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Effect of sodium alginate-calcium chloride coating and glycerol and sorbitol concentration on oxidative stability and fungal growth of Persian walnut (*Juglans regia* L.)

Efecto del recubrimiento de alginato de sodio-cloruro de calcio y la concentración de glicerol y sorbitol sobre la estabilidad oxidativa y el crecimiento fúngico de la nuez persa (*Juglans regia* L.)

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Abstract
The effect of extracted sodium alginate (SA) (0.25, 0.50, 0.75, and 1.00% w/w) from *Sargassum angustifolium* along with sorbitol (S), glycerol (G), and before and after calcium chloride ($CaCl_2$) treatment as a coating material were evaluated on oxidative stability, fungal growth, and sensorial properties of Persian walnut *Juglans regia*. In the second month of the storage period, peroxide and acid values of alginate-Ca^{2+} coated walnuts decreased compared the uncoated sample. Moisture loss was reduced, and the color parameter values were significantly higher in these samples than in uncoated ones (9th week). The fungal growth rate decreased by about 1.5-5.0 Log CFU/g in coated walnuts during the ninth week of storage. SA was an excellent protective barrier to water vapor and oxygen permeability to preserve walnuts against oxidative stress and fungal growth. In addition, SA as a coating material increased oxidative stability and decreased fungal spoilage of walnuts without any adverse changes in their color or sensorial acceptability.
Keywords: Sodium alginate, Persian walnut, *Juglans regia* L., oxidative stability, fungal growth.

Resumen
El efecto del alginato de sodio extraído (0.25, 0.50, 0.75 y 1.00% w/w) de *Sargassum angustifolium* con sorbitol (S), y Glicerol (G) y antes y después del tratamiento de cloruro cálcico como un material de revestimiento fueron evaluados en la estabilidad oxidativa, crecimiento fúngico, propiedades sensoriales de nuez persa *Juglans regia*. En el segundo mes del período de almacenamiento, el peróxido y valores del ácido del alginato de calcio de nueces recubiertas disminuidas en comparación con muestras no recubiertas. La pérdida de humedad se redujo y los valores de color en estas muestras fueron eran significativamente superiores a los no recubiertos (novena semana). La tasa del crecimiento fúngico se disminuyó alrededor de 1.5-5.0 Log CFU/g en nueces recubiertas en la novena semana del almacenamiento. El alginato de sodio fue una excelente barrera protectoraante el vapor de agua y permeabilidad del oxígeno. Para conservar las nueces ante estrés oxidativo y crecimiento fúngico. Además, el alginato de sodio como un material de revestimiento aumentó la estabilidad oxidativa y disminuyó el deterioro fúngico de nueces sin unos cambios adversos de color o aceptabilidad sensorial.
Palabras clave: Alginato de sodio, nuez persa, *Juglans regia* L, estabilidad oxidativa, crecimiento fúngico.

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1 Introduction

Walnuts were the most and second-ranked tree nut in countries with middle and high-income economies in 2016 (Marques, Fuinhas, & Pais, 2018; Nuts, 2018). Walnut kernels have a substantial beneficial impact on human health since they are an excellent source of phenolic compounds, antioxidants, omega-3, 6 fatty acids (FAs), and melatonin (Christopoulos & Tsantili, 2012). Also, walnut contains essential vitamins (niacin, riboflavin, thiamine, pantothenic acid, vitamin B6, and B9) (Sen, 2013). Walnut kernels contain high oil (52 to70 g/100 g) and polyunsaturated FAs, mostly linoleic, oleic, and linolenic acids (Gómez-Estaca, Gómez-Guillén, Montero, Sopelana, & Guillén, 2011; Vickers, 2017). High polyunsaturated FAs content limits the shelf life of walnuts, and lipid oxidation occurs fast during storage, resulting in an unpleasant rancid taste (Gómez-Estaca *et al*., 2011; Vickers, 2017). Also, oxygen concentration, temperature, and light are the main contributing environmental factors to lipid oxidation (Gómez-Estaca *et al*., 2011).

Persian walnut (*Juglans regia* L.) is Iran's most crucial nut crop, with an estimated more than 20 million trees (Hassani *et al*., 2012; Vahdati *et al*., 2019). Due to fresh Persian walnuts' nutritious values, flavor, and moisture content (3.2%), practical experiments for contributing factors to oxidation must be evaluated (Gharibzahedi, Mousavi, Hamedi, & Khodaiyan, 2012).

