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ORIGINAL ARTICLE

The Effect of Various Shelf Life and Conditions on Storage of Walnuts

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ABSTRACT

Background: Walnut production and consumption has shown a steady rise in both amount and value in recent years. According to the seasonal production of walnuts, the storage condition plays a great role in its quality.

Methods: In this cross-sectional Study, effects of storage temperature and removing hard peels of walnut were studied on the shelf life of walnut over 6 months. The aflatoxin levels, lipid oxidation, antioxidant activity, and sensory evaluation were measured every 16 days (d).

Results: The aflatoxin reduction was statistically significant in all stored walnut samples kept in the fridge, freezer, and their hard shell at room temperature after 151 days. The highest lipid oxidation and the lowest antioxidant activity were observed at the 6th month in walnuts preserved at room temperature. Through the sensory evaluation and chemical tests, walnuts preserved at fridge had the highest acceptance among panelist and least chemical changes after 6 months.

Conclusion: The best storage condition for a long time is keeping peeled walnuts in the fridge.

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Introduction

Based on the report by Food and Agriculture Organization of the United Nations (FAOSTAT), the global production of walnuts with shell in 2018 has been 366.25 t/ha, while more than 11% of this production referred to Iran (40.96 t/ha). Walnuts are used in ground or shelled whole kernels being utilized as ingredients of several foodstuffs including confectionery and bakery products, as well as flavoring agents in ice cream and beverages. Walnuts have a lipid content of 60%, protein of 24%, carbohydrates of 12-16%, cellulose of 1.5-2% and finally minerals of 1.7-2% (1).

Walnuts are famous due to their content of high polyunsaturated fatty acids (PUFA) (around 70% of total lipid), while these high levels of PUFAs make walnuts prone to oxidation too. Most of fatty acids noted in walnuts are linoleic alfa linolenic (8.0-15.4%), oleic (13.8-33.0%) and (49.3-62.3%) (2). The high PUFA content confines the shelf life of walnuts based on their susceptibility to oxidation activities. Lipid oxidation is the most prominent quality variable that during storage time can reduce the economic value of walnuts. Oxidation leads to an undesirable rancid taste that is responsible for unacceptable taste of walnuts for the consumers (3).

In this regard, environmental parameters such as storage temperature, oxygen concentration, light, and relative humidity can interfere with lipid oxidation (4). The common method of preserving walnuts is to de-husk and dry the fruit following harvesting and store as in-shell walnuts until consumption, which might takes a year. During that time, kernel deterioration might occur too (5). Bakkalbaşı *et al*. (2012) evaluated the oxidative stability of walnuts preserved in shells and measured the peroxide value of the samples stored at 30˚C reveealing a dramatic increase and led to be unacceptable by panelists (6).

Walnuts are also one of the enriched polyphenol sources which are helpful against cancer, cardiovascular diseases and diabetes type 1 (7, 8). Antioxidant compounds in walnuts are protective against oxidation activities. Walnuts are stronger antioxidant materials in comparison to other nuts (9), and this antioxidant property is due to presence of tocopherols and other phenolic compounds in the walnuts (10, 11). The total phenolic contents of six different walnut varieties grown in Portugal were shown by Pereira et al. (2008) to alter from 58.8 to 95.1 mg $GAEg^{-1}$. The total phenolic content in walnuts was estimated 10.7 $mgGAEg^{-1}$ (11).

Walnuts can be infected by certain kinds of molds such as *Aspergillus* aflatoxins, as one of the secondary metabolites of *Aspergillus* that are potent carcinogens and may affect all organ systems especially the liver and kidneys. Several types of aflatoxins are present in nature, but the four B_1, B_2, G_1, and G_2 are hazardous to animals and man because they are present in all major food crops, especially in contaminated grains and nuts. According to the Institute of Standards and Industrial Research of Iran (ISIRI), 5925, the allowed limit of aflatoxin B_1 in walnuts is 5 ng/g, while for total aflatoxins reaches to 15 ng/g. Aflatoxin elimination in dried crops is complicated due to its resistance; therefore, it becomes a potential threat to human health because of its long-lasting presence in the food chain (12).

