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Interaction effects of natural antioxidants coating and various packaging on walnut kernel during storage at 25 °C

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Abstract

Increasing the walnut shelf life with non-synthetic materials is considered. The effect of edible coating and different packaging methods on physicochemical properties of Persian walnut kernel during 120 days of storage at 25 °C temperature was evaluated. The treatments were: C (control sample, uncoated), K (coated with 1 % chitosan) and K_{500} and K_{1000}, coated with chitosan 1 % containing 500 and 1000 µL L^{-1} of thyme essential oil, respectively, and MP (Mass packaging), PP (packaging in Polypropylene bags) and AP (Active packaging using sachets made by ascorbic acid, sodium bicarbonate and iron oxide). The color properties and oxidation of walnuts have relationship together. The results showed that the amount of a* and b* values were in the following order: $K_{1000} > K_{500} > K > C$. At the end of storage L* value of all samples decreased. Coated samples had less peroxide value and free fatty acid than control. Amount of free fatty acid in the coated samples with mass packaging was less than 0.5 %. The moisture content of coated samples was more than control samples, active packaging and PP packaging had minimum moisture fluctuations respectively, and control sample had less amount of moisture.

Key words: Chitosan, packaging, coating, physicochemical properties, thyme essential oil, walnut

Introduction

Oil yielding crop plants are very important for the economic growth of the agricultural sector. Walnut (*Juglans regia* L.) is one of the famous nuts because of edible kernel. During storage condition, walnut undergoes a series of biochemical, physiological and structural changes which make the kernels unpalatable to the consumer. Walnut kernel is a nutrient-rich food mainly because of its high biological-value proteins (low lysine/arginine ratio), high levels of oil (60 g/100 g average polyunsaturated fatty acids, PUFA) and antioxidants (phytosterols and polyphenols). Although fatty acids in walnuts are important from nutritional point of view, higher amounts of PUFA may result in poorer quality resistance and shorter shelf life of walnut products because of the presence of unsaturated bonds. Important factors influence the quality of walnuts during storage in the following order: temperature > degree of O_2 barrier > lighting conditions (Mexis *et al.,* 2009). The physical and chemical properties of pistachios packed in different gas conditions and high temperature have been associated with the exacerbation of oxidation of pigments and fatty acids (Raii *et al.,* 2007). Lipid oxidation was prevented by using a packaging material with low oxygen penetrance or by storage of walnuts in controlled atmospheres with low oxygen amount (Mate *et al.,* 1996).

Walnut kernels are full of phenolic compounds (Qamar and Sultana, 2011). The reason for darkening of the walnut kernel color can be oxidation of phenolic compounds. Oxygen would be stimulated from a single state to a triple state that would be caused to oxidation of phenolic compounds (Mexis *et al.,* 2009). So discoloration during storage is expected. During storage, oxidation process and changing of color depend on external conditions and important factors in determining the quality of walnut for marketing (Vaidya *et al.*, 2013). A clear, light yellowish-green color of walnut oil is desirable for many food applications, especially for salad dressings (Sze-Tao *et al.,* 2000). One of the famous colour systems is CIE-Lab (Pathare *et al.,* 2013). Lightness (L*), greenness (a*) and yellowness (b*) values were useful to determine the changing of peel color of walnut kernel and could be a useful replacement of subjective method of color charts (Kajuna *et al.*, 1998).

The most common oxidation indicator tests in nuts involve testing the peroxide value (PV) and the amount of free fatty acids (FFA).

Edible coatings such as chitosan forms a thin layer of barrier, against oxygen, moisture and protect the food from undesired chemical damage (Romanazzi *et al.,* 2010). Since preventing the penetration of oxygen and moisture into the crop and trapping metal ions are important features of chitosan; these characteristics prevent the occurrence of adverse enzymatic and non-enzymatic reactions that lead to color changes and oxidation in the product (Chang *et al.,* 2011).