Edible coatings have acquired an interest in recent years due to their benefits in food preservation. Different coatings methods on fresh walnuts are alternatives to modified atmosphere storage during transportation (Hassan, Chatha, Hussain, Zia, & Akhtar, 2018). Applying edible coating can reduce the oxygen flow and provide a barrier to moisture, therefore helping to prevent lipid oxidation and improve the glossy appearance of kernels (Bastani *et al*., 2010; Sabaghi, Maghsoudlou *et al*., 2015). Several studies use chitosan to incorporate green tea extract, whey protein isolate, soy protein, potato starch, pectin- and carboxymethylcellulose-based coatings to preserve walnut kernels (Maté & Krochta, 1997; Mehyar *et al*., 2012).

In the last decades, polysaccharide-based materials like alginates have attracted attention due to their simple accessibility, renewability, and, more importantly, biodegradability (Qi *et al*., 2021). Alginate films tested in pine nuts could slow down lipid oxidation, as indicated by peroxide value and conjugated dienes parameters (Hosseinpouri, Mohammadi, Ehsandoost, Sharafi-Badr, & Obeidi, 2022). Alginate is a polymer of D-mannuronic acid (-M-blocks) and L-guluronic acid (-G-blocks) with the ability to form solid gels and isolated from brown alga (Bastani *et al*., 2010; Hurtado, Estevez, & Barbosa-Canovas, 2000). According to the previuos studies, the oxygen transmission rate of films were limited below the 10^{-4} cm^3 m^{-2} per day (Q.-H. Lu & Zheng, 2018). Alginate has been used in industries because it binds divalent cations. Also, it can become gel formation at low salt concentrations as an encapsulation matrix (Mitchell & Blanshard, 1976). Depending on its molecular weight and -M- and -G- blocks ratio, alginates with different characteristics will be obtained by cross-linking with calcium ions. -G- blocks have more affinity to calcium ions than -M-blocks (Khanna, Moya, Opara, & Brey, 2010; L. Z. Wang, Liu, Holmes, Kerry, & Kerry, 2007). Previous studies have reported that sodium alginate (SA) films have the lowest water vapor and oxygen permeability compared to the films of carboxymethylcellulose, whey protein isolate, potato starch, gelatin, and sodium caseinate. SA films are suitable for coating whole fruits and vegetables due to water, acid, and alkali (L. Z. Wang *et al*., 2007). Increasing the shelf life, minimizing the weight loss and browning, and preserving the firmness during storage were the effects of using alginate-Ca^{2+} coatings for fresh-cut Gala apples (Embuscado & Huber, 2009). Edible coatings create a selective barrier against oxygen, carbon dioxide, and moisture. They improve textural and mechanical properties, prevent flavor loss, and serve as a carrier for various additives (Azarakhsh, Osman, Ghazali, Tan, & Mohd Adzahan, 2012).

The sodium alginate composites provide some additional advantages because of their ability to complex with calcium chloride (Marcotte *et al*., 2000). Calcium chloride has been widely used as a texture firming agent for fruits and vegetables for a long time since it can form complexes with low methoxyl pectin present in the produce tissue, thereby facilitating texture firming (Izumi & Watada, 1994). A similar benefit can potentially be realized with the combination of sodium alginate-calcium chloride combinations (Alonso *et al*., 1997; Marcotte *et al*., 2000).

Plasticizers like glycerol and sorbitol decrease intermolecular attractions between polymeric chains. The incorporation of plasticizers causes modifications in the film's barrier properties. Lipophilic compounds act as emulsifiers and plasticizers increasing film flexibility (Bertuzzi *et al*., 2007).

Javanmard reported that applying alginate coating on pistachio kernel effectively reduced kernel oxidation and improved the coated nuts' shelf-life (Javanmard, 2008). Fresh-cut pineapples with edible coating alginate compared with uncoated samples reduced weight loss and respiration rate (Azarakhsh *et al*., 2012). The effect of concentrations of extracted SA from *Sargassum angustifolium* along with sorbitol (S), glycerol (G), and calcium chloride ($CaCl_2$) treatment as a coating material were evaluated on oxidative stability, fungal growth, and sensorial properties of Persian walnut *Juglans regia*.

