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The Effect of Thermal Pretreatment and Packaging Conditions on the Shelf-life of Walnut Kernels

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A R T I C L E I N F O

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A B S T R A C T

This study aimed to extend the shelf life of walnut kernels by appropriate packaging conditions and physical methods. For this purpose, the kernels were heated at 50 or 70°C for 2 or 10 minutes. About 100g of walnuts were packed in low-density polyethylene (LDPE) packages under ordinary, vacuum or nitrogen conditions. Samples were stored for 18 months at room temperature. Peroxide value and acidy number were determined after 6, 12 and 18 months of storage. The results of the variance analysis showed that the effect of packaging conditions on peroxide value, and the effect of all factors on the acidity of the samples were significant ($p<0.01$) for the 6- month storage. Walnuts packaged under nitrogen conditions had lower peroxide value than others, and the samples preheated at 70˚C had a lower acidity. After 12 months of storage, the effect of all factors on peroxide and the acidy number of walnuts were significant ($p<0.01$). The lowest peroxide value and acidity were observed for walnuts preheated at 50˚C for two minutes. Finally, based on the obtained results, we recommend that walnut kernels should be preheated at 70˚C for two minutes and packaged under nitrogen or vacuum conditions. In this way, they could be stored for six months without perceived oxidation. The oxidation of walnuts would increase during storage for 12 to 18 months gradually, and their peroxide value and the acidity would exceed the desirable limits.

Introduction

Walnuts, with the scientific name of *Juglans*, belong to the *Juglandasse* family. Iran's walnut is one of the most valuable varieties in the world and is an economically valuable product (Hassankhah *et al*., 2017; Farsi *et al*., 2018). The oil content of different walnut varieties is between 60% and 75%, and linoleic acid is the dominant fatty acid (44.84-68.44%). It also contains other nutrients, including vitamins A, B, E, and F, and various minerals, therfore, kernels are prone to rapid degradation by chemical and microbial agents (Tajeddin, 2004; Golzarri *et al*., 2013). According to FAO statistics, in 2012, Iran was ranked second in the world

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with production of 4,500,000 tons of walnuts (FAO, 2014). As mentioned, walnut kernels contain high levels of triglycerides and unsaturated fatty acids and are therefore highly susceptible to hydrolytic oxidation. For example, packaging almonds in non-permeable laminate films could reduce the oxidation of almond oil (Ziaolhagh, 2011). On the other hand, many plants, including walnuts, contain lipoxygenase. It contains iron and oxidizes unsaturated fatty acids (Boranasump *et al*., 2007). Therefore, preventing walnut kernels from exposing to oxygen and inactivating the lipolytic enzymes are among approaches to prevent the oxidation of oils, including those present in walnut kernels. Temperature, degree of O_2 barrier of the packaging and lighting conditions are the main factors influencing the quality of walnuts during storage (Mexis *et al*., 2009).

The pre-storage thermal processing has been used by many researchers to inactivate lipolytic enzymes in peanuts (Mitchell and Malphrus, 1977), hazelnuts (Laskawy *et al*., 1983; Cam and Kilic, 2009), pecan kernels (Senter *et al*., 1984), almonds (Zacheo *et al*., 2000; Buranasompob *et al*., 2003) and walnuts (Buranasompob *et al*., 2003; Ling *et al*., 2014). Besides, heating treatment could significantly improve the nutritional qualities of walnuts (Han *et al*., 2018).

Han *et al*. (2018) showed that the contents of protein, soluble sugar, minerals, and carbohydrates were increased with the rise of temperature to 105°C. They demonstrated that total flavonoids and total polyphenols of the thermally processed walnuts were increased at 140°C.

Hosseini *et al*. (2014) investigated the oxidative stability of walnuts over a one-year storage period under different conditions. They did not observe any significant difference between the oxidative stability of walnuts stored in vacuum packages and those containing carbon dioxide gas. Ziaolhagh (2013) stored almond kernels for 20 months at room temperature and showed that the best packaging material and packaging conditions for the storage of almond kernels were PA-PE-PE-PA laminate in vacuum conditions.

