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RESEARCH ARTICLE

Evaluation of shelf life of walnut kernel coated by antioxidants in combination with packaging under different storage conditions

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ABSTRACT

The interaction effects of edible coating and packaging on Persian walnut kernel to extend shelf life by reducing oxidation and changing of color were evaluated. Coating with chitosan (1%) in combination with thyme essential oil (TEO) at different concentrations (500 and 1000 μL/L) (CT_{500}, CT_{1000}) or chitosan alone (CT) in corporate with different packaging methods (Loose packaging, packaging in polypropylene bags and Active packaging) (LP, PP, AP) compared with control walnut (C) were used. Storage conditions: (darkness; 55% RH, 4ºC and 45% RH, 25ºC). The results showed that amounts of L*, a*, b* values in treated samples decreased and samples which stored at 4ºC have acceptable amount. The minimum moisture fluctuations in all packaging related to 4°C. The peroxide and conjugated diene values at 4°C were lower amount. Treatments did not have positive effect on free fatty acids of walnut kernels.

Keywords: Antioxidants, Packaging, Shelf life, Walnut.

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INTRODUCTION

Persian walnut (*Juglans regia* L.) is famous nut because of edible kernel. During storage condition, walnut undergoes a series of biochemical, physiological and structural changes which make the kernels unacceptable to the consumer. Walnut kernel is a nutrient-rich food majorly because of its high biological-value proteins (low lysine/ arginine ratio), high levels of oil (60 g/ 100 g in average mainly polyunsaturated fatty acids, PUFA and antioxidants (phytosterols and polyphenols) (Savage 2001). Although fatty acids in walnuts are good from a nutritional value, higher amounts of PUFA may result in poorer quality resistance and shorter shelf life of walnut products because of the presence of unsaturated bands. A number of experiments have been carried out on the oxidation stability of walnut. Temperature, light, moisture and exposure to oxygen have been found to be the main contributing factors to oxidation (Salcedo et al., 2010). Oxidative rancidity results from the changes that occur from reactions with atmospheric oxygen. Lipid oxidation was prevented by using a packaging material with low oxygen penetrance or by storage the walnuts in controlled atmospheres with low oxygen amount (Mate et al., 1996). Hydrolytic rancidity is most often caused by a combination of moisture and enzymes interacting with the fat or oil. The walnut kernel has

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bioactive compounds such as phenols, tocopherols and phytosterols (Trandafir et al., 2017). Polyphenols, were subjected to oxidation, their concentrations decreased more in the presence of O_2 (Vidrih et al., 2012). The reason for darkening of the walnut kernel color can be oxidation of phenolic compounds. During storage, oxidation process and changing of color depend on external conditions (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 color systems is CIE-L*a* b*. Lightness (L*), greenness (a*) and yellowness (b*) values are useful to determine the changing of peel color of walnut kernel and can be a useful replacement of subjective method of color charts (Pathare et al., 2013). Peroxide value (PV), Free fatty acids (FFA), and Conjugated diene value (CDV) are useful methods of detecting oxidized and/or hydrolyzed fats in foods. Moisture is one of the important factors of the quality of nuts (Nelson and Labuza 1994). The moisture content (MC) of nuts has a profound influence on their physical, chemical, mechanical, aerodynamic, and thermal properties (Seyed and Taghizadeh 2007).

Safe methods with no toxic substances are needed for food safety issue. In recent years, edible coatings appear to be one of the most innovative ways to improve the commercial shelf life of fruits. An edible coating such as chitosan makes a barrier against moisture and oxygen (Ana Rita et al., 2018). 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 all have antioxidant agent (Ozcan and Chalchat 2004). According to Martinez et al. (2013) walnut oil which is kept in ambient temperature has a greater shelf life if antioxidants are added into the oil. Although edible films create a barrier against oxygen and moisture, but they aren't perfect replacement of synthetic packaging (Bourtoom 2008). A large variety of active packaging systems has been developed and, to date, numerous reviews have emphasized the potential of active packaging technologies to supply safer, "healthier," and higher-quality foods to the consumer (Brockgreitens and Abbas, 2016). Active packaging has been characterized by changing the inside atmosphere of the packed food (Kuorwel et al., 2015). In this study the effect of different types of coating materials containing natural antioxidants and packaging methods on physicochemical properties of walnut kernel was investigated during 120 days of storage under two temperatures storage.

MATERIALS AND METHODS

In-shell Walnuts (*Juglans regia* L) were purchased from local market. Dried walnuts were shelled manually. Chemicals used in this study were supplied by Merck and AppliChem Companies.