Table 1. Ingredients and compositions of coating formulations

Formulation	Sodium alginate (g/100 mL solvent)	Sorbitol (g/100 mL solvent)	Glycerol (g/100 mL solvent)
C (Control)	0	0	0
T1	0.75	0	1
T2	0.75	1	0
T3	0.75	0.25	0.5
T4	0.75	0.25	0.75
T5	0.75	1	0.5
T6	0.75	1	0.75
T7	0.75	0.75	0.75

2 Materials and methods

2.1 Materials, samples

Sargassum angustifolium was collected from the seashores of Bushehr, Iran. Sodium alginate (SA; MW = 371 $\times 10^3$ (g/mol), % G = 38-41, intrinsic viscosity [η] = 7.92 ± 0.24 and pH = 6.5-8.0 for 1% SA solution in water), extracted from *Sargassum angustifolium*, was provided by the collaboration of edible company (Persian Gulf Algae Development Technology Company) (Bushehr, Iran) (Borazjani, Tabarsa, You, & Rezaei, 2017; Labowska, Michalak, & Detyna, 2019). Glycerol (G; purity 87%), sorbitol (S), starch, $CaCl_2$, sodium chloride, acetic acid, chloroform, acetone, saturated potassium phosphate, n-hexane, magnesium nitrate, saturated potassium iodide, and Yeast Extract Glucose Carbonate (YGC) culture medium were purchased from Merck Company (Darmstadt, Germany). Persian Walnuts (*Juglans regia* L.) were obtained from the Bavanat region, Fars, Iran. Their nuts were manually cracked, shelled, and stored at 30 ºC for further analysis.

2.2 Coating preparation

The film-forming solutions were prepared by dissolving different concentrations of sodium alginate (SA) (0.25, 0.50, 0.75, and 1.00% (w/v) in distilled water by magnet stirring at 300 rpm. SA solution was mechanically stirred for 15 min at 25 ºC, and plasticizers (glycerol or sorbitol) were added at different concentrations (0.25, 0.50, 0.75, and 1.00% w/v). One kg of covered walnut kernels with polyvinyl chloride stretch film (PVC films) was placed in a fabric mesh and dipped entirely into the sodium alginate solution (5 min, 25 ºC) until the gel layer on the surface of the fruit became firm and did not have a liquid and soft state (Table 1, samples T1-T7).

Due to the effects of calcium chloride as a firming agent, high-water solubility, and anti-browning, this component of 2% (w/v) was added to each coating formulation, and peroxide value, acid value, and color measurement were evaluated before and after the addition of calcium chloride salts. Therefore, calcium chloride salt was shaken to dissolve the salt thoroughly in a volumetric flask with distilled water (Valdés *et al*., 2017). Then walnut kernels were drained in a plastic mesh and dipped again in the calcium chloride solution (2%) for 5 min. The sample was removed, drained, and left on filter paper at room temperature (10 min, 27ºC) to remove the surface excess of the coating solution. Uncoated walnut kernels were used as the control sample. All assays were triplicated at room temperature (27ºC) for two months.

2.3 Sample storage

Seven walnut kernel samples (coated and controlled) were covered with polyvinyl chloride stretch film (PVC film). PVC films were punctured (1-2 mm each), and plastic containers were stored in the 4 ºC for 15 days. Further tests were performed every three days (days 0, 3, 6, 9, 12, and 15).

2.4 Moisture content measurement

The drying method to constant weight was used to measure the moisture content of walnuts. Chopped kernels were dried in an oven (Memmert, UN55, Germany) at 105ºC for 72 h (Salcedo, de Mishima, & Nazareno, 2010). The control and coated samples were tested the same as before. The moisture content of kernels was calculated by following Eq. (1):

$$\text{Moisture content}(\%) = \frac{(\text{Nuts weight before drying-Nuts weight after drying})}{\text{nuts weight before drying}} \times 100 \quad (1)$$

