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Evaluation of Shelf Life of Walnut Kernels Treated by Antioxidants and Different Packaging under Two Temperatures

Roghieh Talebi Habashi[1], Shahin Zomorodi[*2], Alireza Talaei [3], Sepideh Kalateh Jari[1]

[1]*Department of Horticultural Science and Agronomy, Science and Research Branch, Islamic Azad University, Tehran, Iran*

[2]*Department of Engineering Research, West Azerbaijan Agricultural, Education and Natural Resources Research Center, AREEO, Urmia, Iran*

[3] *Department of Horticulture, University of Tehran, Tehran, Iran*

A R T I C L E I N F O

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A B S T R A C T

In this study, the effects of coatings made of chitosan (Chi) incorporating thyme essential oil (TEO) on lipid oxidation and changing color indexes of walnut kernels were investigated. Chi: pure, in accompany with TEO at concentrations of 500 and 1000 microliter per liter, in an aqueous coating solution, with different packaging methods: Gunny sack, polypropylene and active packaging, compared with control walnut, were used and stored at 4ºC and 25ºC. The study was performed as factorial experiment based on a complete randomized design. The results showed that amounts of color indexes in treated samples decreased. The samples stored at 4°C contained minimum moisture fluctuations in all packaging methods. The peroxide values and conjugated diene values at 4°C were lower than those at 25°C. Treatments did not have positive effect on free fatty acids of walnut kernels. During the storage, shelf life of walnut kernels prolonged with active packaging and chitosan coating at 4ºC.

Introduction

Walnut (*Juglans regia* L.) is a valuable nut crop grown in most of the temperate zone climates of the world (Hassankhah *et al.*, 2017). Nuts of walnut have a high nutritional and therapeutic values (Chatrabnous *et al.*, 2018b; Jahanbani *et al.*, 2016; Jahanbani *et al.*, 2018). Study of oxidation in oils is important in evaluation of nuts quality as well as the outcomes of lipid oxidation bear carcinogenic and mutagenic effects on humans. Walnut kernels possess nearly saturated (6.13 g/100g walnut), monounsaturated (8.93 g/100g walnut) and polyunsaturated (47.17 g/100g walnut) fatty acids (Shah *et al*., 2018). Although fatty acids in walnuts are beneficial, indeed unsaturated fatty acids in nuts and inappropriate environmental situations cause the susceptible to lipid oxidation and they may cause weak resistance and shorter shelf life of walnut kernels. Numerous environment factors such as light, temperature, exposure to O_2 and H_2O strongly affect oxidation of walnuts (Salcedo *et al*., 2010). Oxidative rancidity results from the changes that happening from reactions with O_2 in atmosphere. Lipid oxidation was inhibited by using a packaging material with minimum

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O_2 permeability or through storing the walnuts in controlled atmospheres with minimum O_2 content (Mate *et al*., 1996). A chemical combination of moisture and enzymes interaction with fat or oil caused hydrolytic rancidity. The walnut kernels have bioactive compounds such as phenols, tocopherols and phytosterols (Cheniany *et al.*, 2013; Trandafir *et al.*, 2017). Polyphones were subjected to oxidation: their concentrations decreased more under the exposure of O_2. Oxidation of phenolic elements is one of the main reasons for opaque of the walnut kernel color. During storage, oxidation process and color change depend on outside conditions (Vaidya *et al*., 2013). A clear, light yellowish-green color of walnut oil is acceptable for many food applications, especially for salad dressings (Sze-Tao *et al*., 2000). CIE-L^*a^* b^* is One of the famous color systems. Lightness (L^*), greenness (a^*) and yellowness (b^*) values are useful in analyzing the color change of peel of walnut kernel and it can be an advantageous alternative for subjective method of color charts (Pathare *et al*., 2013). Peroxide value, free fatty acids, and conjugated diene value are ways helping detection of oxidized and/or hydrolyzed oils in products. Moisture is one of the important factors for the quality of nuts. Amount of moisture content (MC) of walnut kernels strongly affects their properties (Seyed and Taghizadeh, 2007).

Coating is a form of films directly used on the external layer of crops, becoming as a part of the final product. Edible active coatings are biodegradable and biocompatible, maximizing shelf life of crops due to antioxidant agents, and acting as an oxygen barrier that can bound oil oxidation (Ana Rita *et al*., 2018). Since preventing the penetration of oxygen and moisture into the crop and trapping metal ions are important characteristics of Chi, these features prevent adverse enzymatic and non-enzymatic reactance that leading to color changes and oxidation in the product (Chang *et al*., 2011).