Thus, it is necessary to provide a proper storage condition to control aflatoxin production during preservation. Following harvesting and removing walnuts green husk, storing walnuts are mostly done through four methods including; preserving walnuts within their hard shell at ambient temperature, and storing peeled walnuts at ambient temperature, in the fridge or in the freezer. Through the HPLC test, respectively, 14.6% and 19.2% of analyzed pistachios and peanuts of Turkey were infected with aflatoxins (13).

Consequently, it was shown that more aflatoxin contamination has been detected in peeled nuts or nuts that their shell has been physically damaged (7). According to the high contamination rate of walnuts to aflatoxins and its vulnerability to lipid oxidation, the aim of this study was to compare the effect of temperature of fridge and freezer conditions on aflatoxin level changes and lipid oxidation in walnuts during storage.

Material and Methods

All chemical materials were prepared from Merck, Germany with high purity HPLC or analytical grade. The study was cross-sectional and interventional and sampling was undertaken as a randomized method from produced green raw walnuts provided from a specific garden in Fars Province, southern Iran. The walnuts green shell was traditionally removed by hand and sun-dried for 14 days. All samples are divided into two groups; the first group was peeled off (hard wooden peel) and the second group was kept within its hard peel. This study was approved by the Local Ethics Committee.

The moisture content was 12-14%. To investigate the effect of temperature on peeled walnuts (1^{st} group), samples were mixed and kept in nylon bags at ambient temperature (25-35˚C), fridge (5±2˚C) and freezer (–19±2˚C) in dark boxes. Simultaneously, walnuts within their hard peel (2^{nd} group) were preserved in nylon bags at room atmosphere and temperature (25-35˚C). As the tests were conducted two times per month through 6 months with two repetitions, 104 sample bags were provided.

To measure aflatoxins by HPLC, 150 g of walnut samples were grounded and mixed with 150 mL of distilled water to make the slurry. Fifty grams of the slurry was mixed with 5 g NaCl and extracted with 120 mL of methanol. The extract was filtered, defatted, diluted with water, and utilized to an affinity column containing antibodies specific for aflatoxins B_1, B_2, G_1, and G_2 (Agilent 1100-HPLC includes C18 column and FLD detector). The reverse-phase high-performance liquid chromatography (HPLC) with fluorescence detection and post-column isolation were used to quantify the aflatoxins (14).

Lipid oxidation measurement by thiobarbituric acid method (TBA) was carried out based on the method described by Sarooei *et al*. (2019). Two and a half grams of ground walnut were mixed with 50 mL of acetic acid-water solvent (1:1, v/v) and were shaken at room temperature for 1 h. The mixture was centrifuged (model: K2042, made by Centurion Scientific Ltd, UK) at 10000 rpm for 5 min, and further TBA reagent was added to the supernatant and incubated at 95°C for 1 h to complete the reaction.

The TBA was evaluated by spectrophotometer (PD-303, Japan) by calculating the mixture absorption

at 530 nm. The lipid oxidation was expressed as ppm of malondialdehyde (MDA) and the calibration curve was determined measuring the absorbance of 1-8 ppm of malondialdehyde (15). The lipid oxidation was presented as ppm of MDA through TBA test by equation (1): TBA (ppm MDA)=absorbance at 530 nm×7.8.

Anti-oxidant activity was assessed by the FRAP method as described by Shaghaghian *et al.* (2014) using Ferric Reducing Anti-oxidant Power (FRAP). Briefly, 5 grams of ground sample was mixed with 50 mL of a solvent containing methanol-water (4:1, v/v). The extraction was performed by an orbital shaker for 3 h at ambient temperature (20±2°C). The extract was centrifuged (model: K2042, made by Centurion Scientific Ltd, UK) for 20 min at 4000 rpm. The supernatant was reacted by FRAP reagent and incubated at 37°C for 5 min. Using a spectrophotometer (PD-303, Japan) at 593 nm wavelength, the complex was analyzed for the antioxidant property. The antioxidant property was presented as mmol $Fe^{2+}L^{-1}$. Finally, the calibration curve was drawn with aqueous solutions of Fe (II) concentrations ranging from 100 to 2000 µM ($FeSO_4.7H_2O$) (16).