2.5 Peroxide value (PV)

The peroxide value (PV) of walnut kernels was determined according to (Kang, Kim, You, Lacroix, & Han, 2013), with some modifications. Oil of chopped kernels (20 g) was extracted using n-hexane (100 mL) in a shaking incubator for 3 h. After filtering the solution with a filter paper (Whatman no. 1), hexane was removed using evaporator equipment (Heidolph Instruments, R206D, Germany). Then, consecutively added 30 mL of chloroform/acetic acid 2:3 (v/v) and 0.5 mL of saturated potassium iodide to 5 g of

extracted oil. The solution was stirred in the darkroom for 1 min, after which 30 mL of distilled water was added and titrated with sodium thiosulfate ($Na_2S_2O_3$; 0.01 N). Starch solution (0.5 mL of 1 g/100 mL of water) was used as an indicator. The disappearance of the starch solution's purple color showed the endpoint of the titration every 30 and 60 days.

PV was calculated by following Eq. (2):

$$\text{Peroxide value}\left(\frac{\text{meq oxygen}}{\text{Kg oil}}\right) = \frac{(S-B)\times N\times 100}{w} \quad (2)$$

S and B were the used volume of sodium thiosulfate for sample and blank, respectively, N was the normality of sodium thiosulfate solution, and W was the weight of the sample.

2.6 Acid value measurement (AV)

The acid value (AV) was determined by the standard AOCS Cd 3d-63 method (Firestone, 2009).

2.7 Color measurement

The color of walnut kernels was evaluated instrumentally using a Lab CIE system colorimeter (Lovibond colorimeter, RT500, Amesbury UK). Hunter system parameters, including lightness-darkness (L*) and yellowness-blueness (b*), were determined (Gómez-Fernández *et al*., 2021; Labuckas, Maestri, & Lamarque, 2014). The test was done on days 0, 21, 42, and 63.

2.8 Fatty acids (FAs) determination

Gas chromatography (GC-17, Shimadzu, Japon) with a 100 m capillary column (0.25 mm inner diameter, 0.33 mm outer diameter) was used to evaluate the FAs composition of control walnut oil. The column temperature program was started at 90 °C for 5 minutes, rising to 230 °C at a rate of 2 °C/min for 85 minutes. The temperature of the detector and injector was 250 °C. FAs were detected and calculated by comparing the retention times of sample peaks with those of standard FAs.

2.9 Total Yeast and Mold Counts (TYMC)

10 g of each sample were powdered, and sodium chloride 0.9 % solution was added to a bag and mixed in a blender (uncoated and coated) (Seward Laboratory, UK). Each dilution (0.1 mL, 10^{-2} and 10^{-3}) were added to the PCA solid culture medium (Arian Company, Artin Azma, Iran). After a few minutes, the plates were heated upside down at 20 to 25ºC. Then, the plates were heated upside down at 20 to 25ºC (Sabaghi *et al*., 2015). The test was done on the control and coated samples on days 0, 21, 42, and 63. The number of grown mold and yeast were counted and reported in the CFU/g unit for both the control and coated samples.

2.10 Sensory evaluation

Sensorial properties of walnut kernels were evaluated by ten panelists from students of the Food Science and Technology Department, Faculty of Agriculture, Shiraz University, Iran, to evaluate flavor, rancidity, taste, texture, and overall acceptance on a 5-point hedonic scale, where 1 represented "dislike extremely" and 5 represented "like extremely". They consumed the whole kernel samples and rinsed their mouth with drinking water between different samples. The samples were randomly coded with three-digit numbers and presented in random order.

2.11 Statistical analysis

Data were analyzed using SPSS software version 17 and carried out by two-way analysis of variance. Significant mean differences were compared using post hoc analysis (Duncan's test) at a 5% significant level. Mean values and standard error of the mean were expressed. The diagram was designed by Graphpad Prism 6.1.

3 Results and discussion

3.1 Moisture content measurement

An essential qualitative factor in dried food products is moisture content (Maghsoudlou *et al*., 2012). The effect of alginate coating and $CaCl_2$ treatment was investigated on the moisture content of walnut kernels for 2 months (Fig. 1). Coating with SA and treating with $CaCl_2$ reduced moisture loss ($P < 0.05$) compared to uncoated and no cross-linked samples. This result indicates the good preventing effect of SA and cross-linking with $CaCl_2$ against moisture-transferring between walnut kernels and the surrounding atmosphere. Many studies have also reported the water loss reduction of coated drumsticks (Mountney & Winter, 1961), Galla apples (Embuscado & Huber, 2009), and fish fillets (F. Lu, Liu, Ye, Wei, & Liu, 2009) with SA and $CaCl_2$ solution, coated pistachio nuts with chitosan, and coated citrus fruit with low molecular weight chitosan (Chen, Shen, & Lin, 2007; Maghsoudlou *et al*., 2012).