Thyme essential oil (TEO) contains high levels of phenolic compounds, such as thymol and carvacrol, and the main component of its non-phenolic compounds is para-cymin, which act as antioxidant agent (Ozcan and Chalchat, 2004). According to Martinez *et al.* (2013), walnut oil kept in the dark warehouses and at ambient temperature had a greater shelf life and a significant reduction in lipid oxidation, if synthetic antioxidants are added into the oil.

Although the use of edible films creates a barrier against oxygen, it does not mean that it is the complete replacement of synthetic packaging materials, however secondary containment is required to limit the migration of gases (Bourtoom, 2008).

The shelf life of the products can be increased by removing oxygen from the package by oxygen absorbers and releasing carbon dioxide, in this consideration, active packaging is one of the emerging and exciting fields in packaging technology of food industries products. Active packaging has been characterized as packaging, which changes the inside atmosphere of the packed food (Ahvenainen, 2003).

This study evaluates the correlations established between coating and packaging of walnuts in delaying oxidation process that lead to physicochemical changes during storage at 25 ºC.

Materials and methods

Walnuts were purchased from the local market. Nuts were cracked and shelled. The composition of the walnut kernels is shown in Table 1. Chemicals used in this study were supplied by Merck and AppliChem Companies. Active packaging's sachets with ascorbic acid, sodium bicarbonate, iron powder in 1:1:1 ratio was prepared.

Table 1. Physico-chemical indicators of walnut kernels used in the analysis

Parameter	Content
Carbohydrate (%)	20.39±0.56
Protein (%)	15.07±0.65
Total oil (%)	59.14±0.66
Moisture (%)	3.16±0.03
Ash (%)	1.79±0.11

Mean values ± standard deviation over three replicates

Stages of the treatments: The chitosan solution (1 % w/v) was prepared by dissolving chitosan powder in glacial acetic acid 1 % (v/v) (Badawy and Rabea, 2011). Then the TEO (thyme essential oil) was added at concentration of 500 and 1000 µL L^{-1} to the solution. The walnut kernels were soaked in the coating solution for 60s. The treatments included: Control (uncoated) (C), coated with chitosan (K), coated with chitosan containing 500 µL L^{-1} TEO (K_{500}), and coated with chitosan containing 1000 µL L^{-1} TEO (K_{1000}). Then they were packed as following: Mass packaging (M), Packaging in polypropylene bags (PP) and Active packaging (polypropylene bags containing sachets of ascorbic acid, sodium bicarbonate and iron oxide) (A).

Packets were stored for 120 d at 25±1 °C with 45 % relative humidity (environment temperature) and tested every 60 d.

Physicochemical analyses: The color coordinates were recorded on the surface of the walnut kernel using Hunter LAB colorimeter (model D65/10). Two measurements were taken for each walnut kernel at two different places, and average value was recorded. The color parameters corresponding to the uniform color space CIELAB were obtained directly from the apparatus. a* is degree of redness to greenness, b* is degree of yellowness to blueness, L* is degree of lightness to darkness (Leahu *et al.*, 2013).

The oil was extracted from the walnut kernels using a hexane solvent without additional heat treatment (Vanhanen and Savage, 2006).

For the chemical analysis, each group of walnuts was homogenized thoroughly and then analyzed to determine the amount of protein by using the micro-Kjeldahl procedure, using 5.4 as conversion factor, fat (as an extractable component in Soxhlet apparatus) and protein (as crude nitrogen x 6.25), ash (after drying at 105 °C, and carbonizing first at 250°C, then gradually ramping up the temperature to 600 °C overnight) (Leahu *et al.*, 2013).

Peroxide value (PV) and moisture of the samples were determined according to the AOAC methods (AOAC, 1995). Free fatty acids of the samples were determined according to the oleic acid percentage (Standard EN ISO 660).

Statistical analysis: A factorial method in the form of a complete randomized design was used for the statistical design of the present study. The results were analyzed using SPSS 20 statistical software.

