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Effects of chitosan coating enriched with thyme essential oil and packaging methods on a postharvest quality of Persian walnut under cold storage

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

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

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

[3] Horticultural Department , University of Tehran, Karaj, Iran

* e-mail: s.zomorodi@areeo.ac.ir

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Abstract: This study evaluated the effects of edible coatings and different packaging methods on the shelf-life and quality of walnut kernels. It focused on the coatings with chitosan (1%) and thyme essential oil (TEO) at concentrations of 500 and 1,000 µl L^{-1} (CT_{500}, $CT_{1,000}$) or with chitosan alone (CT). The effects of the coatings was assessed for different packaging methods (LP, loose packaging; PP, packaging in polypropylene bags; and AP, active packaging) as contrasted to control walnuts (C). Walnuts were stored for 120 days in darkness, with relative humidity of 55%, at 4°C. The results showed that the L* index and moisture content of the samples in the chitosan with 500 and 1,000 µl L^{-1} thyme essential oil in active packaging were maximum, whereas peroxide and conjugated diene values were minimum. The lowest rate of mold growth was observed for the chitosan samples with 500 µl L^{-1} thyme essential oil in active packaging. The best overall acceptability score was related to the samples with chitosan alone and the chitosan with 500 µl L^{-1} thyme essential oil in active packaging. The chitosan alone and the chitosan with 500 µl L^{-1} thyme essential oil in active packaging are recommended for storage of kernels at 4°C.

Keywords: Active packaging, chitosan, thyme essential oil, quality, walnut

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INTRODUCTION

Walnuts play an important part in human diet since ancient times. The walnut (*Juglans regia* L.) is quite widespread in Iran. During storage, kernels undergo a series of biochemical, physiological, and structural changes, which make them unacceptable to consumers. Walnut 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 in average mainly polyunsaturated fatty acids, or PUFA) [1]. Although fatty acids in walnuts have nutritional value, higher amounts of PUFA (owning unsaturated bands) may cause a poorer quality resistance and a shorter shelf-life [2]. Low oxygen prevent lipid oxidation. The most common oxidation indicators in oils are peroxide value (PV) and conjugated diene value (CDV) [3]. Walnut kernels contain bioactive compounds such as phenols, so polyphenols are subject to oxidation [4, 5]. The walnut kernel can darken due to oxidation of phenolic compounds. L* index shows brightness of products [6]. Moisture is one of the important factors of the quality of nuts [7]. Moisture content (MC) of nuts has a profound effect on their physical, chemical, mechanical, aerodynamic, and thermal properties [8]. Postharvest operations are expected to have a major impact on the microbial contamination of nuts [9]. Among various microbes, fungi are known to play a significant role in the spoilage and loss of stored plant products [10].

Food safety issue requires safe methods with no toxic substances. In recent years, edible coatings have been 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, oxygen, and dissolved materials and protects foodstuff from microbial, chemical, and mechanical damages [11, 12]. Chitosan has a higher expansion and elasticity, as well as anti-viral, anti-bacterial, anti-fungi, and antioxidant effects due to different amounts of free amine groups. It can participate in the reactions by forming hydrogen and ionic bonds [13]. Chitosan reduced the growth of *Aspergillus flavus*, the absorption of moisture, and the rate of oxidative reactions [14]. Essential oils (EOs) have been extensively studied as additives in bio-based emulsified coatings. One example is the study by Campos-Requena *et al*. based on carvacrol and thymol, both included in HDPE/modified montmorillonite nanocomposite films [15]. Thyme essential oil (TEO) contains high levels of phenolic compounds, such as thymol and carvacrol. The main component of non-phenolic compounds in TEO is paracymin. Thymol, carvacrol, and paracymin are all antioxidant agents [16]. Although edible coatings create a barrier against oxygen and moisture, they are not perfect replacement of synthetic packaging [17]. A large variety of active packaging systems have been developed and. Today, numerous reviews have emphasized the potential of active packaging technologies to supply safer, 'healthier', and higher-quality foods to consumers [18]. Active packaging is characterized by changing the inside atmosphere of the packed food [19]. Unfavorable flavors, caused by rancidity during storage of the product, did not appear when oxygen adsorbents were applied [2].