3.2 Peroxide value (PV)

High oil and polyunsaturated FAs content, high surface area, and injuries resulting from the breaking process are the reasons for high walnut susceptibility to lipid oxidation (Maté & Krochta, 1997). Table 2 shows walnut kernels' PV, lipid rancidity standard indicator, during storage. PV was increased significantly ($P<0.05$) during storage time in all samples. Maximum PV (5.93 and 10.80 meq O_2/Kg oil in the first and second months, respectively) was related to the uncoated walnut kernel during storage, and it was

Table 2. Peroxide value (meq O_2/ Kg oil) changes of walnut samples during two months.

Sample	1st month		2nd month	
	With calcium	Without calcium	With calcium	Without calcium
C†	$5.93^{a*} \pm 0.04$		$10.80^{a} \pm 0.03$	
T1	$2.59^{k} \pm 0.04$	$4.03^{d} \pm 0.02$	$5.91^{g} \pm 0.03$	$7.21^{d} \pm 0.05$
T2	$1.58^{n} \pm 0.01$	$2.91^{i} \pm 0.02$	$4.92^{k} \pm 0.06$	$6.83^{e} \pm 0.08$
T3	$2.07^{m} \pm 0.05$	$3.42^{g} \pm 0.01$	$3.58^{l} \pm 0.04$	$5.51^{i} \pm 0.04$
T4	$3.52^{f} \pm 0.04$	$4.79^{b} \pm 0.04$	$5.30^{j} \pm 0.05$	$6.67^{f} \pm 0.07$
T5	$2.44^{l} \pm 0.02$	$3.11^{h} \pm 0.08$	$5.75^{h} \pm 0.01$	$7.34^{c} \pm 0.03$
T6	$3.63^{e} \pm 0.07$	$4.34^{c} \pm 0.02$	$7.20^{d} \pm 0.04$	$8.56^{b} \pm 0.01$
T7	$2.84^{j} \pm 0.03$	$4.03^{d} \pm 0.05$	$5.82^{h} \pm 0.07$	$7.38^{c} \pm 0.03$

†*C*=control, T1=0.75% sodium alginate+1.00% glycerol; T2= 0.75% sodium alginate+1.00% sorbitol; T3=0.75% sodium alginate+0.25% sorbitol+0.50% glycerol; T4=0.75% sodium alginate+0.25% sorbitol+0.75% glycerol;T5=0.75% sodium alginate+1.00% sorbitol+0.50% glycerol; T6=0.75% sodium alginate+1.00% sorbitol+0.75% glycerol; T7=0.75% sodium alginate+0.75% sorbitol+0.75% glycerol; * Mean value ± SD; Same letters indicate no significant difference in each month ($p < 0.05$).

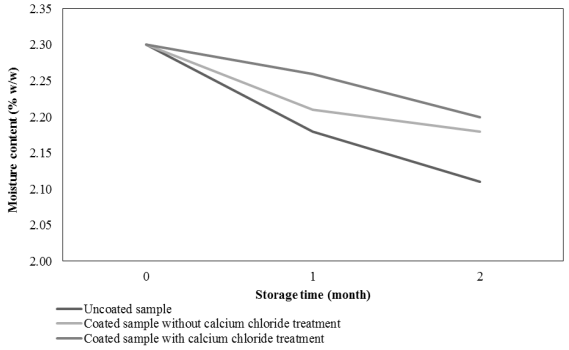

Fig. 1. Moisture content changes of walnut kernels during storage.

significantly (P<0.05) different from the PV of coated samples. In the first month, the PV of coated samples was in the ranges of 1.58- 4.79 meq O_2/Kg oil that reduced by about 19-73% as compared to the uncoated sample, and in the second month, this reduction was in the range of 20-67% for PVs between 3.58- 8.56 meq O_2/Kg oil.

