none of the nuts could be confirmed as safe according to the EU, Iranian and Australian/New Zealand food standard codes (Fig. 1).

These data agree with those reported by Ozay *et al.*[31], who found that high temperatures, humid conditions and extreme temperature gradients during storage and transport increase the susceptibility of nuts to spoilage. Spoilage can occur whether the nuts are raw or previously treated by thermal or non-thermal processes. The Codex Alimentarius Commission[14] mentions the opportunity for moisture migration if storage temperatures and humidity are not held constant or if storage containers are exposed to sunlight, with these conditions leading to higher water activity levels that can result in mold growth and spoilage. Pacheco and Scussel[26] reported that the moisture content and total fungi counts were high in raw Brazil nuts being sent to the factory, with a mean water content of 22.4% (range: 15.4-31.6%) and mean total fungi count of 11.5×10^{2} CFU g^{-1}. Aflatoxin

Table 5: Mean aflatoxin levels±SD and mold/yeast counts in the nuts after 6 months of storage at 45°C

	Variables				
	Nuts stored for 6 months at 45°C				
	Aflatoxins (µg kg^{-1})				
Samples	AFB1	AFB2	AFG1	AFG2	Mold/yeast count (CFU g^{-1})
Pistachio	5.65±0.50^{c}	5.69±0.70^{b}	5.71±0.60^{c}	6.20±0.63^{ab}	7×10^{5}
Cashew	5.95±0.64^{c}	5.77±0.90^{b}	5.96±0.44^{bc}	5.98±0.70^{b}	7×10^{5}
Walnut	4.80±0.61^{cd}	5.63±0.50^{bc}	5.71±0.42^{c}	5.93±0.52^{b}	8×10^{5}
Almonds	6.78±0.70^{b}	5.60±0.70^{b}	6.85±0.62^{ab}	6.79±0.75^{ab}	7×10^{5}
Hazelnut	7.82±0.63^{a}	6.51±0.80^{a}	7.19±0.83^{a}	7.08±0.77^{a}	8×10^{5}

Mean values in each column with different superscripts (a, b, c, d) differ significantly

Table 6: Mean aflatoxin levels±SD and mold/yeast counts in the nuts after 3 months of storage under refrigeration (4°C)

	Variables				
	Nuts stored for 3 months at 4°C				
	Aflatoxins (µg kg^{-1})				
Samples	AFB1	AFB2	AFG1	AFG2	Mold/yeast count (CFU g^{-1})
Pistachios	3.58±0.30^{c}	2.96±0.50^{b}	2.99±0.43^{b}	3.94±0.50^{b}	6×10^{3}
Cashews	3.87±0.55^{c}	2.85±0.43^{b}	3.95±0.44^{a}	2.82±0.40^{c}	5×10^{3}
Walnuts	2.88±0.44^{d}	2.95±0.50^{b}	2.96±0.42^{b}	3.99±0.51^{b}	5×10^{3}
Almonds	4.96±0.57^{b}	3.99±0.66^{a}	3.82±0.45^{a}	4.77±0.55^{a}	7×10^{3}
Hazelnuts	5.66±0.66^{a}	3.68±0.55^{a}	3.77±0.59^{a}	4.88±0.55^{a}	6×10^{3}

Mean values in each column with different superscripts (a, b, c, d) differ significantly

contamination was detected in 4 out of the 16 samples (25%) collected at this stage, with levels ranging from as low as 4.8 µg kg^{-1} to as high as 19.2 µg kg^{-1}. The high moisture content of the nuts suggested that environmental factors such as the high temperature and humidity of the extraction site (forest) and the river during transport could have led to the fungal proliferation and aflatoxin formation.