Sensory analysis by the hedonic method was conducted two times a month through the 6 months of storage. Ten trained panelists (3 males, 7 females) with an average age of 31 years old were assembled for this study. Each panelist completed a training session. The selected and used sensory attributes by the panelists were unshelled walnuts preserved at room temperature, peeled walnuts kept at ambient temperature, fridge, and freezer, using a 5-point scale hedonic method ranging from 1 to 5, where 1= very good and 5= very bad used to evaluate acceptability of samples. Samples were coded using 3-digit random numbers and expressed for panelists in random order at room temperature with water and paper ballots on a tray. All panels were conducted in duplicate (6, 17).

Descriptive statistics such as mean and standard deviation were used to describe the data. Statistical analysis was carried out using SPSS software (version 22, Chicago, IL, USA) and One-way ANOVA to determine the significant difference between the means. A *p* value less than 0.05 was considered statistically significant.

Results

The aflatoxin B_1 content of walnut samples during 6 months were shown in Figure 1. On the first day, the aflatoxin B_1 of dried walnuts of all four treatments was 1.93±0.38 ngg^{-1}, while it became zero after 15 days. The aflatoxin B_1 content of samples kept in the fridge, freezer, and unshelled walnuts was trace during 6 months; however, the aflatoxin B_1 in peeled walnuts kept at room temperature reached to 0.53±0.035 ngg^{-1}, 0.83±0.31 ngg^{-1}, and 1.2±0.38 ngg^{-1}, on days 61, 106, and 151, respectively.

According to the test results on the first day, the aflatoxin B_2 content of dried walnuts was 1.46±0.007 ngg^{-1}. Tests were conducted twice every 16 days for 6 months. The aflatoxin B_2 in samples based on determined sampling methods became nearly zero in all walnuts. The results indicated that aflatoxin G_1 and G_2 of dried walnuts on the first day was trace and this amount remained zero during 6 months of preserving under tested conditions.

To assess lipid oxidation index, control samples (dried walnuts on the first day), as well as stored walnuts, were examined for malondialdehyde content. The TBA index of control samples was 0.282±0.007 ppm MDA. Figure2 indicated that the TBA index showed a statistically significant increase during 6 months among all studied sample

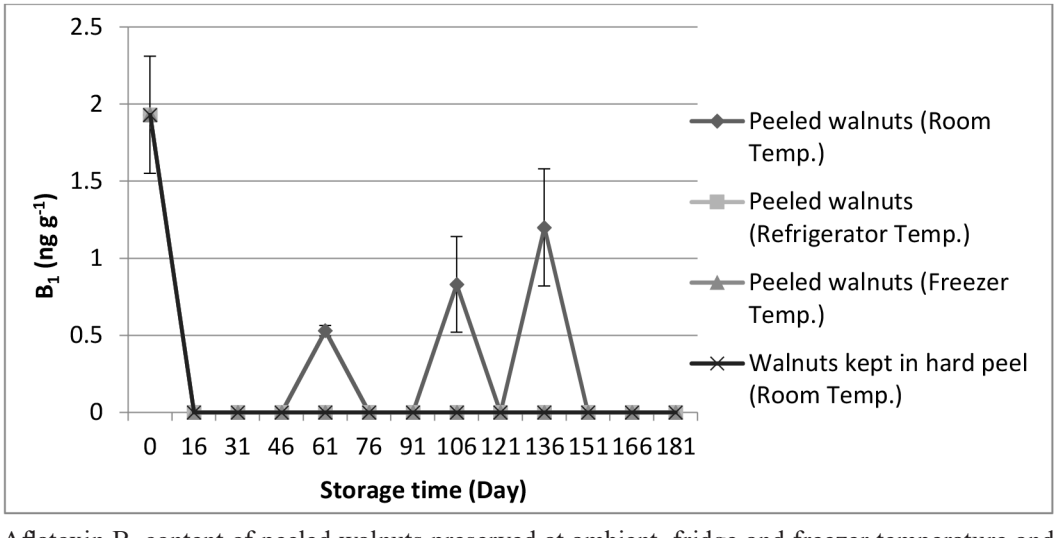

Figure 1: Aflatoxin B_1 content of peeled walnuts preserved at ambient, fridge and freezer temperature and unshelled walnuts at ambient temperature during 6 months.