John *et al*. (1988) showed that walnuts packed in nitrogen at 5°C maintained their quality after 16 months of storage. Prabhakar & Hamavathi (1979) investigated the effect of antioxidants, neutral atmosphere, and polyethylene packaging on walnut shelf life and showed that the oxidation rate was increased by using polyethylene bags compared to wooden boxes. They found CO_2-containing cans to be more effective than nitrogen-containing or vacuum cans for long-term storage. Mexis (2009) examined the effect of packaging and storage conditions on walnut quality. In this study, the peroxide value for fresh walnut kernels was 0.3 and for walnuts packed in PE bags kept for 12 months, was 31.4 meq O_2 / kg oil. The results showed that the acceptable shelf life of walnuts in PE bags with air was about 2 months, in PET/PE bags with nitrogen atmosphere was 4 to 5 months, and in PETSiOx / PE bags with nitrogen was at least 12 months at 20°C. Young *et al*. (1991) reported that almond, macadamia, and pistachio kernels were acceptable at 38°C after 6 to 9 months of storage, but that of hazelnut, peanut, and walnut were preserved only 2 to 5 months at this temperature and subsequently oxidized.

In the first stage of kernel oxidation, peroxides and hydroperoxides are produced. Peroxide value is used to study oxidative deterioration in the kernels. Walnut kernel oil peroxide value of less than 3 meq O_2/ kg indicates acceptable walnut kernel quality (Gutteridge, 1986). The percentage of free fatty acids in walnut kernels heated at 55 or 60°C after storage for 60 days was less than 1.5% based on oleic Acid (Buranasompob *et al*., 2003). Koyuncu *et al*. (2003) showed that the decline in quality of walnut kernel was greater than the whole walnut and walnuts that were dried in the sun could be stored for up to 12 months at 21°C and 50-65% relative humidity.

Little work has been done on walnuts' quality and shelf life in Iran (Habibie et al., 2019), and most studies on walnut in Iran are concerned with the chemical composition of walnut and its gardening issues. However, the effects of different processing conditions on the quality of walnuts have been investigated by many researchers in the world (Christopoulos and Tsantili, 2011; Leahu *et al*., 2016; Tatarov *et al*., 2017; Han *et al*., 2018; Talebi Habashi, 2019; Grosso *et al*., 2020).

By increasing the shelf life and proper packing of walnut kernels, it can be exported overseas with favorable quality and reasonable price and significant exchange can be obtained thereby. The purpose of this research was to extend the shelf life of the walnuts by pre-storage thermal processing and packaging them in appropriate packages. In this way, the added-value walnut kernels could be sold for a longer time and the income of the gardeners, exporters or producers would increase. In addition, it can be valuable as part of the non-oil exports of the country.

Materials and Methods

The walnuts used for the project was prepared by a gardener in Dibaj village in Damghan in mid-November and transferred to the Agricultural Engineering Department laboratory of the Agricultural and Natural resources research center of Shahrood, Iran. All walnuts were collected from one tree of an orchard. Walnuts were enucleated in the laboratory and preliminary tests including moisture content, oil content, peroxide value and acid number were measured. Walnut kernels were uniformly mixed and heated at 50 and 70°C for 2 and 10 minutes. This temperature range was selected based on enzyme inactivation (Zacheo *et al*., 2000; Ling *et al*., 2014; Han *et al*, 2019). The kernels were kept at room temperature for about 20 minutes to cool down and then they were packed into polyethylene-polyamide-polyethylene bags with a thickness of 0.1 mm. One-third of them were packaged under normal atmospheric conditions, one-third under vacuum (using the vacuum packing machine BOXER42 made in the Netherlands), and one-third under nitrogen atmosphere. Experiments included peroxide value, acidity, as well as sensory test. The measurements were carried out after 6, 12 and 18 months of storage.