This study investigates the effects of chitosan coating enriched with thyme essential oil and types of packaging on the postharvest quality of Persian walnut under cold storage.

STUDY OBJECTS AND METHODS

The study was conducted with walnuts (*Juglans regia* L.) purchased from the local market. Walnuts were shelled manually. The kernels were dried at room temperature till moisture contents of 3.16 ± 0.03%. The chemicals were supplied by Merck and AppliChem Companies. Sachets for active packaging were prepared with ascorbic acid, sodium bicarbonate, and iron powder with the 1:1:1 ratio.

The chitosan solution (1%, w/v) was prepared by dissolving chitosan powder in glacial acetic acid (1%, v/v). The solution was heated, and then glycerol was added as a plasticizer [20]. Tween 80 was used to achieve uniform distribution of essential oil inside the coating solution. TEO (500 and 1,000 µl L^{-1}) was added to the solution; finally the uniform solutions were exposed to UV light for 1 hour for sterilization.

First, the kernels were soaked in the coating solutions for 60 s. Second, the samples were dried at room temperature. The treatments resulted in four samples: control, i.e. uncoated (C); coated with chitosan (CT); coated with chitosan containing 500 µl L^{-1} of thyme essential oil (CT_{500}); and coated with chitosan containing 1,000 µl L^{-1} of thyme essential oil ($CT_{1,000}$). Third, each sample was divided into three equal parts, and then they were packed as follows: loose packaging (LP), packaging in polypropylene bags (PP), and active packaging in polypropylene bags containing sachets (AP). At the end, packets were stored in a dark cold room (55% RH, 4°C for 120 days) and tested every 60 days.

Compositional analysis. The kernels were ground using a home grinder (La Moulinette; Moulinex, Lyon, France). The protein was determined by means of the micro-Kjeldahl procedure, using 5.4 as a conversion factor. The fat contents were evaluated by Soxhlet extraction. The total ash was determined by weighing the dry mineral residue of the samples obtained at 500–550°C. The total amount of carbohydrate was measured by subtracting the amount of ash, protein, and fat from the total dry matter. The moisture contents of the kernels were determined by oven-drying at 103 ± 2°C [21].

Oil extraction and quality analyses. 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. The n-hexane extract was filtered, and the solvent was removed under reduced pressure using a rotavapor (RE 111; Büchi, Flawil, Switzerland) [22].

Peroxide value (PV). First, the acetic acid-chloroform solution and the saturated potassium iodide (KI) solution were added to the oil sample. Second, 30 ml of distilled water was added, then 0.01-N sodium thiosulfate was slowly titrated while shaking the flask vigorously near the end point which was indicated by a faint blue color. Third, the sodium thiosulfate ($Na_2S_2O_3$) was added dropwise until the blue color disappeared. Finally, the peroxide value (meq/kg^{-1}) of oil was calculated according to the following equation:

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

where V is the volume of the applied sodium thiosulfate, (ml); N is the normality of the thiosulfate, and W is the oil weight, g [23].

Conjugated diene value (CDV). The CDV was determined at a wavelength of 233 nm, using isooctane as an oil solvent. 0.1–0.3 g of oil was mixed with the isooctane solution. The amount of solution adsorption was determined at a wavelength of 233 nm with a spectrophotometer (Pharmacia, England) [24].

L* index. L* index (black/white) of the kernel was measured using a HunterLab colorimeter (model D65/10) [25].

Mold count. 5 g of each sample was transferred into a sterile stomacher bag under aseptic conditions and diluted 1:10 (w/v) with sterile peptone water (0.1%, w/v, Sigma-Aldrich, Darmstadt, Germany). Then the samples were homogenized for 2 min by means of a stomacher (Seward Laboratory, London, UK). The series of dilutions were prepared by adding 1 ml of each concentration to 9 ml of sterile peptone water (0.1% w/v). In order to count mold, 0.1 ml of each dilution was transferred onto the potato dextrose agar (PDA) medium using the surface culture method and was incubated at 25°C for 5 days [21].