Thyme essential oil (TEO) has maximum amounts of thymol, carvacrol, and para-cymin, all having antioxidant effects. Several studies were done to realize the antioxidant activities of different *Thymus* species extracts (Chatrabnous *et al.*, 2018; Raal *et al*., 2004). According to Martinez *et al*. (2013), walnut oil kept in ambient temperature has a vast shelf life if antioxidants are added into it.

The in-shelled walnut is generally packed in gunnysacks, wooden boxes, and baskets for local market, while kernels are packed in cardboard boxes of different capacity for international market. One of the new methods to control oxygen is the use of active packaging, based on applying of absorber or releaser compounds (Brockgreitens and Abbas, 2016).

In this study the effect of different types of coating materials containing natural antioxidants and packaging methods on physicochemical properties of walnut kernels were investigated at two different storage temperatures during 120 days.

Materials and Methods

In-shell walnuts (*Juglans regia* L.) were purchased from local market of Urmia, Iran in September 2018 and dried walnuts were manually shelled.

According to the method of Maghsoudlou *et al*., (2012) Chi (deacetylated P95%, and viscosity <630 mPa s) solutions at concentrations of 1% w/v in aqueous coating solutions, were prepared by dissolving Chi powder in an aqueous solution of glacial acetic acid 1% (v/v). Aqueous coating solutions were in three groups: 1) Ch: coated with Chi alone, 2) Chi and TEO were blended to obtain the final concentrations of Ch-$T_{500:}$ coated with Chi containing 500 ml/L TEO, 3) Ch-T_{1000}: coated with Chi containing 1000 ml/L TEO in aqueous coating solutions. Kernels were immersed in the coating solutions for 1min. Then, the coated samples and control samples were packed in 50-g packages as following; Gunny sack (G), polypropylene (PP) and active packaging in polypropylene containing sachets (AP). The packets were then stored for 120 day at ambient

temperature (Darkness, 25ºC at 45% RH) and refrigerator temperature (Darkness, 4ºC at 50% RH) and examined every 60 days.

Moisture content (MC) was measured by oven-drying (AOAC, 2012). The color indexes of kernels were measured using Hunter LAB colorimeter (model D65/10). About 20g of kernels were put on the transparent glass container that was put on top of the machine, indicating its color based of black/white (L^*), redness/greenness (a^*) and yellowness/ blueness (b^*) (Leahu *et al.,* 2013). N-hexane solvent was applied for extracting kernel's oil without heat treatment (Vanhanen and Savage, 2006). The PV was determined by AOCS (2003). At first, a mixture of oil and chloroform acetic acid 2:3 (v/v) with saturated potassium iodide solution was made in darkness; subsequently, the formed iodine was titrated with 0.1 N sodium thiosulphate until the yellow color disappeared: after adding starch indicator, titration was continued until the blue color just disappeared, too. Peroxide value (meq kg^{-1}) was calculated according to the formula:

PV = volume of sodium thiosulphate × 0.1 N × 1000/mass of oil.

The CDV were determined at 234 nm wavelength by means of a spectrophotometer (IUPAC 1992).

The FFA analyses were determined according to Nielson (2003). After data analysis with SPSS software 20.00**,** means were compared by Duncan's Multiple Range Test at significance level of 5%.

Results

Color

The effect of coated treatments and temperature as well as the effect of coated treatments and packaging methods and temperature on a^* value of walnut kernels were significant ($P<0.05$). During the storage, control samples showed a significant increase at both temperatures (Table 1). After 120 days of storage, the coated samples had a significant decrease in a^*, the least amount was observed in Chi at 25ºC and Ch-T_{1000} at 4°C. The samples coated with Ch-T_{500} with AP had the lowest values of index a^* at both temperatures (Table 2).