To measure the moisture content, an oven (Froilabco, France) was used at 105°C. Five grams of sample (with three replications) were placed in the oven, their moisture content was calculated after fixing the weight (equation 1), and the mean of three replicates was reported as the average moisture content (Parvaneh, 2006).

$$\text{Moisture percentage} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100 \qquad (1)$$

Soxhlet method was used to measure walnut oil content. The walnuts were first ground using a homemade grater and then sifted with an 18-mesh sieve. About five grams of the milled sample was poured into a filter paper cup and placed into the extractor unit. The hexane solvent was used to extract the oil, and the extraction was continued for eight hours. After the solvent was evaporated, and the weight of the balloon containing oil was fixed, the percentage of oil samples was obtained by weight difference (Parvaneh, 2006).

Since heat reduces the produced peroxide in the oil, cold extraction was used to measure the peroxide value and acidity. Each sample was then unpacked, and ground by a hand mill. After passing through an 18-mesh sieve, about 50 to 60g of the specimens were poured into a 1-litre beaker containing about 300 cm^3 of n-hexane. The beaker was coated with aluminum foil to prevent solvent evaporation. The specimens were kept in the dark for 72 hours and stirred continuously. About 150 cm^3 of fresh solvent was also added to increase the solvent extraction power. The aluminium foil was then removed from the beaker, and the samples were filtered using mesh and filter paper to separate the solvent and oil mixture from the pulp. The filtered samples were kept in the dark until the weight was fixed. Then the residual oil was used for experiments (Ziaolhagh, 2011& 2017).

Peroxide value was measured by weighting approximately five grams of the extracted oil sample into a 250 mL Erlenmeyer flask and adding 30 cm^3 acetic acid-chloroform solution to it in a ratio of three to one. After shaking to dissolve the oil in the solvent, about 0.5cm^3 of saturated potassium iodide was added and stirred well. After two minutes, 30 cm^3 of distilled water was added and titrated with 0.1N sodium hyposulphite in the presence of starch as an indicator. The peroxide value was obtained from equation 2:

$$\text{Peroxide Value} = \frac{S \times N \times 1000}{M} \quad (2)$$

S is the titration of the sample, N is the normality of sodium hyposulphite, and M is the sample weight (Parvaneh, 2006).

Acidity was measured by adding 20 cm^3 of alcohol and 20 cm^3 of chloroform into an Erlenmeyer and neutralizing it in the presence of phenolphthalein reagent. It was then added to another Erlenmeyer flask containing ten grams of walnut oil, and after stirring to dissolve the oil, it was titrated with 0.1N NaOH, and the acidity was obtained from equation 3:

$$\text{Acidity (\%)} = \frac{N \times 0.0282}{M} \times 100 \quad (3)$$

N is the titration of the sample and M is the sample weight (Parvaneh, 2006).

The five-point hedonic method was used for sensory examination. For this purpose, 15 untrained employees of Shahroud Agricultural Research Center were selected and asked to consume walnut samples and give them a score of 1 to 5 for their taste and odour.

Statistical analysis of the project was done using MSTATC software and completely randomized design in factorial format.

Results

In this study, the percentage of walnut oil was 73.36%, and its moisture content was 3.082%. The results of the variance analysis (Table 1) showed that only the effect of packing conditions on the peroxide value of the samples was significant and the thermal treatments had no significant effect on the peroxide value. As shown in Fig. 1, samples kept under normal conditions had the highest peroxide value, and samples kept under N_2 atmosphere had the lowest peroxide values during six months of storage. This shows that the nitrogen conditions could prevent the production of peroxide, which is one of the signs of oxidation of oils, more than vacuum or atmospheric conditions. Besides, it has shown that the peroxide value has increased as the storage time extended from 6 months to 18 months (Fig. 1). As can be seen in table 1, all treatments had significant effects (p <0.01) on the acidity of the samples.