Sensory evaluation. A panel of 10 members evaluated the overall acceptance using the 9-point Hedo-

Table 1. Compositional analysis of walnut kernels

Ash, %	Moisture, %	Fat, %	Protein, %	Carbohydrate, %
1.79 ± 0.11	3.16 ± 0.03	59.14 ± 0.66	15.07 ± 0.65	20.39 ± 0.56

Note: Mean values ± standard deviation over three replicates

Table 2. Effect of coatings and packaging methods on moisture content of kernels

Moisture content, %				
Packaging	Coatings	Storage time, days		
		1	60	120
LP	C	2.25^{Ca}	2.06^{Cab}	1.30^{Db}
	CT	5.56^{Ba}	4.21^{Bb}	2.97^{Cc}
	CT_{500}	5.97^{ABa}	4.35^{Bb}	3.53^{BCc}
	$CT_{1,000}$	6.86^{Aa}	4.81^{Bb}	3.73^{Bc}
PP	C	2.25^{Ca}	2.09^{Ca}	2.02^{CDa}
	CT	5.56^{Ba}	4.95^{Ba}	4.68^{Ba}
	CT_{500}	5.97^{ABa}	5.89^{ABa}	5.46^{Aa}
	$CT_{1,000}$	6.86^{Aa}	6.04^{Aba}	5.96^{Aa}
AP	C	2.25^{Ca}	2.21^{Ca}	2.18^{Ca}
	CT	5.56^{Ba}	5.42^{Ba}	5.20^{ABa}
	CT_{500}	5.97^{ABa}	5.73^{ABa}	5.85^{Aa}
	$CT_{1,000}$	6.86^{Aa}	6.62^{Aa}	6.29^{Aa}

Note: C is the control sample; CT is coated with 1% chitosan; CT_{500} and $CT_{1,000}$ are coated with 1% chitosan containing 500 and 1,000 µl L^{-1} TEO, respectively. LP, PP, and AP are loose packaging, packaging in polypropylene bags, and active packaging, respectively. Superscript lower letters (a–d) beside mean values in the same row and superscript upper letters (A–D) beside mean values in the same column show the difference in Duncan's multiple range test ($p < 0.05$). Standard error mean = 0.35

nic scale: 1 = dislike extremely; 2 = dislike very much; 3 = dislike moderately; 4 = dislike slightly; 5 = neither like nor dislike; 6 = like slightly; 7 = like moderately; 8 = like very much; 9 = like extremely. The panelists had sensory evaluation experience and were trained in descriptive evaluation of nuts.

Statistical analysis. The research employed a factorial method in the form of a complete randomized design with four replications. Data were subjected to analysis of variance (*ANOVA*) followed by LSD test ($p < 0.05$) to distinguish differences among the treatments. Statistical analyses and Pearson correlation coefficients between traits were analyzed using SPSS software 20.00.

RESULTS AND DISCUSSION

The chemical compositions of the kernels were shown in Table 1.

Moisture. As a result of the analysis of variance, the interaction effect of coating treatments and packaging methods on the moisture content was significant ($p < 0.05$). At the end of storage, the coated samples had the highest moisture. The minimum and maximum moisture content was observed in LP and AP, respectively (Table 2). The hydrophobicity characteristic of chitosan is conditioned by the acetyl groups in its structure, which has not been completely deacetylated. Besides, residual acetyl groups in chitosan play a role in preventing water vapor transmission [26]. Chitosan also decreases weight loss of guava [27].

Moisture absorption in the coating can be effectively decreased due to the hydrophobic characteristics of TEO, which were placed in empty spaces between the polymer chains [28]. In agreement with the previously reported data, the water vapor permeability of coatings was reduced by adding coriander, citronella, tarragon, and TEO [29]. Active packaging ensures a high concentration of carbon dioxide and a high relative humidity inside the package atmosphere [30].

Peroxide value (PV) and conjugated diene value (CDV). The analysis of variance showed the significant interaction effects ($p < 0.05$) for coating treatments, packaging methods, and time of storage. Coating treatments and time of storage, packaging methods and time of storage, coating treatments and packaging methods influenced the peroxide value and conjugated diene value considerably. The trend of CDV was similar to trends obtained for PV. There is a positive correlation between peroxide value and conjugated diene content in walnut [31].