The mean aflatoxin levels and mold and yeast counts in the experimental nuts after 6 months of storage at 45°C are shown in Table 5 and Fig. 1. The raw walnuts had the lowest levels of AFB1 (4.80±0.61 µg kg^{-1}), while the pistachios, cashews, walnuts and almonds had the lower AFB2 levels (range: 5.60-5.77 µg kg^{-1}). The pistachios and walnuts had the lower AFG1 levels (5.71±0.60 and 5.71±0.42 µg kg^{-1}, respectively), while the cashews and walnuts had the lower AFG2 levels (5.98±0.70 and 5.93±0.52 µg kg^{-1}, respectively). The hazelnuts had the highest levels of all four aflatoxins tested (AFB1, AFB2, AFG1 and AFG2). The mold and yeast counts ranged from 7×10^{5} to 8×10^{5} CFU g^{-1}. After 6 months of storage at 45°C, none of the nuts could be confirmed as safe according to the EU, Iranian and Australian/New Zealand food standards for acceptable total aflatoxin levels (Fig. 1).

These results agree with those of Kaaya and Kyamuhangire[8], Gurses[32], Paramawati *et al.*[33] and Waliyar *et al.*[34]. They contrast with the results of Pacheco and Scussel[26], who reported that a drying process in a rotary heater at temperatures above 70°C (70-102°C) for 48 h achieved an average moisture content of 4.7% and a low total fungi count of only 0.28×10^{2} CFU g^{-1}. As far as the aflatoxins are concerned, only two (12.5%) of the samples in this study tested positive, each having levels of 4.8 µg kg^{-1}. During storage, the moisture content increased to 7.9%, which still safely reduces fungi proliferation.

The statistical analysis shown in Table 6 demonstrates the effect of 3 months of storage at 4°C on aflatoxin levels and mold and yeast counts in the experimental nuts. Pistachios had low AFB2 and AFG1 levels (2.96±0.50 and 2.99±0.43 µg kg^{-1}, respectively), while cashews had low AFB2 and AFG2 levels (2.85±0.43 and 2.82±0.40 µg kg^{-1}, respectively). The walnuts had low AFB1, AFB2 and AFG1 levels (2.88±0.44, 2.95±0.50 and 2.96±0.42 µg kg^{-1}, respectively). Almonds had high AFB2, AFG1 and AFG2 levels, while hazelnuts had high levels of all four aflatoxins measured. The mold and yeast counts ranged between 5×10^{3} and 7×10^{3} CFU g^{-1}. The pistachios, cashews and walnuts met the food safety standards of the EU, Iran and Australia/New Zealand after 3 months of storage at 4°C, but the total aflatoxin levels in the almonds and hazelnuts were too high to be deemed safe, as seen in Fig. 1.

Table 7: Mean aflatoxin levels±SD and mold/yeast counts in the nuts after 6 months of storage under refrigeration (4°C)

	Variables				
	Nuts stored for 6 months at 4°C				
	Aflatoxins (µg kg^{-1})				
Samples	AFB1	AFB2	AFG1	AFG2	Mold/yeast count (CFU g^{-1})
Pistachios	3.95 ± 0.60^{b}	2.99 ± 0.48^{c}	2.98 ± 0.54^{b}	3.99 ± 0.65^{b}	6×10^{4}
Cashews	3.98 ± 0.64^{b}	2.79 ± 0.47c	3.98 ± 0.44^{a}	2.93 ± 0.64^{c}	5×10^{4}
Walnuts	2.98 ± 0.56^{c}	2.97 ± 0.57^{c}	2.99 ± 0.51^{b}	4.09 ± 0.65^{ab}	6×10^{4}
Almonds	5.11 ± 0.60^{a}	4.12 ± 0.76^{a}	3.97 ± 0.53^{a}	4.90 ± 0.64^{a}	6×10^{4}
Hazelnuts	5.49 ± 0.59^{a}	3.95 ± 0.61^{ab}	3.89 ± 0.64^{a}	4.98 ± 0.63^{a}	6×10^{4}

Mean values in each column with different superscripts (a, b, c, d) differ significantly

These results were in accordance with those of Paramawati *et al.*[33], who mentioned that aflatoxin formation in nuts is dependent on such conditions as the number of fungal species contaminating the nuts, the relative humidity, storage conditions and hygienic factors. When mold is present, the interaction between water activity and temperature is the most important determinant of fungal growth and aflatoxin production. Moreover, The ICMSF[16] reported that refrigerated storage is adequate for most foods including nuts because it can provide protection against recontamination or prevent the growth of pathogenic bacteria and fungal toxin production. The humidity can be increased either by evaporating more water, reducing the temperature, or a combination thereof. Lower temperatures and increased humidity can lower the multiplication of fungi and thus, the aflatoxin production. Danyluk *et al.*[35] found that consumers usually store nuts at room temperature for less than 6 months or in the freezer for longer storage.