Table 1. Mean squares of the effect of different factors on peroxide and acidic numbers of walnut kernels after 6, 12 and 18 months.

Source Deviation	Degree of freedom	Mean Squares for 6 months		Mean Squares for 12 months		Mean Squares for 18 months	
		Peroxide value	Acidity	Peroxide value	Acidity	Peroxide value	Acidity
A	1	0.321^{ns}	0.514^{**}	36.14^{**}	4.027^{**}	10.737^{ns}	0.013^{ns}
B	1	0.49^{ns}	0423^{**}	41.92^{**}	0.548^{**}	1.596^{ns}	0.026^{ns}
A×B	1	1.361^{ns}	0.034^{**}	304.095^{**}	1.941^{**}	10.912^{ns}	0.692^{ns}
C	2	36.13^{**}	0.302^{**}	141.118^{**}	0.095^{ns}	9.371^{ns}	1.646^{ns}
A×C	2	0.354^{ns}	0.212^{*}	48.387^{**}	3.141^{**}	2.773^{ns}	0.808^{ns}
B×C	2	1.021^{ns}	0.141^{ns}	60.833^{**}	5.942^{**}	2.025^{ns}	1.153^{ns}
A×B×C	2	2.55^{ns}	0.009^{ns}	103.876^{**}	3.309^{**}	29.839^{ns}	0.712^{ns}
error	26	2.78	0.046	2.664	0.123	13.613	1.39

A: temperature, B: time, C: Packaging conditions, ns not significant, ** significant at p<0.01, * significant at p<0.05

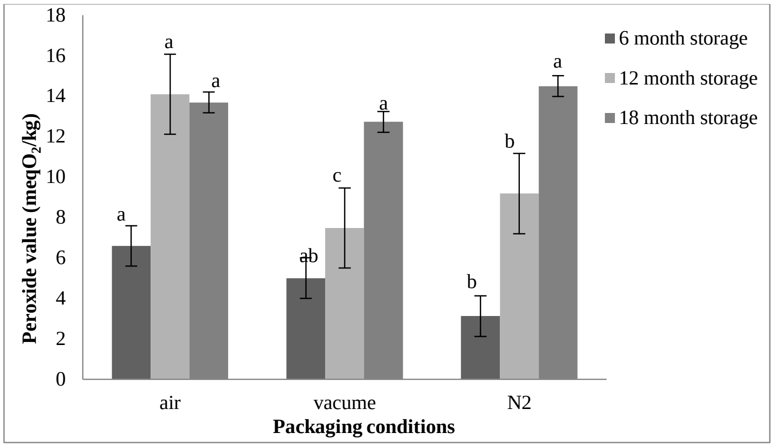

Fig. 1. Effect of packing conditions on walnut peroxide value. Different letters indicate significant differences (Duncan test, $P<0.01$).

According to Table 1, after 12 months of storage, the effect of all treatments on the peroxide value of the samples, and the effect of all treatments except the packing conditions on the acid number of samples was significant ($p<0.01$). After 18 months of storage, the effect of different factors on the peroxide and acid numbers of the samples was not significant ($p>0.01$). However, the peroxide value of all samples was above the permissible limit, and the sensory evaluator group refused to use them.

Regarding the effect of heating temperature on the acid number of samples, it can be seen from Fig. 2 that the acid value of the samples treated at 50°C was higher than the acid value of the sample treated at 70°C. This result suggests that the temperature of 70°C is more effective in preventing the oxidation of walnut fat.