The initial PV in the walnut was about 0.04 meq/kg^{-1}. The fresh walnut kernels in different cultivars had the peroxide values between 0.015–0.29 meq/kg^{-1}; the diversity can be related to the variety of the walnut trees and the weather conditions they grew up in [1]. During storage, the PV and CDV increased in all samples, the maximum amounts were detected in the C and the minimum amounts, in the CT_{500} and $CT_{1,000}$ samples (Table 3). Chitosan prevents the reactive oxygen species (ROS) and the lipid oxidation in food and biological systems because of its antioxidant capacity [32]. Chitosan has antioxidant activity against free radicals [33]. The low PV and CDV of CT_{500} and $CT_{1,000}$ samples can be attributed to antioxidant properties of TEO. Baldwin *et al.* also reported similar results in reducing the PV in oil of pecans [34]. The increasing of PV in the walnut during storage has also been reported [35].

Table 3. Effects of coating treatments and time of storage on PV and CDV of kernels

Test	Coating treatments	Storage time, days		
		1	60	120
Peroxide value, meq/kg oil	C	0.04^{Ac}	1.15^{ABb}	2.82^{Aa}
	CT	0.04^{Ac}	1.45^{Ab}	2.09^{Ba}
	CT_{500}	0.04^{Ac}	1.03^{Bb}	1.62^{Ba}
	$CT_{1,000}$	0.04^{Ac}	0.97^{Bb}	1.25^{Ca}
Conjugated diene value, µmol/g	C	4.88^{Ac}	5.94^{ABb}	7.543^{Aa}
	CT	4.88^{Ac}	6.231^{Ab}	6.844^{Ba}
	CT_{500}	4.88^{Ac}	5.828^{Bb}	6.393^{BCa}
	$CT_{1,000}$	4.88^{Ac}	5.771^{Ba}	6.039^{Ca}

Note: C is the control sample; CT is coated with 1% chitosan; CT_{500} and $CT_{1,000}$ are coated with 1% chitosan containing 500 and 1,000 µl L^{-1} TEO, respectively. Superscript lower letters (a–c) beside mean values in the same row and superscript upper letters (A–C) beside mean values in the same column show the difference in Duncan's multiple range test ($p < 0.05$). Standard error mean = 0.15

Table 4. Effects of packaging methods and time of storage on PV and CDV of kernels

Test	Packaging	Storage time, days		
		1	60	120
Peroxide value, meq/kg oil	LP	0.04^{Ac}	1.33^{Ab}	2.92^{Aa}
	PP	0.04^{Ac}	0.95^{Bb}	1.99^{Ba}
	AP	0.04^{Ac}	0.58^{Cb}	1.30^{Ca}
Conjugated diene value, μmol/g	LP	4.88^{Ac}	6.11^{Ab}	7.639^{Aa}
	PP	4.88^{Ac}	5.752^{Bb}	6.748^{Ba}
	AP	4.88^{Ac}	5.395^{Bb}	6.08^{Ba}

Note: LP, PP, and AP are loose packaging, polypropylene bags, and active packaging, respectively. Superscript lower letters (a–c) beside mean values in the same row and superscript upper letters (A–C) beside mean values in the same column show the difference in Duncan's multiple range test ($p < 0.05$). Standard error mean = 0.13 for PV and 0.15 for CDV

Tables 4 and 5 showed that the maximum PV and CDV values were with LP and the minimum, with AP. In fact, oxygen is an oxidation resonator; the degree of rancidity was reduced by increasing the amount of carbon dioxide inside the package. The mixture of ascorbic acid and sodium bicarbonate is used in active packaging, so carbon dioxide is produced by combining them. This system is used to increase the shelf-life of fresh meat and fish [36].

L* index. As a result of the analysis of variance, the effect of coating treatments and packaging methods on the L* index was significant ($p < 0.05$). The maximum value was in the $CT_{1,000}$ samples with active packaging and the minimum, in the control sample with loose packaging (Table 6).