Table 1. The effect of coated treatments and temperature on a* value of kernels

Treatments	Storage time(d)	Temperature (ºC)	
		4	25
C	**1**	6.35±0.33^{e}	6.35±0.33^{e}
	60	11.7±0.5^{d}	14.80±0.12^{cd}
	120	12.30±0.67^{d}	17.10±0.26^{b}
Ch	**1**	18±0.09^{b}	18±0.09^{b}
	60	15.7±0.87^{bc}	14.72±0.03^{bc}
	120	2±0.83^{f}	0.2±0.07^{g}
Ch-T_{500}	**1**	12±0.44^{d}	12±0.44^{d}
	60	15.8±0.03^{c}	12.80±0.56^{d}
	120	2.8±0.13^{t}	5.9±0.93^{d}
Ch-T_{1000}	**1**	22.4±0.05^{a}	22.40±0.05^{a}
	60	16.90±0.88^{b}	17.45±0.23^{b}
	120	0.65±0.02^{g}	7.75±0.98^{e}

Notes: C, Control; Ch, coated with 1% chitosan; Ch-T_{500} and Ch-T_{1000}, coated with 1% chitosan containing 500 and 1000 ml/L TEO, respectively ($P < 0.05$).

Table 2. The effect of coated treatments and packaging and temperature on a* value

Treatments	Packaging	Temperature (ºC)	
		4	25
C	**G**	10.57±0.73^{de}	9.36±0.13^{e}
	PP	13.62±0.83^{c}	11.89±0.10^{d}
	AP	6.16±0.22^{e}	17.46±0.11^{ab}
Ch	**G**	10.44±0.40^{de}	11.09±0.63^{d}
	PP	15.72±0.19^{b}	9.80±0.84^{e}
	AP	9.29±0.43^{e}	11.95±0.93^{d}
Ch-T_{500}	**G**	12.46±0.03^{cd}	11.43±0.66^{d}
	PP	10.32±0.85^{de}	11.82±0.34^{d}
	AP	7.25±0.93^{e}	7.30±0.43^{e}
Ch-T_{1000}	**G**	17.82±0.23^{ab}	18.51±0.83^{a}
	PP	14.83±0.53^{bc}	16.32±0.63^{b}
	AP	7. 75±0.08^{e}	12.73±0.43^{cd}

Notes: C, Control; Ch, coated with 1% chitosan; Ch-T_{500} and Ch-T_{1000}, coated with 1% chitosan containing 500 and 1000 ml/L TEO respectively; G, PP and AP, gunnysacks, Packaging in polypropylene and active packaging, respectively ($P < 0.05$).

The effect of packaging methods and temperature on b^* values of walnuts were significant (P<0.05). In all samples, the b^* value was significantly reduced during storage, so that at the end of storage, the minimum values related to PP packaging at 4ºC and 25ºC (Table 3).

The L^* value of samples kept at 4ºC showed a significant increase, on the contrary, the samples kept at 25ºC showed a significant decrease (Fig. 1).

Table 3. The effect of packaging methods and temperature on b* values of kernels

Packaging	Storage time (d)	Temperature (˚C)	
		4	25
G	**1**	38±0.03^{a}	38±0.03^{a}
	60	12±0.45^{f}	20±0.54^{d}
	120	-3±0.13^{i}	-6.5±0.44^{h}
PP	**1**	38±0.33^{a}	38±0.33^{a}
	60	32.40±0.65^{b}	10.3±0.65^{g}
	120	-12.3±0.09^{f}	-1.45±0.63^{k}
AP	**1**	38±0.63^{a}	38±0.63^{a}
	60	22±0.05^{c}	16.25±0.93^{e}
	120	0.4±0.83^{j}	3±0.43^{i}

Notes: G, PP and AP, gunnysacks, Packaging in polypropylene and active packaging, respectively. ($P < 0.05$). Standard Error Mean=5.81.

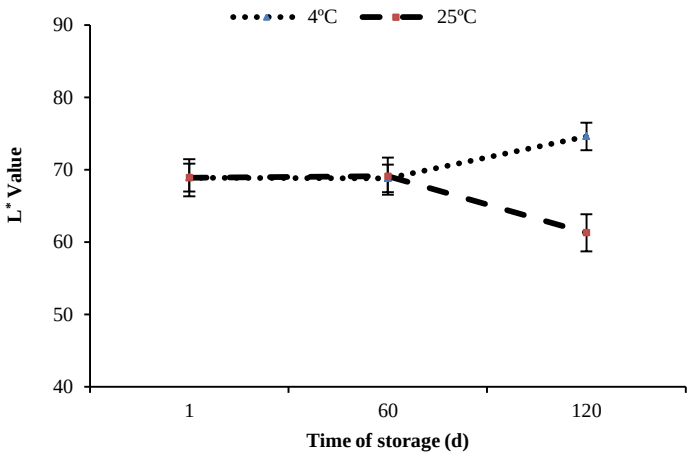

Fig. 1. Effects of two temperatures on L* value of kernels during storage ($P < 0.05$)

Moisture Content (MC)

Moisture content of walnut kernels in various packaging were significant at two temperatures (P<0.05). After 120 days of storage at 4ºC and 25°C, the coated samples exhibited the highest moisture, but no significant difference was observed between them (Fig. 2). The highest MC was related to AP, and the lowest MC was related to G (Fig. 3). The MC of active packaging was higher than polypropylene and gunnysacks, respectively.