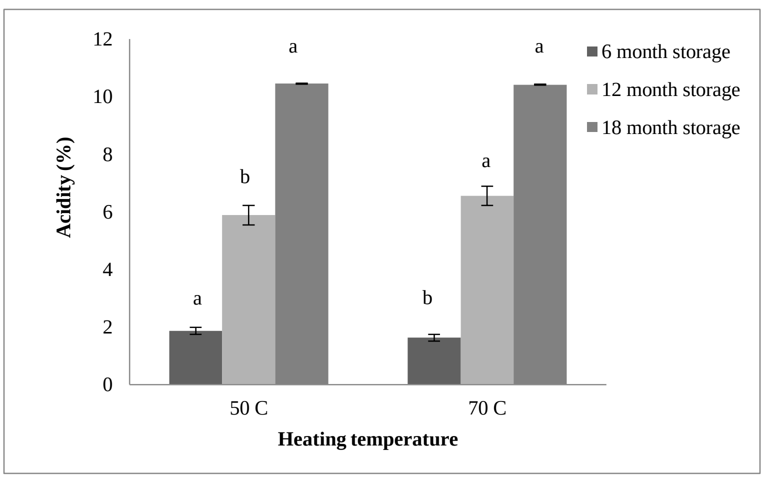

Fig.2. The effect of heating temperature on the acidic value of walnut. Different letters indicate significant differences (Duncan test, $P<0.01$).

The effect of the heating period on the oxidation of the samples was shown in Fig. 3. The effect of 2 minutes of heating in preventing oxidation was greater than 10 minutes of heating. The acidity value of the samples heated for 2 minutes was less than the acidity value of samples heated for 10 minutes.

Comparison of means by Duncan's method showed that the temperature of 70°C for 2 minutes significantly prevented oxidation compared to other treatments and the corresponding samples had a less acidic number, as shown in Fig. 4.

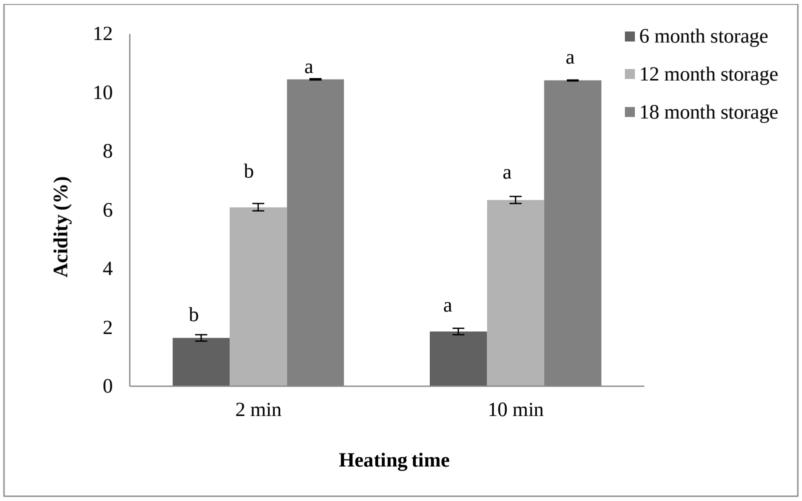

Fig. 3. Effect of heating time on the acidic value of walnut. Different letters indicate significant differences (Duncan test, P<0.01).

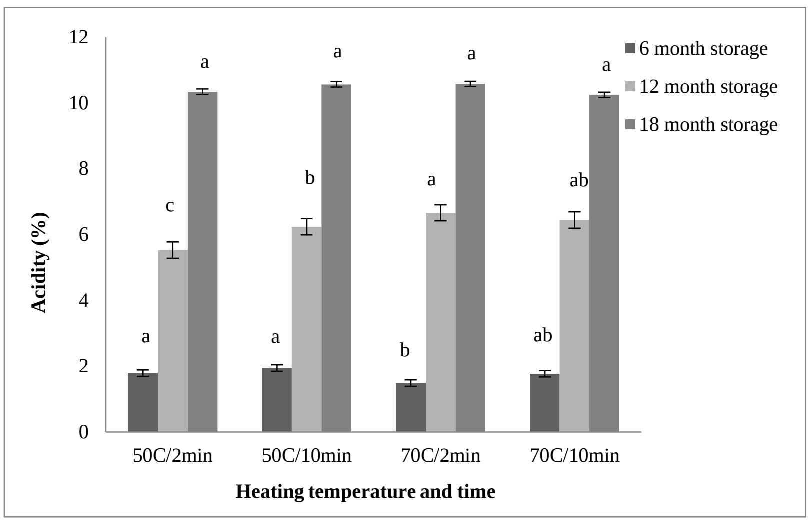

Fig. 4. Interaction of temperature and heating time on the acidic value of walnut. Different letters indicate significant differences (Duncan test, P<0.01).