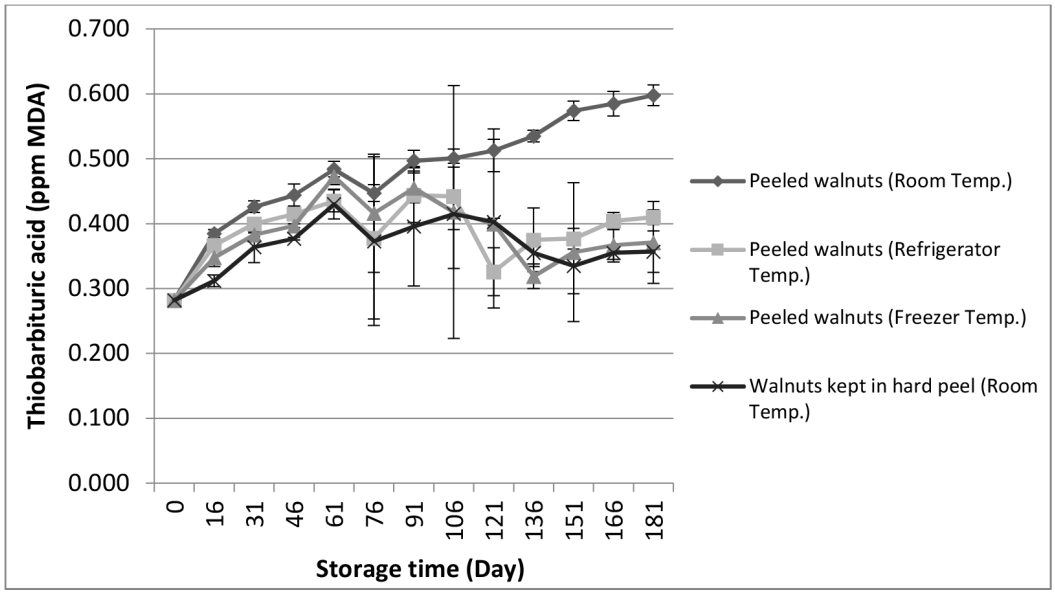

Figure 2: Malondialdehyde measurement of peeled walnuts preserve at ambient, fridge and freezer temperature and unshelled walnuts at ambient temperature during 6 months through TBA.