The walnut kernel has bioactive compounds such as phenols. The dark color of the walnut kernel in the control sample may be caused by the enzymatic oxidation of carotenoids and phenolic compounds in the kernels [37]. Enzymatic browning of phenols in peanuts correlated with the decrease in the L* index during storage [38]. The reason of high L* in CT_{500} and $CT_{1,000}$ samples can be attributed to the antioxidant properties of chitosan and TEO which inhibited the oxidation of phenolic compounds. The highest L* index in AP resulted from the low oxygen content in the package. The acceptable value of the L* of walnut color was above 40 [39]. The L* indices of all samples were higher than 40, and the coated and actively packed samples had the highest L* index.

Table 5. Effects of coating treatments and packaging methods on PV and CDV of walnut

Test	Coatings	Package		
		LP	PP	AP
Peroxide value, meq/kg oil	C	3.48^{Aa}	2.91^{Ab}	2.48^{Ac}
	CT	2.95^{Ba}	2.25^{ABb}	2.01^{Ab}
	CT_{500}	2.52^{BCa}	2.03^{BCb}	1.16^{Bc}
	$CT_{1,000}$	2.34^{Ca}	2.06^{Cb}	1.06^{Bc}
Conjugated diene value, μmol/g	C	8.175^{Aa}	7.629^{Ab}	7.217^{Ab}
	CT	7.668^{Ba}	6.997^{Bb}	6.767^{Ab}
	CT_{500}	7.256^{Ba}	6.784^{Bb}	5.95^{Bc}
	$CT_{1,000}$	7.08^{Ba}	6.815^{Bb}	5.857^{Bc}

Note: C is the control sample; CT is coated with 1% chitosan; CT_{500} and $CT_{1,000}$ are coated with 1% chitosan containing 500 and 1,000 μl L^{-1} TEO, respectively. Superscript lower letters (a–c) beside mean values in the same row and superscript upper letters (A–C) beside mean values in the same column show the difference in Duncan's multiple range test ($p < 0.05$). Standard error mean = 0.15 for PV and 0.17 for CDV

Table 6. Effects of coating treatments and packaging methods on L* index of walnuts

Packaging	Coatings	Storage time, days		
		1	60	120
LP	C	74.87^{Aa}	70.09^{Aab}	59.76^{Cb}
	CT	62.73^{Bb}	68.42^{Ab}	70.03^{Ba}
	CT_{500}	76.34^{Aa}	74.23^{Aa}	72.74^{Ba}
	$CT_{1,000}$	61.61^{Bb}	72.94^{Aa}	75.72^{Ba}
PP	C	74.87^{Aa}	72.95^{Aa}	65.05^{BCb}
	CT	62.73^{Bb}	69.08^{Ab}	74.46^{Ba}
	CT_{500}	76.34^{Aa}	78.18^{Aa}	79.85^{ABa}
	$CT_{1,000}$	61.61^{Bb}	74.86^{Aa}	79.43^{ABa}
AP	C	74.87^{Aa}	67.81^{Bb}	72.58^{Bab}
	CT	62.73^{Bb}	69.80^{ABab}	78.87^{Ba}
	CT_{500}	76.34^{Aa}	77.85^{Aa}	83.17^{Aba}
	$CT_{1,000}$	61.61^{Bb}	65.44^{Cb}	86.25^{Aa}

Note: C is the control sample; CT is coated with 1% chitosan; CT_{500} and $CT_{1,000}$ are coated with 1% chitosan containing 500 and 1,000 μl L^{-1} TEO, respectively. LP, PP, and AP are loose packaging, packaging in polypropylene bags, and active packaging, respectively. Superscript lower letters (a–d) beside mean values in the same row and superscript upper letters (A–D) beside mean values in the same column show the difference in Duncan's multiple range test ($p < 0.05$). Standard error mean = 2.13

Mold count. As shown in Fig. 1a, the counts of mold in all samples increased during storage, but the control sample count had the highest. In all the treatments, the growth of molds increased within 60 days, but after that in the coated samples, no significant change was observed in the growth of fungi. To the contrary, in the control sample, the number of fungi increased to $\log_{10}$ 3.67 CFU/g^{-1} by the end of storage.