Discussion

Packaging under nitrogen conditions prevented the production of peroxide better than packaging under vacuum or atmospheric conditions. The reason for the low peroxidation value in our study is probably because the nitrogen atmosphere prevents the loss of the phenolic compounds of walnut. Phenolics are compounds that are important in preventing oxidation (Christopoulos *et al.* 2008; Cheniany et al., 2010). This is in line with the results of Jensen *et al*. (2003) who showed that walnut kernels could be stored in nitrogen conditions, without altering their quality significantly. Salagegheh and Tajeddin (2020) who studied on the effect of modified atmosphere packaging on walnut kernel shelf life also showed that the peroxide value of kernels packed in laminated films containing 92% N_2 was much lower than those packed in atmosphere conditions or 80% N_2. Lipid oxidation could be inhibited by using a packaging material with low oxygen permeability or by packaging walnuts in controlled atmospheres with low oxygen content (Mate *et al*. 1996). Lin *et al*. (2014) suggested that vacuum packages could be used to keep the quality of walnut paste at 4°C during two years of storage.

Jaleh Rezaei (1999) showed that oxidation intensity was directly correlated with the amount of oxygen in the environment, and packing almonds in the vacuum increased its storage life. Mazinani *et al*. (2011) showed that the total amount of phenolic compounds in walnut kernels was higher than those in pistachio and almond kernels and that walnut oil had lower thermal stability despite the higher amount of phenolic compounds. Also, tocopherols in the walnut kernels protect them against lipid oxidation and extend their storage life. Alpha-tocopherol is a natural antioxidant that inactivates free radicals, thereby delays oxidative corruption (Zacheo *et al*., 2000).

The temperature of 70°C was more effective in preventing the oxidation of walnut fat due to the inactivation of more related enzymes such as lipoxygenase at this temperature. This is in line with Lin *et al.* (2014), who showed that the FFA values of the heated walnut paste were the highest compared with unheated walnut kernels. Boranasumbo *et al*. (2003) also showed that heating the walnut kernel prevents their oxidation during storage. Boranasumbo *et al*. (2007) showed that a temperature of 55°C for 2 minutes or more decreased the activity of lipoxygenase and thereby delayed walnut and almond kernel oxidation. Severini *et al*. (2003) also showed that peroxide value and oxidation rate of almond triglycerides after roasting were low. They attributed this to the reaction between peroxide radicals or carbonyl compounds derived from peroxides and the products of the Millard reaction.

Heating the walnuts in a long period increased the oxidation and thus the acidity value, because heating may accelerate the oxidation of oils (thermal oxidation).

Conclusions

Walnut kernels are oxidized rapidly during storage due to their high unsaturated fatty acids content. In this research, we aimed to extend the shelf life of walnut kernels by inactivating the oxidizing enzymes and by proper packaging. In this way, we were able to store the walnuts for 12 months. The oxidation of walnut kernels stored for more than this period increased gradually and the walnuts developed off odor and flavor. In this research, it was shown that the pre-storage thermal processing of walnut kernels was an effective way to prevent lipolysis reactions. In addition, the gas composition in the packages was important in extending the storage life of walnut kernels. The kernels were preserved best when packaged under vacuum or N2 atmosphere. Finally, to store walnut kernels for 6-12 months, it is recommended to preheat the walnut kernels at 50°C for 2 minutes before packaging them in polyethylene-polyamide-polyethylene bags under nitrogen atmosphere.

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