Results and discussion

The walnut samples on the 60th and 120th days of storage at 25 °C showed changes in colour parameters. The changes in a* value are given in Fig. 1. As a result of the analysis of variance, the interaction effect of time of storage and coating treatments on the a* value of walnut kernels during 120 d of storage were significant (P<0.05). After 120 d storage; coated samples had a significant decrease in a*, the least amount was observed in

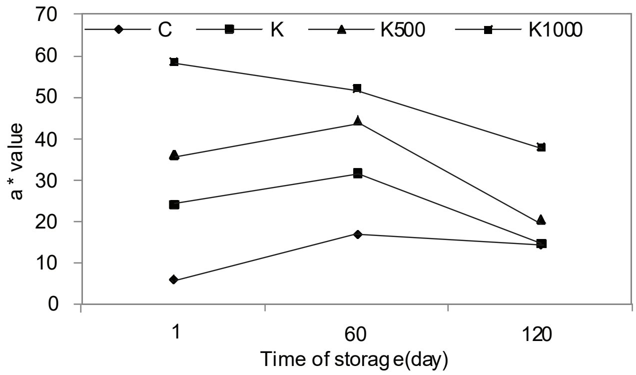

Fig.1. Interaction effect of time of storage and coating treatments on a* value of walnut ; C, Control, uncoated; K, coated with 1 % chitosan; K_{500} and K_{1000}, coated with 1 % chitosan containing 500 and 1000 µL L^{-1} TEO, respectively (P < 0.05).

coated samples with chitosan alone at 25 °C. The coated samples with 1000 (µL/L) TEO had the highest a* value. a* value of coated samples was higher than control. The samples which have tendency to the red spectrum will own the most suitable a* value.

The changes in b* value during the storage are given in Fig. 2. As a result of the analysis of variance, the interaction effect of time of storage and coating treatments on b* value of walnuts during storage were significant (P<0.05). In all samples, during storage, the b* value was significantly reduced. In walnuts, decline in a* and b* value results appearance of a pale green-yellow color spectrum, developed during storage at different temperatures and walnut loose color intensity (Leahu *et al.*, 2016). A decline in b* value was reported in the color of pectin-coated peaches

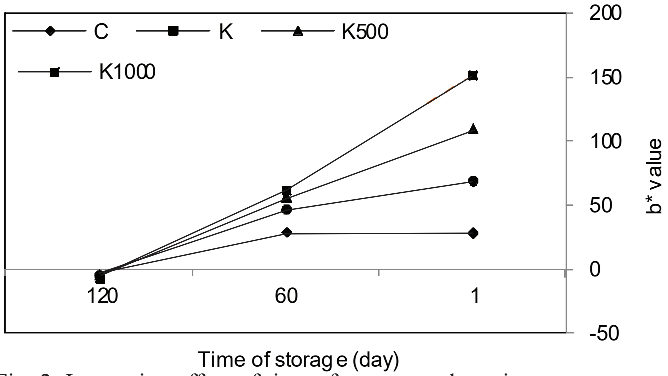

Fig. 2. Interaction effect of time of storage and coating treatments on b* value of walnut; C, Control, uncoated; K, coated with 1 % chitosan; K_{500} and K_{1000}, coated with 1 % chitosan containing 500 and 1000 µL L^{-1} TEO, respectively; (P < 0.05).

Table 2. Effect of time of storage on L* value of walnut kernels

	L* value	
1 (day)	60 (day)	120 (day)
68.90±0.03^{A}	69.07±0.73^{A}	61.30±0.33^{B}

Superscript upper letters (A-B) beside mean values in columns show the difference in Duncan's multiple range test (P < 0.05). Standard Error Mean=1.84.

(Maftoonazad *et al.*, 2008). A reduction in the color properties of avocado fruit was reported when covered with methyl cellulose (Maftoonazad *et al.*, 2005).