Chitosan extends the product shelf life directly affecting the growth of fungi and other defense operations, such as chitinase accumulation, which reduces the inhibitory effect of fungal cell wall proteinase. Chitosan inhibits microorganisms such as gram-positive and gram-negative bacteria and fungi [40]. According to [41] and [42], chitosan coating in artichoke seeds reduced the activity of various fungi and microorganisms on tomatoes. Antifungal effect of chitosan coating on pears was reported by Xianghong *et al.* [43].

TEO in combination with chitosan coating decreased the fungi count: $CT_{1,000}$ and CT_{500} samples had less microbial load than the others did. The main constituents of TEO are thymol and carvacrol, which have antimicrobial effects [44].

As shown in Fig. 1b, with the LP method, mold growth was significantly higher than with other packaging methods, but there was no significant difference between PP and AP methods.

As shown in Fig. 1c, with the LP method, the highest mold growth was observed in the control sample, but there was no significant difference between the other treatments. The maximum and minimum growth

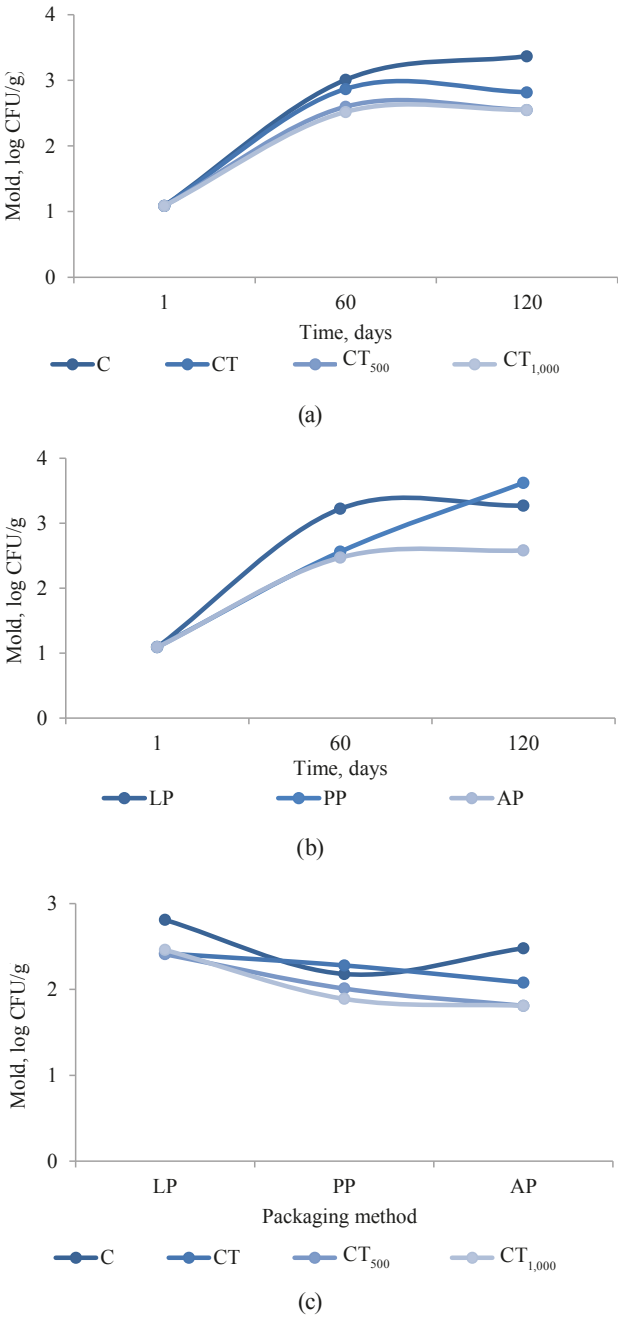

Fig. 1. Effects of coating treatments (a) and packaging methods (b) during storage and the effect of coating treatments and packaging methods (c) on the growth of molds in the kernels. C is the control sample; CT is coated with 1% chitosan; CT_{500} and $CT_{1,000}$ are coated with 1% chitosan containing 500 and 1,000 µl L^{-1} TEO, respectively. LP, PP, and AP are loose packaging, packaging in polypropylene bags, and active packaging, respectively ($p < 0.05$).