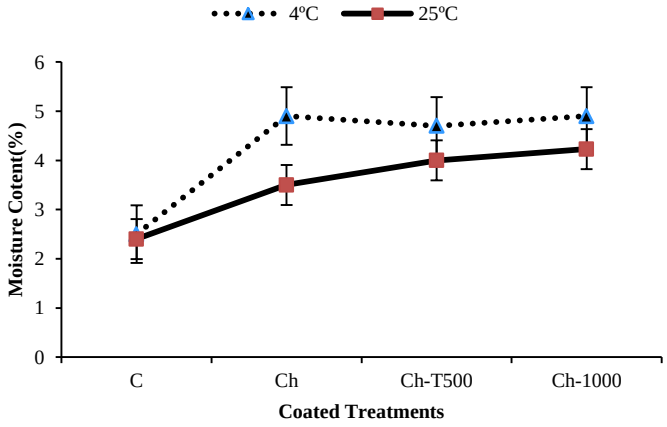

Fig. 2. Interaction effects of coated treatments and temperature on moisture content of kernels; C, Control; Ch, coated with 1% chitosan; Ch-T500 and Ch-T1000, coated with 1% chitosan containing 500 and 1000 microliter per liter TEO, respectively ($P < 0.05$)

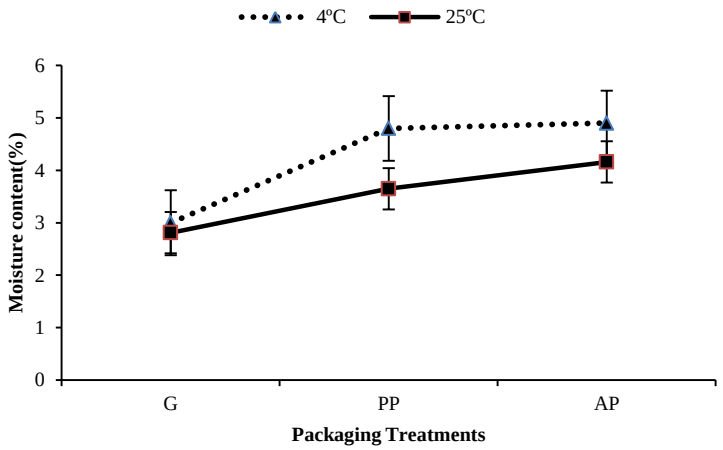

Fig. 3. Interaction effects of packaging treatments and temperature on moisture content of kernels; G, PP and AP gunnysack, Packaging in polypropylene, and active packaging, respectively ($P < 0.05$)

Peroxide Value (PV) and Conjugated Diene Values (CDV)

The effects of temperature on PV and CDV during storage were significant (P<0.05). During the storage, PV and CDV increased in both temperatures (Table 4), and the amounts of PV and CDV at 25ºC were more than those at 4ºC.

Table 4. The effect of time of storage and temperature on peroxide and conjugated diene values of kernel's oil

Test	Storage time (d)	Temperature (ºC)	
		4	25
Peroxide Value (meq/kg oil)	**1**	0.04 ± 0.01^{e}	0.04 ± 0.01^{e}
	60	0.22 ± 0.05^{d}	2.07 ± 0.04^{b}
	120	1.21 ± 0.03^{c}	6.02 ± 0.13^{a}
Conjugated diene value (µmol/g)	**1**	4.88 ± 0.01^{c}	4.88 ± 0.11^{c}
	60	5.04 ± 0.04^{c}	6.8 ± 0.02^{b}
	120	5.98 ± 0.03^{b}	10.55 ± 0.01^{a}

Notes: Superscript lower letters (a-e) beside mean values in rows and Superscript upper letters (a-c) beside mean values show the difference in Duncan's multiple range test ($P < 0.05$).

Free Fatty Acid (FFA)

During storage at two temperatures, the effect of coated treatments on free fatty acid of walnut were significant (P<0.05). During the storage, FFA increased at both temperatures, coated samples had high FFA compared to the control (Table 5). At the end of storage, free fatty acids values of samples at 4°C were more than those 25°C.