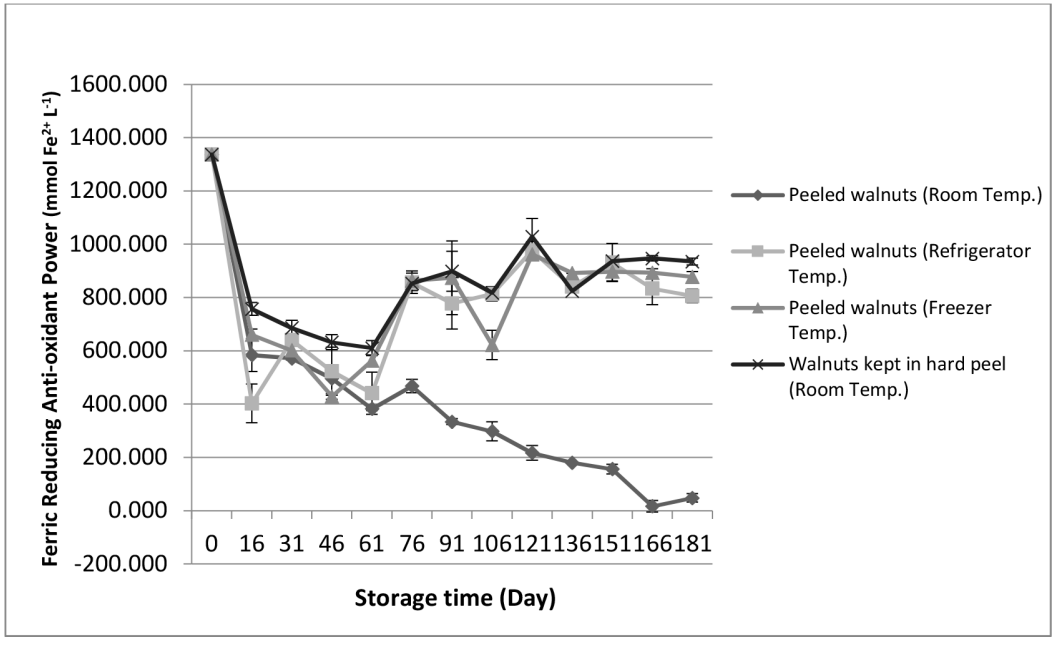

Figure 3: Antioxidant activity measurement of peeled walnuts preserved at ambient, fridge and freezer temperature and unshelled walnuts at ambient temperature during 6 months through FRAP test.

groups. Respective limit values for TBA were 1-2 mg malondialdehyde/kg walnut. The walnuts preserved within their hard peel had the lowest TBA index after 16 days (0.312±0.009 ppm MDA). After two months, peeled walnuts preserved in the freezer had the least lipid oxidation changes, but they did not show any statistical difference in comparison to unshelled walnuts (p>0.05). Regarding the TBA test results, peeled walnuts kept at room temperature had the highest lipid oxidation and the difference between MDA content of the other 3 groups became statistically insignificant after 76 days.

To determine the influence of preserving condition on antioxidant activity of walnuts, FRAP test was undertaken revealing that by increasing the storage time, the antioxidant activity of walnuts decreased significantly. According to Figure 3, on the first day, dried walnuts' antioxidant activity was 1337.27± 6.37 mmol $Fe^{+2}L^{-1}$, while on the 16th day, walnuts kept in their hard peel showed the highest antioxidant activity (757.19±24.19 mmol $Fe^{+2}L^{-1}$) in comparison with walnuts under other preserving conditions during 6 months. The results showed the lowest antioxidant activity in the peeled walnuts kept at ambient temperature after a long storage time of 6 months.

Peeled walnuts kept at the ambient, fridge, and freezer temperature and unshelled walnuts were evaluated by trained panelists through the 5-point scale hedonic method during 6 months. While