Coating method of walnut kernels

The chitosan solution (1% w/v) was prepared by dissolving of chitosan powder in glacial acetic acid (1% v/v). The solution was heated. Glycerol was added as a plasticizer (Badawy and Rabea, 2011). Tween 80 to achieve uniform distribution of essential oil inside the coating solution was used. TEO (500 and 1000 µL/L) was added to the solution, finally uniform solutions exposed to UV light 1 hr for sterilization. First, Kernels were soaked in the coating solutions for 60s. Secondary, samples were dried at room temperature. The treatments included: Control, uncoated (C), coated with chitosan (CT), coated with chitosan containing 500 µL/L Thyme Essential Oil (CT_{500}), and coated with chitosan containing 1000 µL/L Thyme Essential Oil (CT_{1000}). Third, each treatment was divided into 3 equal parts and then they packed as following; Loose packaging (LP), Packaging in polypropylene bags (PP) and Active packaging in polypropylene bags containing sachets (AP). At last, the packets were then stored for 120 day at ambient temperature (Darkness, 25ºC at 45% RH) and refrigerator temperature (Darkness, 4ºC at 55% RH) tested every 60 days.

Color and moisture content

The color indexes of the walnut kernel were measured using Hunter LAB colorimeter (model D65/10). About 20g of kernels were put on the transparent glass container and were put on top of the machine which states the color of it on the basis of black/white (L*), redness/greenness (a*) and yellowness/ blueness (b*) (Leahu et al., 2013). Moisture content (MC) of the kernels was determined by oven-drying at 103±2 °C (AOAC 2012).

Oil extraction

The oil was extracted from kernels using n-hexane solvent without additional heat treatment. About 50 g of ground walnut was mixed with 50 mL of n-hexane (J.T. Baker, Deventer, Holland) and stirred for 30 min. Then-hexane extract was filtered, and the solvent was removed under reduced pressure using a rotavapor (RE 111; Büchi, Flawil, Switzerland) (Vanhanen and Savage, 2006).

Peroxide Value (PV)

First, acetic acid–chloroform solution and saturated potassium iodide (KI) solution added to oil sample. Secondary, after adding 30 mL distilled water, slowly titrate with 0.01-N sodium thiosulfate while shaking the flask vigorously near the end point which is a faint blue color. Third, the sodium thiosulfate ($Na_2S_2O_3$) dropwise until the blue color just disappears was added. Finally, the Peroxide Value (meq/ kg) of oil was calculated according to following equation:

$$PV = \frac{V \times N \times 1000}{W}$$

Where: V is volume of applied sodium thiosulfate (mL), N is the normality of thiosulfate and W is the oil weight (g) (AOCS 2003).

Conjugated Diene Values (CDV) and Free Fatty Acid (FFA)

The CDV were determined at 233 nm wavelength using isooctane as an oil solvent. 0.1-0.3 g of oil was mixed with the isooctane solution and the amount of adsorption of the solution at 233 nm using a spectrophotometer (Pharmacia, England) (IUPAC 1992). Free fatty acid (FFA) analyses were determined according to Nielson (2003).

Statistical analysis

The study was carried out as factorial experiments based on a complete randomized design with 4 replicates. Data were subjected to analysis of variance (ANOVA) followed by LSD test ($P<0.05$) to distinguish differences among the treatments. Data were analyzed with SPSS.

RESULTS AND DISCUSSION

Color

As a result of the analysis of variance, the effect of coated treatments and temperature as well as the effect of coated treatments and packaging methods and temperature on the 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 d storage; Coated Samples had a significant decrease in a*, the least amount was observed in CT at 25 ºC and CT_{1000} at 4°C. At both temperatures the samples coated with CT_{500} with AP had the lowest values of index a* (Table 2).

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

Treatments	Storage time(d)	Temperature (ºC) 4	25
C	1	6.35 ± 0.33^{Ea}	6.35 ± 0.33^{Ea}
	60	14.80 ± 0.12^{Ca}	11.7 ± 0.54^{Db}
	120	17.10 ± 0.26^{Ba}	12.30 ± 0.67^{Db}
CT	1	18 ± 0.09^{Ba}	18 ± 0.09^{Ba}
	60	14.72 ± 0.03^{Cb}	15.7 ± 0.87^{Ca}
	120	0.2 ± 0.07^{Fb}	2 ± 0.83^{Fa}
CT_{500}	1	12 ± 0.44^{Da}	12 ± 0.44^{Da}
	60	12.80 ± 0.56^{Db}	15.8 ± 0.03^{Ca}
	120	5.9 ± 0.93^{Ea}	2.8 ± 0.13^{Fb}
CT_{1000}	1	22.40 ± 0.05^{Aa}	22.4 ± 0.05^{Aa}
	60	17.45 ± 0.23^{Ba}	16.90 ± 0.88^{Cb}
	120	7.75 ± 0.98^{Ea}	0.65 ± 0.02^{Gb}

Notes: C, Control, uncoated; CT, coated with 1% chitosan; CT_{500} and CT_{1000}, coated with 1% chitosan containing 500 and 1000 µL/L 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=2.86.