Variations in L* value during the storage are given in Table 2. The L* value of samples kept at 25 °C showed a significant decrease. The decreased L* value at the end of the storage resulted in reduction of walnut transparency. Walnuts packed with oxygen adsorbent at 4 and 21 °C during 12 months showed color variations by increasing of temperature and light that caused increase in a* and b* and decrease in L* values (Mexis *et al.*, 2009). The acceptable value of the L* for the walnut color was above 40 (Hill *et al.*, 1997). During storage, along with decrease in the L* value, the amount of darkness in the kernels was increased. In this experiment, samples kept at 25 °C were acceptable. The positive values of the index L* highlight the brightness of the product, low values of the index a* indicate that the product has a greenish color and negative values of the index b* indicate yellow coloring.

Moisture is one of the important factors of the quality of nuts. As a result of the analysis of variance, the interaction effect of time of storage and coating treatments was as significant as interaction effect of time of storage and kind of packaging on moisture of walnut after 120 d (P<0.05). After 120 days of storage at 25 °C, the highest humidity was related to coated samples, and the lowest moisture content was related to mass packing (Fig. 3). At the end of storage, the moisture content of coated samples was measured between 2.23- 2.38 %, and the moisture content of samples which were packed in PP and active packaging was 2.14 and 2.92 %, respectively which is within the acceptable range for walnut kernels (2-6 %) (Jensen *et al.*, 2001). Vanhanen and Savage (2006) found that walnut flour can be stored in plastic bags for 26 weeks at temperatures below 23 °C without major changes in moisture content. The main task of food packaging is often the prevention or reduction of the exchange of moisture between the food and the surrounding atmosphere, so the amount of water vapor permeability factor in the film used in packaging should be as low as possible (Gontard *et al.*, 2007).

The initial Peroxide Value (PV) in the walnut was about 0.04 meq/kg (Fig. 4). Interaction effect of time of storage and coating treatments on peroxide value during storage were significant

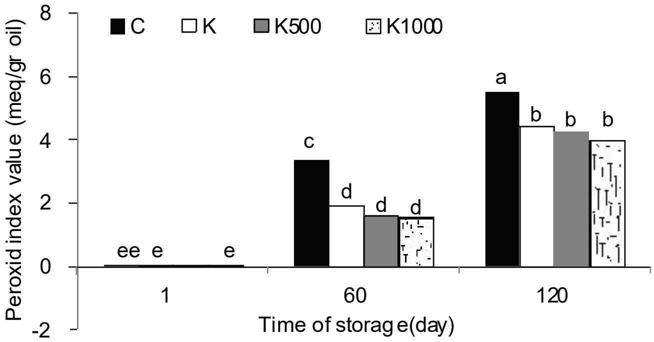

Fig. 4. Interaction effect of time of storage and coating treatments on peroxide value of walnut ; C, Control, uncoated; K, coated with 1 % chitosan; K_{500} and K_{1000}, coated with 1 % chitosan containing 500 and 1000 µL L^{-1} TEO, respectively; (P < 0.05).

(P<0.05). The peroxide index indicates the oxidation degree of lipids by means of oxygen, temperature and light action (Avramiuc, 2009). During the storage, the amount of peroxide increased so coated samples stored at 25 °C had less peroxide than control. There wasn't any significant difference between coating treatments.

In walnut, the oleic fatty acid content is an index for the measurement of free fatty acids. The effect of time of storage and coating treatments and packaging on free fatty acid of walnut