was in CT and $CT_{1,000}$ samples, respectively. Active packaging had a better effect on controlling the growth of molds. This could be due to creating an atmosphere with higher CO_2, which inhibits growth of microorganisms. CO_2 is the only gas that has a direct antimicrobial effect and increases the lag phase and growth time during the logarithmic growth stage [45]. Packaging with the modified atmosphere (with a low oxygen and high carbon dioxide content) was effective in controlling fungal rot and protecting the quality in the post-harvest period of fruits [46].

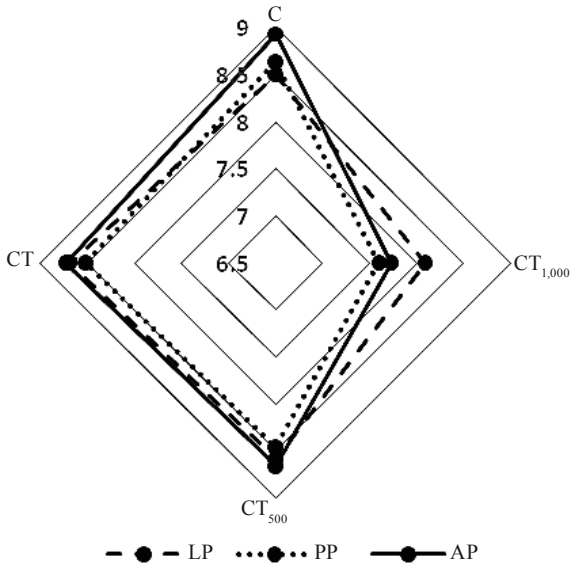

Fig. 2. Effects of treatments and packaging methods on overall acceptability score of walnut kernels. C is the control sample; CT is coated with 1% chitosan; CT_{500} and $CT_{1,000}$ are coated with 1% chitosan containing 500 and 1,000 µl L^{-1} TEO, respectively. LP, PP, and AP are loose packaging, packaging in polypropylene bags, and active packaging, respectively ($p < 0.05$).

Sensory evaluation. As shown in Fig. 2, the AP and LP control samples had the highest and lowest overall acceptability score, respectively. The high overall acceptability score in active packaging (AP) could be attributed to the low peroxide content in the samples. Although peroxides themselves do not directly play a role in off-flavour, but the ingredients of their decomposition produce an undesirable flavour. Hydroperoxides break down to form short-chain compounds, including aldehydes, ketones, alcohols, acids, esters, lactones, ethers and hydrocarbons, which contribute to odor and taste [47].

There was no significant difference in CT and CT_{500} samples in all three packages. The results indicated that chitosan did not adversely affect the general acceptance score of the walnut kernel, which was similar to the previous studies on walnut kernels, strawberries, and peeled lychee [35, 48, 49].

$CT_{1,000}$ treatment resulted in the lowest sensory properties with all three kinds of packaging. Therefore, treatments containing high levels of TEO were evaluated as undesirable. This can be related to a high concentration of the essence oil. The overall acceptance score for this treatment was higher with LP than with other packaging methods. With LP, because of volatile characteristics of TEO, some of the TEO evaporated. It made the flavour score of the sample comparable to those with other packaging methods.

CONCLUSION

The shelf-life and quality of the walnut kernel can be affected by some environment factors during storage. Coating materials and packaging methods can be useful for prolonging the postharvest quality of crops. The study results showed that chitosan

and thyme essential oil coating combined with active packaging had a significant effect on reducing oil oxidation and growth of molds. They also prevented the loss of moisture and a decrease in L* value, improving the sensory properties of the samples during storage. An increase in the essential oil up to 500 µl L^{-1} also improved the functional properties of chitosan coating. Compared with loose packaging, polypropylene packaging was also effective in protecting the qualitative properties of walnut. Active packaging offered the considerable potential and proved the most efficient.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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ORCID IDs
Roghieh Talebi Habashi https://orcid.org/0000-0003-1420-5819