Furthermore, treating with $CaCl_2$ had a significantly (P<0.05) better effect on reducing PV than no $CaCl_2$ treating in all coating formulations and both months. These results accord with (Kang *et al*., 2013) findings, in which PV was between 2.93 (on day 1) to 4.88 (on day 14) meq O_2/Kg oil for an uncoated sample, and it was in the range of 2.43-4.72 for coated samples with various coating formulations. Also, reported PVs for six different walnut cultivars were 2-18 meq O_2/Kg oil (Amaral, Casal, Pereira, Seabra, & Oliveira, 2003). Walnut coating by whey protein isolate, pea starch, and their combinations with carnauba wax reduced the PV and acidity of walnuts compared to uncoated samples (Mehyar *et al*., 2012). Also, chitosan and green tea extract in coating formulations decreased significantly (P<0.05) PV of walnut kernels as compared to the uncoated walnut kernel (Sabaghi *et al*., 2015).

The lowest peroxide value was related to the T2 sample (0.75% SA+ 1% S) with $CaCl_2$ treatment. S has shown activity as a good barrier to oxygen permeability in starch- S-water-based films. The peroxide value decreased from 1.43 $\times 10^{-16}$ cm^3.cm/cm^2.s.Pa (sample without S) to 0.15×10^{-16} cm^3.cm/cm^2.s.Pa (sample containing<9% S) as a result of starch and S connections that reduced the oxygen motions (Gaudin, Lourdin, Forssell, & Colonna, 2000).

3.3 Acid value measurement (AV)

Enzymatically hydrolysis of oils with heat and moisture as catalysts produces free FAs. Highly reactive molecules may be generated due to free FAs autoxidation, creating undesirable and noxious flavors and odors (Mehyar *et al*., 2012). AV changes in walnut kernels were investigated for two months, and the results are indicated in Table 3. The Maximum AV (0.43 and 0.59 in the first and second months, respectively) was related to the uncoated sample in both months, and it was significantly ($P < 0.05$) different from the AV of coated samples. There is an overall increase in acidity of all samples during storage. In the first month, SA coating without $CaCl_2$ treating reduced AV of walnut kernels by about 11-37%, while this coating and $CaCl_2$ treating decreased the AV by about 23-46% compared to the uncoated sample. Also, this reduction was 30-49% in the second month for all treatments. The lowest AVs were related to coated samples with T2 and T3 coating formulations and $CaCl_2$ treatment. They were significantly (P<0.05) different from the AV of other coated samples, but there were no significant (P<0.05) differences between the AV of many coating formulations.

Table 3. Acid value changes of walnut samples during two months.

Sample	1st month		2nd month	
	With $CaCl_2$	Without $CaCl_2$	With $CaCl_2$	Without $CaCl_2$
C†	$0.43^{a*} \pm 0.00$		$0.59^{a} \pm 0.02$	
T1	$0.33^{bcd} \pm 0.06$	$0.38^{ab} \pm 0.03$	$0.36^{bcd} \pm 0.01$	$0.40^{b} \pm 0.04$
T2	$0.25^{de} \pm 0.04$	$0.27^{cde} \pm 0.02$	$0.30^{d} \pm 0.03$	$0.35^{bcd} \pm 0.02$
T3	$0.23^{e} \pm 0.05$	$0.28^{cde} \pm 0.01$	$0.30^{d} \pm 0.07$	$0.37^{bcd} \pm 0.05$
T4	$0.28^{cde} \pm 0.03$	$0.31^{bcde} \pm 0.00$	$0.31^{cd} \pm 0.02$	$0.36^{bcd} \pm 0.08$
T5	$0.31^{bcde} \pm 0.09$	$0.34^{bc} \pm 0.04$	$0.36^{bcd} \pm 0.01$	$0.39^{bc} \pm 0.03$
T6	$0.32^{bcd} \pm 0.02$	$0.35^{bc} \pm 0.07$	$0.37^{bcd} \pm 0.04$	$0.41^{b} \pm 0.06$
T7	$0.29^{cde} \pm 0.01$	$0.32^{bcd} \pm 0.04$	$0.34^{bcd} \pm 0.05$	$0.36^{bcd} \pm 0.04$

†*C*=control, T1=0.75% sodium alginate+1.00% glycerol; T2= 0.75% sodium alginate+1.00% sorbitol; T3=0.75% sodium alginate+0.25% sorbitol+0.50% glycerol; T4=0.75% sodium alginate+0.25% sorbitol+0.75% glycerol;T5=0.75% sodium alginate+1.00% sorbitol+0.50% glycerol; T6=0.75% sodium alginate+1.00% sorbitol+0.75% glycerol; T7=0.75% sodium alginate+0.75% sorbitol+0.75% glycerol; * Mean value ± SD; Same letters indicate no significant difference in each month ($p < 0.05$).