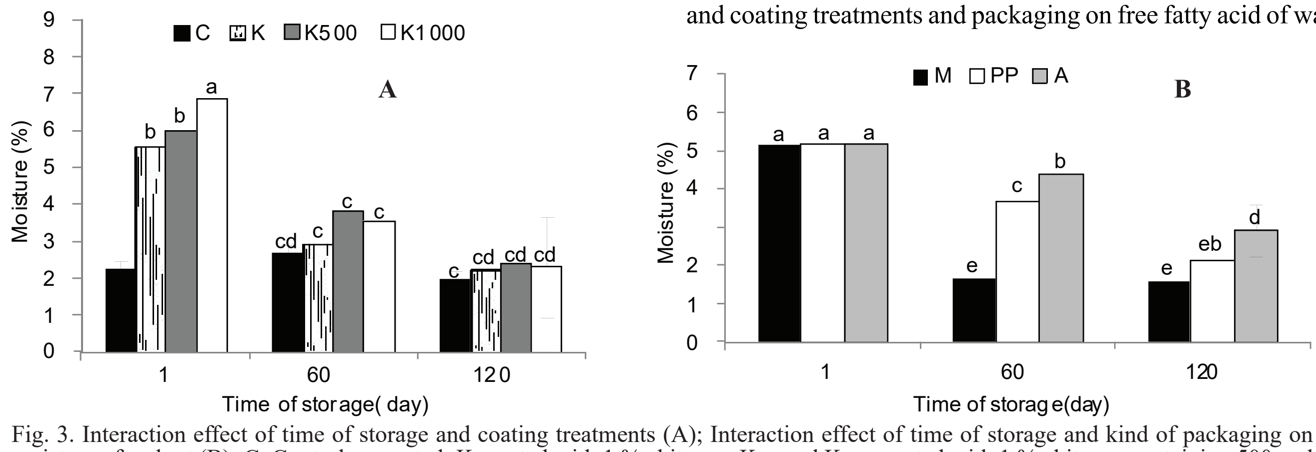

Fig. 3. Interaction effect of time of storage and coating treatments (A); Interaction effect of time of storage and kind of packaging on moisture of walnut (B); C, Control, uncoated; K, coated with 1 % chitosan; K_{500} and K_{1000}, coated with 1 % chitosan containing 500 and 1000 µL L^{-1} TEO, respectively, M, PP and A, mass packaging, Packaging in polypropylene bags and Active packaging, respectively; Mean values with the same letter are not significantly different at Duncan's multiple range test (P < 0.05).

Table 3. Interaction effect of time of storage and coating treatments and packaging on free fatty acid value of walnut

Packaging	Treatments	Storage time (day)		
		1	60	120
M	C	0.20^{Ca}	0.20^{Ea}	0.26^{Ga}
	K	0.50^{Ba}	0.13^{Fc}	0.34^{Gb}
	K_{500}	0.56^{Aa}	0.14^{Fc}	0.40^{Fb}
	K_{1000}	0.56^{Aa}	0.29^{Dc}	0.40^{Fb}
PP	C	0.20^{Ca}	0.14^{Fb}	0.27^{Ga}
	K	0.50^{Bb}	0.74^{Ca}	0.54^{Eb}
	K_{500}	0.56^{Ac}	1.38^{Ba}	0.96^{Bb}
	K_{1000}	0.56^{Ac}	0.84^{Cb}	1.17^{Aa}
A	C	0.20^{Cb}	0.54^{Da}	0.58^{Ea}
	K	0.50^{Bc}	0.6^{Cb}	0.73^{Da}
	K_{500}	0.56^{Ac}	1.7^{Aa}	0.93^{Bb}
	K_{1000}	0.56^{Ab}	0.82^{Ca}	0.81^{Ca}

Notes: C, Control, uncoated; K, coated with 1 % chitosan; K_{500} and K_{1000}, coated with 1 % chitosan containing 500 and 1000 µL L-1 TEO respectively. Superscript lower letters (a-d) beside mean values in rows and Superscript upper letters (A-D) beside mean values in columns show the difference in Duncan's multiple range test ($P < 0.05$). Standard Error Mean=0.133.

during storage at 25 °C was significant (P<0.05). At the end of storage, free fatty acids increased more than the first day (Table 3). Coated samples showed high amount of free fatty acids than the control sample. The fatty acid content of less than 0.5 % (oleic fatty acid) is acceptable for walnuts (Swarthout *et al.*, 1958). Amount of free fatty acid in the coated samples with mass packaging is less than 0.5 %.

The study revealed that effect of edible coating and different packaging methods influenced the physico-chemical properties of Persian walnut kernel during 120 days of storage at 25 °C. The treatments had favourable effect on color properties and oxidation of walnuts.

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