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

Treatments	Packaging	Temperature (ºC) 4	25
C	LP	9.36 ± 0.13^{Cb}	10.57 ± 0.73^{Da}
	PP	11.89 ± 0.10^{Bb}	13.62 ± 0.83^{Ca}
	AP	17.46 ± 0.11^{Aa}	6.16 ± 0.22^{Fb}
CT	LP	11.09 ± 0.63^{Ba}	10.44 ± 0.40^{Db}
	PP	9.80 ± 0.84^{Cb}	15.72 ± 0.19^{Ba}
	AP	11.95 ± 0.93^{Ba}	9.29 ± 0.43^{Db}
CT_{500}	LP	11.43 ± 0.66^{Bb}	12.46 ± 0.03^{Ca}
	PP	11.82 ± 0.34^{Ba}	10.32 ± 0.85^{Db}
	AP	7.30 ± 0.43^{Da}	7.25 ± 0.93^{Ea}
CT_{1000}	LP	18.51 ± 0.83^{Aa}	17.82 ± 0.23^{Ab}
	PP	16.32 ± 0.63^{ABa}	14.83 ± 0.53^{Bb}
	AP	12.73 ± 0.43^{Ba}	7.75 ± 0.08^{Eb}

Notes: C, Control, uncoated; CT, coated with 1% chitosan; CT_{500} and CT_{1000}, coated with 1% chitosan containing 500 and 1000 µL/L TEO respectively; MP, PP and AP, Mass packaging, Packaging in polypropylene bags and Active packaging 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=2.86.

As a result of the analysis of variance, the effect of packaging methods and temperature on b* values of walnuts were significant (p<0.05). In all samples, during storage, the b* value was significantly reduced, so that at the end of maintenance, the minimum values related to PP packaging at 4ºC and 25ºC (Table 3). The L* value of samples kept at 4 ºC showed a significantly increase, in the contrary, the samples which kept at 25ºC showed a significant decrease (Table 4).

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

Packaging	Storage time(d)	Temperature (ºC) 4	25
LP	1	38±0.03^{Aa}	38±0.03^{Aa}
	60	20±0.54^{Ba}	12±0.45^{Db}
	120	-6.5±0.44^{Gb}	3±0.13^{Fa}-
PP	1	38±0.33^{Aa}	38±0.33^{Aa}
	60	10.3±0.65^{Db}	32.40±0.65^{Ba}
	120	-1.45±0.63^{Fa}	-12.3±0.09^{Gb}
AP	1	38±0.63^{Aa}	38±0.63^{Aa}
	60	16.25±0.93^{Cb}	22±0.05^{Ca}
	120	3±0.43^{Ea}	0.4±0.83^{Eb}

Notes: LP, PP and AP, Loose packaging, Packaging in polypropylene bags and Active packaging 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=5.81.

Table 4: The effect of temperature on L* values of kernels during 120 d of storage

Temperature (ºC)	Time of storage (d) 1	60	120
4	74.60±0.67^{Aa}	68.8±0.01^{Ab}	68.9±0.44^{Ab}
25	61.30±0.41^{Bb}	69.09±0.53^{Aa}	68.9±0.44^{Aa}

Notes: 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=1.54.

Table 5: The effect of coated treatments and temperature on MC of kernels

Coated treatments	MC (%) Temperature (ºC) 4	25
C	2.5±0.05^{Ba}	2.4±0.06^{Ca}
CT	4.9±0.11^{Aa}	3.5±0.08^{Bb}
CT_{500}	4.7±0.09^{Aa}	4±0.15^{Ab}
CT_{1000}	4.9±0.07^{Aa}	4.23±0.18^{Ab}

Notes: C, Control, uncoated; CT, coated with 1% chitosan; CT_{500} and CT_{1000}, coated with 1% chitosan containing 500 and 1000 µL/L 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=0.18.

Moisture content (MC)

As a result of the analysis of variance, MC of walnut kernels in various packaging at two temperatures were significant (p<0.05). After 120 days of storage at 4ºC and 25°C, the highest moisture was related to coated samples, but there aren't significant difference between them (Table 5). The highest MC was related to AP, and the lowest MC was related to LP (Table 6). The MC of active packaging was higher than polypropylene and loose packaging, respectively

Table 6: The effect of packaging treatments and temperature on MC of kernels

Packaging treatments	MC (%)	
	Temperature (ºC)	
	4	25
LP	3±0.15^{Ba}	2.81±0.18^{Ca}
PP	4.8±0.09^{Aa}	3.65±0.16^{Bb}
AP	4.9±0.01^{Aa}	4.16±0.04^{Ab}

Notes: LP, PP and AP, Loose packaging, Packaging in polypropylene bags and Active packaging 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=0.15.