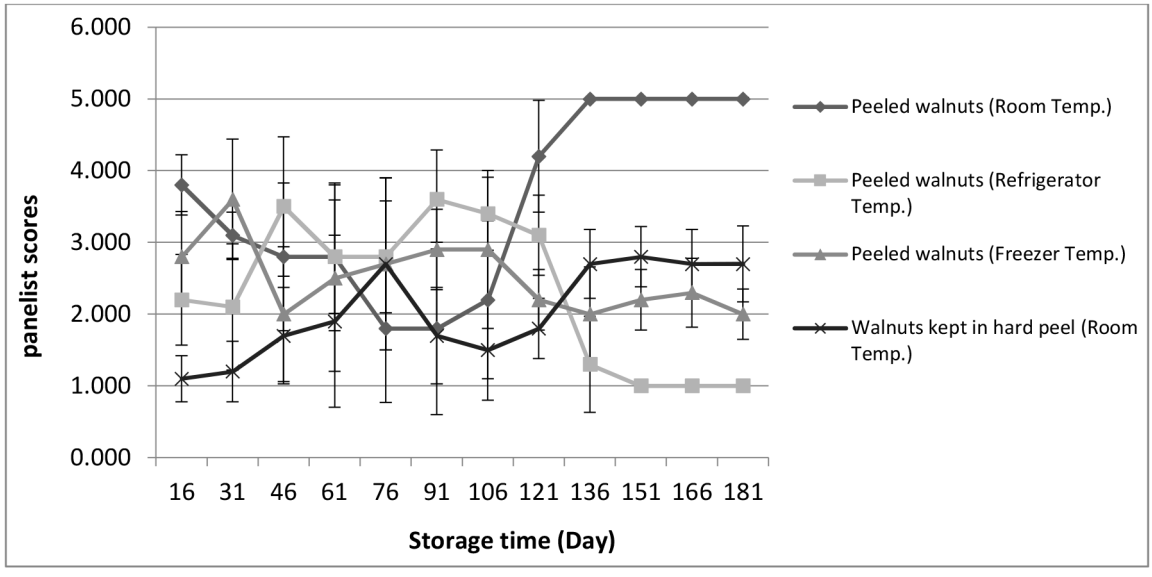

Figure 4: Sensory evaluation of peeled walnuts preserved at ambient, fridge and freezer temperature and unshelled walnuts at ambient temperature during 6 months.

the unshelled walnuts had the average acceptance after 136 days (2.7±0.48), peeled walnuts at room temperature reached the lowest acceptance degree (5.0±0.0) (Figure 4). After 3 months (61 and 76 d), sensory evaluation results showed no statistically significant difference between the unshelled walnuts and other samples. The greatest sensory acceptance after 6 months was related to peeled walnuts preserved at fridge temperature.

Discussion

In dried walnuts, on the first day, the aflatoxin B_1 (1.93±0.38 ngg^{-1}), B_2 (1.46± 0.007 ngg^{-1}), G_1 and G_2 (0 ngg^{-1}) were lower than the allowed standard limits. Gallo *et al*. (2016) determined the influence of different combinations of water activity and temperature on fungal growth and production of aflatoxin B_1 in *Aspergillus flavus* present on an almond medium. Maximum accumulation of fungal biomass and aflatoxin B_1 production was noted at 28°C and 0.96 a.w.; while no fungal growth and aflatoxin B_1 production was visible at 20°C and 0.90-0.93 a.w. (18).

Schroeder and Hein (1968) found that temperatures of 25°C and 10°C lead to respectively increase and stop of the *Aspergillus* growth and aflatoxin production (19). Lahouar *et al*. (2016) reported the highest aflatoxin production in sorghums at 37°C with 0.99 a.w., and found no aflatoxin production at temperatures below 15°C (20). The present study outcomes confirm the previous study results. While unshelled walnuts at room temperature and the peeled walnuts preserved at the fridge and freezer showed no aflatoxins contamination during storage, peeled walnut samples kept at the ambient temperature after 61, 106, and 136 days, respectively as 0.53±0.035 ngg^{-1}, 0.83±0.31 ngg^{-1}, and 1.2±0.38 ngg^{-1} aflatoxin B_1. Test results emphasize that temperatures above 25°C (room temperature) set the stage for fungal growth and aflatoxins production in long term storage. Likewise, temperatures below 10°C (fridge and freezer) stop the aflatoxin production, probably due to the low growth rate of *Aspergillus*.

In comparison with the first day, the lower amount of aflatoxins in the last days in peeled walnut samples at the ambient temperature may be due to the sample homogeneity, packaging condition in zip-keeps (limited oxygen content), low contamination of samples with aflatoxins, and lower preserving temperature than the drying temperature. Absence of any aflatoxin contamination of walnut samples kept within their hard peel is probably because of the controlling role of hard peel in curbing oxygen, temperature, and a.w., which leads to less *Aspergillus* growth and halting aflatoxin production.

Lipid oxidation is a critical quality control factor in walnuts because of the high lipid content of walnuts (60%), which mostly consists of unsaturated fatty acids (70%). In this regard, determining free malondialdehyde through TBA test can provide a context to measure oxidation progress (21). While the respective limit values for TBA is 1-2 mg MDA/kg of walnut, our study results indicated that as time passes, the TBA index increases significantly in all walnut samples (p<0.05). According to Raisi *et al*. (2015), peroxide index of almond kernels increased by storage time in different atmosphere packaging (air, vacuum, and CO_2) at 4 and 23°C (22).