The statistical analysis in Table 7 shows the effect of 6 months of storage at 4°C on the aflatoxin levels and mold and yeast counts in the experimental nuts. Pistachios had low AFB2 and AFG1 levels (2.99 ± 0.48 and 2.98 ± 0.54 µg kg^{-1}, respectively), while cashews had low AFB2 and AFG2 levels (2.79 ± 0.47 and 2.93 ± 0.64 µg kg^{-1}, respectively). Walnuts had low levels of AFB1, AFB2 and AFG1 (range: 2.97-2.99 µg kg^{-1}), while the almonds and hazelnuts had high levels of all tested aflatoxins. The mold and yeast counts in the nuts ranged from 5×10^{3} to 6×10^{3} CFU g^{-1}. After 6 months of storage at 4°C, the aflatoxin levels in the pistachios, cashews and walnuts remained within the safe limits set by the EU, Iranian and Australian/New Zealand food standard codes, but the aflatoxin levels in the almonds and hazelnuts were too high to be considered safe (Fig. 1).

This finding is in line with the results obtained by Rahmianna *et al.*[36], Lee *et al.*[30] and Kimatu *et al.*[37], who reported that the length of storage time of nuts significantly affects the physical damage they incur, with the number of damaged and shriveled seeds increasing with increased time in storage. Senesi *et al.*[38] reported that the kernels of peeled almonds could be stored for up to 9 months without a serious loss of quality when packaged in high barrier packaging, regardless of the storage temperature (4°C or ambient). However, for longer storage times of more than 9 months, refrigeration (4°C) and storing of the nuts under nitrogen and within a metalized film was used. Abdulkadar *et al.*[39] and Cheraghali *et al.* [40] analyzed various nuts marketed in Saudi Arabia and Qatar, respectively and found that none of the in-shell or shelled almond samples were contaminated. All pistachio-producing countries (Iran, USA and Turkey, among others) face an aflatoxin contamination problem. Surveys and monitoring programs performed in several countries have attempted to discern a general pattern regarding the extent of aflatoxin contamination in dried nuts, with pistachios routinely showing the highest incidence of contamination Abdulkadar *et al.*[39], Juan *et al.*[41] and Luttfullah and Hussain[42].

CONCLUSION AND FUTURE RECOMMENDATIONS

This study showed that storing nuts at low temperature (refrigeration) can be beneficial for reducing the presence of aflatoxins and the mold and yeast counts for 3-6 months, allowing the total aflatoxin levels in the samples to remain below the permissible limits of the EU, Iranian and Australian/New Zealand food standard codes, which have set a maximum aflatoxin limit of 15 µg kg^{-1} for nuts.

The results of the present study can be used to guide and educate consumers on the risks associated with nut consumption should they become contaminated. Based on this evaluation of aflatoxins levels and mold and yeast counts, nut quality depends on both the storage period (3 or 6 months) and the temperature at which they are stored. The rates of aflatoxin contamination in different nuts should not be neglected, as they can negatively affect various health factors. It can be predicted that as the duration of sample storage increases, aflatoxin contamination levels will also increase. Further research must be undertaken systematically and to continue to monitor aflatoxins in nuts. Proper inspections in retail markets are necessary to control the mycotoxin levels in nuts and consumers should be made increasingly aware of aflatoxins as a pathogen source as well as the storage conditions required for maintaining the safety of nuts.

SIGNIFICANCE STATEMENT

This study will aid researchers in uncovering the critical parameters affecting aflatoxins production in other consumed nuts that have not yet been explored. Thus, a new theory on the effects of these storage periods and temperatures may be formed.

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