Table 5. The effect of coated treatments and temperature on free fatty acid of kernel's oil

Coated treatments	Storage time (d)	Temperature (ºC)	
		4	25
C	**1**	0.20±0.01^{f}	0.20±0.01^{f}
	60	0.37±0.02^{e}	0.29±0.03^{e}
	120	0.58±0.01^{d}	0.36±0.02^{e}
Ch	**1**	0.20±0.03^{e}	0.20±0.00^{e}
	60	0.41±0.00^{e}	0.48±0.01^{e}
	120	0.63±0.04^{d}	0.50±0.02^{de}
Ch-T_{500}	**1**	0.20±0.01^{f}	0.20±0.03^{f}
	60	3.14±0.07^{a}	1.07±0.01^{c}
	120	0.95±0.04^{c}	0.76±0.02^{d}
Ch-T_{1000}	**1**	0.20±0.01^{f}	0.20±0.03^{f}
	60	1.73±0.06^{b}	0.64±0.02^{d}
	120	0.95±0.05^{c}	0.79±0.00^{d}

Notes: C, Control; Ch, coated with 1% chitosan; Ch-T500 and Ch-T1000, coated with 1% chitosan containing 500 and 1000 ml/L TEO respectively ($P < 0.05$).

Discussion

Leahu *et al.* (2016) showed a pale green-yellow color spectrum appearance during storage of walnut oil, due to reduced amounts of a^* and b^*; After storing at various temperatures and lights for several months, oxidation of carotenoid and phenolic compounds happen and oil loosed its color intensity. Similar results have also been reported in peaches and avocado (Maftoonazad *et al*., 2005 and 2008). Reducing the L^* value at the end of the storage is a reason for reduced transparency the walnut. Walnuts packed with oxygen adsorbent at 4 and 21ºC over 12 months showed color variations by increasing temperature and light, causing the increase of a^* and b^* and decrease in L^* values (Mexis *et al*., 2009). The acceptable amount of the L^* value for the walnut kernel's color was more than 40 (Hill *et al*., 1997). During storage, along with decreasing in the L^* value, the amount of darkness in the kernels was increased. In this experiment, both 4ºC and 25ºC were acceptable. The positive values of L^* value highlight the brightness of the product and low values of the index a^* value indicate that the product has a greenish color; whereas, negative values of the b^* value indicate yellow color.

In all treatments, amounts of moisture content at 4 ºC are more than those at 25ºC. A high MC of a fat is associated with free fatty acid formation and hydrolytic rancidity. However, low MC accelerates oxidation due to the absence of water activity sending the fat into "hydration protection" mode (Gunstone and Padley, 1997). Acceptable amount of moisture of walnut kernels is 2-6% (Jensen *et al*., 2001). Vanhanen and Savage (2006) also suggested that walnut could be stored at temperatures below 23°C without major changes in MC.

Peroxide value (PV) indicates the oxidation degree of lipids with oxygen, temperature and light (Avramiuc, 2009). There is a positive correlation between peroxide value and conjugated dienes (CDV) content in walnut (Eliseeva *et al.*, 2017). 2meq.O_2/g is the standard point for peroxide value (Kanner, 2007). According to Kanner, at the end of the storage, PV of 4ºC was acceptable.

In nuts, the oleic fatty acid content is an index for measurement of free fatty acids. The rate of hydrolysis is determined by the free fatty acid content of the oil, the type of oil, the amount of dissolved water in the oil, as well as the storage conditions to which the oil is exposed

(Gunstone and Padley, 1997). At both temperatures, the coated samples showed high amounts of free fatty acids compared to the control sample. It can be related to their amount of moisture. The fatty acid content of less than 0.5% (oleic fatty acid) is acceptable for walnuts (Gunstone and Padley, 1997).

Conclusions

During storage, the shelf life and quality of walnut kernel can be affected by some environment factors. Coating materials and packaging methods can be useful for prolonging the postharvest quality of nuts. During the storage of walnut kernels, the speed and rate of rancidity increased by increasing temperature. Compared to gunny packaging, polypropylene packaging was also effective in protecting the qualitative properties of walnut. Active packaging offered considerable potential and it was proved the most efficient. Therefore, shelf life of walnut kernels at 4ºC with active packaging and Chi coated offered a considerable potential and proved the most efficiency.

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