Likewise, an increases in peroxide value and hexanal content of shelled walnuts was illustrated by passing time (6). Almonds kept within their hard peel showed higher quality and lower peroxide

value in comparison with roasted almond kernels after 9 months (23). Storing macadamia nuts in the shell at different temperatures (-18, 10, and 15°C) in vacuum-packed and net bags for a period of 2 months displayed increases in peroxide index and malondialdehyde production (5).

Despite increasing TBA in all walnut samples through this study, the walnuts kept in their hard peel showed lower MDA comparing with walnut kernel samples. This may be due to curbing parameters such as oxygen, light, and water activity responsible for lipid oxidation (17). Although the MDA produced in all the stored walnuts was below the respective limit value, it is obvious that the less MDA in walnuts, the higher quality and taste they have. The statistically significant differences between TBA values of walnut kernels kept at fridge and freezer with ones preserved at room temperature confirmed the effect of temperature on lipid oxidation.

The antioxidant activity of all four groups of walnut samples investigated in the current study decreased significantly through the time ($p<0.01$). The antioxidant capacity of pistachio was assessed and a decline was shown through the storage time. It was also demonstrated that low temperature had a greater impact on protecting pistachios' antioxidants than the oxygen limitation (24). The antioxidant property and phenolic content of hazelnuts kept at the room, fridge, and freezer showed significant decreases through the time. As a result, fridge temperature was recommended for annual hazelnuts storage (25).

According to a previous report, there was a close reverse correlation between peroxide value and tocopherols (23). The present study confirmed the important role of storage time in the decline of the antioxidant activity, which can be a reason for the lipid oxidation. The antioxidant activity of pistachios, walnuts and hazelnuts showed that the removal of seed coat could decline the total antioxidant activity of pistachios, walnuts and hazelnuts to about 36, 90 and 55%, respectively (26). The current study indicated that the FRAP test results of walnuts kept in their peel denoted to a 29% decrease in antioxidant activity after 6 months, while the test result for the peeled walnuts at the same time and temperature condition was 98%. Thus, removing walnuts' hard peel provided a context for oxidizing the antioxidant components.

One of the crucial quality control factors in long term storage is sensory evaluation. Sensory evaluation of four walnut sample groups during 6 months showed that unshelled walnuts had the highest panelist acceptance from the first to the fourth month. However, during the fifth and sixth month, walnut kernels preserved at the fridge mostly gained higher acceptance. The lowest panelist acceptance was related to the walnut kernels preserved at room temperature. Lopez *et al*. (1995) during their study on the effect of cold storage condition on the quality of peeled walnuts through a year demonstrated that temperature of 12-14°C was the best range for walnut storage and preserving in 40% RH was not suitable due to any weight loss (27).

Keeping hazelnuts at low temperatures (in the fridge) helps to protect phenolic compounds, antioxidant activity, and sensory properties through a year (28). The sensory evaluation for walnut kernels preserved at 10, 20, and 30°C over 12 months storage period, showed a statistically significant differences between storage temperatures. However, there was no difference between 10°C and 20°C, except for 30°C. Although walnuts were edible in the 2nd, 4th and 6th months of storage, there was a sharp reduction in panelist acceptance after six months and all walnut kernels were very inedible and rancid (6).

Whilst the shelf life of almond kernels in the fridge is over 10 months, keeping them at the room temperature decreased their acceptance to 9 months (22). Lower lipid oxidation and higher antioxidant activity of walnuts kept in their hard peel is probably the reason for its high acceptance among panelists. Lower relative humidity and more weight loss occurred in the freezer than the fridge, through the 5th and 6th months, may be the proof of lower acceptability of walnut kernels preserved in the freezer.

Conclusion

Findings of the present study showed that during the storage period, the antioxidant activity decreased, while TBA value increased in all stored walnuts. Evaluation of lipid oxidation, level of aflatoxins, and sensory assessment showed that the best storage condition for a short time was preserving walnuts within their hard shell at room temperature. However, for the long-term storage of 6 months, preserving walnuts at room temperature resulted in great lipid oxidation and reduction in antioxidant activity, while, through the sensory evaluation and chemical tests it, was concluded that the best storage condition for a long time was keeping peeled walnuts in the fridge.

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Conflict of Interest

None declared.

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