Amounts of moisture content at 4ºC are more than 25ºC in all treatments. 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 in water activity sending the fat into 'hydration protection' mode (Gunstone and Padley, 1997). Acceptable range of moisture of walnut kernels is 2-6% (Jensen et al., 2001). Vanhanen and Savage (2006) also suggested that walnut can be stored at temperatures below 23°C without major changes in MC.

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

The peroxide value (PV) indicates the oxidation degree of lipids with oxygen, temperature and light (Avramiuc, 2009). There is positive correlation between peroxide value and conjugated dienes (CDV) content in walnut (Eliseeva et al., 2017). As a result of the analysis of variance, 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 7). During the storage amounts of PV and CDV at 25ºC were more than 4ºC. Different Standard points for Peroxide value is mentioned in the nuts: Naturland (2002) = 1 meq.O2/g; UNECEF Standard (2002) = 5 meq.O2/g; Kanner (2007) =2meq.O2/g. according to Kanner at the end of the storage PV of 4 °C was acceptable.

Table 7: The effect of time of storage and temperature on PV and CDV of kernel's oil

Test	Time(d)	Temperature (ºC)	
		4	25
Peroxide Value (meq/kg oil) SEM=0.4	1	0.04±0.01^{Ca}	0.04±0.01^{Ca}
	60	0.22±0.05^{Bb}	2.07±0.04^{Ba}
	120	1.21±0.03^{Ab}	6.02±0.13^{Aa}
Conjugated diene value (µmol/g) SEM=0.52	1	4.88±0.01^{Ca}	4.88±0.11^{Ca}
	60	5.04±0.04^{Bb}	6.8±0.02^{Ba}
	120	5.98±0.03^{Ab}	10.55±0.01^{Aa}

Notes: 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).

Free Fatty Acid (FFA)

In nuts, the oleic fatty acid content is an index for measurement of free fatty acids. As a result of the analysis of variance, the effect of coated treatments on free fatty acid of walnut during storage at two temperatures were significant ($p<0.05$). During the storage, FFA increased at both temperatures, coated samples had high FFA in comparison to the control (Table 8). At the end of storage, free fatty acids of samples at 4°C and were more than 25°C.

Table 8: 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^{Ca}	0.20 ± 0.01^{Da}
	60	0.29 ± 0.03^{BCa}	0.37 ± 0.02^{CDa}
	120	0.36 ± 0.02^{BCab}	0.58 ± 0.01^{CDa}
CT	1	0.20 ± 0.00^{Ca}	0.20 ± 0.03^{Da}
	60	0.48 ± 0.01^{BCa}	0.41 ± 0.00^{CDa}
	120	0.50 ± 0.02^{BCa}	0.63 ± 0.04^{CDa}
CT_{500}	1	0.20 ± 0.03^{Ca}	0.20 ± 0.01^{Da}
	60	1.07 ± 0.01^{Ab}	3.14 ± 0.07^{Aa}
	120	0.76 ± 0.02^{Ba}	0.95 ± 0.04^{Ca}
CT_{1000}	1	0.20 ± 0.03^{Ca}	0.20 ± 0.01^{Da}
	60	0.64 ± 0.02^{BCb}	1.73 ± 0.06^{Ba}
	120	0.79 ± 0.00^{Ba}	0.95 ± 0.05^{Ca}

Notes: C, Control, uncoated; CT, coated with 1% chitosan; CT_{500} and CT_{1000}, coated with 1% chitosan containing 500 and 1000 µL/L 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=0.24.

The rate of hydrolysis is determined by the free fatty acid (FFA) content of the oil, the type of oil, the amount of dissolved water in the oil, and the storage conditions to which the oil is exposed (Gunstone and Padley, 1997). At both temperatures, coated samples showed high amount of free fatty acids in relative to the control sample. It can be related to amount of moisture of them. The fatty acid content of less than 0.5% (oleic fatty acid) is acceptable for walnuts (Swarthout et al., 1958).

CONCLUSION

After 120 d of storage, with increasing of temperature, the speed and the rate of rancidity increased. During the storage amounts of PV and CDV at 25ºC were more than 4ºC. At both temperatures, the moisture content of active packaging was higher than polypropylene and mass packaging, respectively. The color of walnut kernels at 4ºC is better than 25ºC. So shelf life of walnut kernels at 4ºC with active packaging and chitosan coated are the best.

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