Coatings made an excellent barrier against water vapor and oxygen permeability. This finding follows the results (Mehyar *et al*., 2012). In their study, the acidity of uncoated walnut and pine nuts increased throughout the storage period compared to the coated ones, and the 3-component coatings containing whey protein isolate, pea starch, and carnauba wax had the best effect on lipolysis prevention. Also, (Yildirim & Barutçu Mazi, 2017) reported AV increasing in all roasted hazelnut samples during storage and decreasing the rate of AV rising by incorporating skin extract and α-tocopherol into the coating formulation. The α-Tocopherol was more effective than the prevention of free FAs formation.

3.4 Antifungal activity

Nuts are susceptible to fungal attacks. *Aspergillus* and *Penicillium* species, producers of aflatoxins and ochratoxins, are two species of many fungi that attack nuts. Moisture content and storage conditions are intrinsic and extrinsic factors influencing fungal growth and mycotoxin production. Fungal spoilage affects nuts' sensorial and nutritional properties (Nkwonta, Medina, del Carmen Alamar, & Terry, 2015), so it should be prevented. Influences of SA coating before and after $CaCl_2$ treatment were evaluated (Fig. 2). The rate of yeasts and mold growth was constantly increased after three weeks of storage at ambient temperature, and this was significantly (P<0.05) higher in the control sample than in coated and cross-linked ones via calcium during storage completely exposed to oxygen. The fungal growth rate reduction was in the ranges of 1.50-2.65, 1.50-4.00, and 1.50-5.00 Log CFU/g at the third, sixth, and ninth weeks, respectively. Therefore, SA coating has an excellent preventing effect on fungal growth. In some treatments, cross-linked modification showed a significant (P<0.05) effect on the growth reduction of yeasts and molds. For example, $CaCl_2$ treatment in the sample coated with 0.75% SA, 0.75% S, and 0.75 % G (T7) indicated significantly (P<0.05) lower yeasts and molds count than no cross-linked related sample (T7). In the studies of (Sabaghi *et al*., 2015) and (Maghsoudlou *et al*., 2012). Chitosan coating reduced the growth of molds and yeasts on walnut kernels and pistachio nuts, respectively, and this reduction effect increased by increasing chitosan concentration. Edible coatings containing walnut green husk extract with or without ascorbic acid inhibited the growth of yeast and mold in fresh walnut kernels compared to uncoated samples (Habibie, Yazdani, Saba, & Vahdati, 2019). The result is that coated formulations' antifungal activity was time-dependent and significant with plasticizers and $CaCl_2$ addition.

3.5 Color measurement

Color is an essential parameter in accepting a food product. The color parameters (L* and b*) of walnut kernels were measured, and the results are shown in Table 4. In all samples, L* decreased slightly during storage. Lightness and b* parameters of coated and $CaCl_2$ treated walnut kernels were significantly (P<0.05) higher than lightness and b* of uncoated and no- $CaCl_2$ treated samples. It may be related to their higher moisture content (Fig. 1). Coated walnut kernels with T2 and T5 coating formulations and $CaCl_2$ treatment showed maximum lightness in all weeks. According to walnut industry standards, walnut kernels' acceptable L* values are > 40 (S. Wang, Monzon, Johnson, Mitcham, & Tang, 2007). Many coating formulations without $CaCl_2$ treatment showed b* values similar to uncoated samples. Therefore, SA coating and $CaCl_2$ treatment had a more protective effect on color parameters. (Yildirim & Barutçu Mazi, 2017) Reported no visible changes in roasted hazelnuts' L*, a*, and b* color parameters after coating with zein solutions compared to the uncoated sample. In a

study by (Bastani *et al*., 2010), the shining appearance of carboxymethylcellulose-coated walnut kernels was higher than in other samples, and b* showed no significant difference during storage.

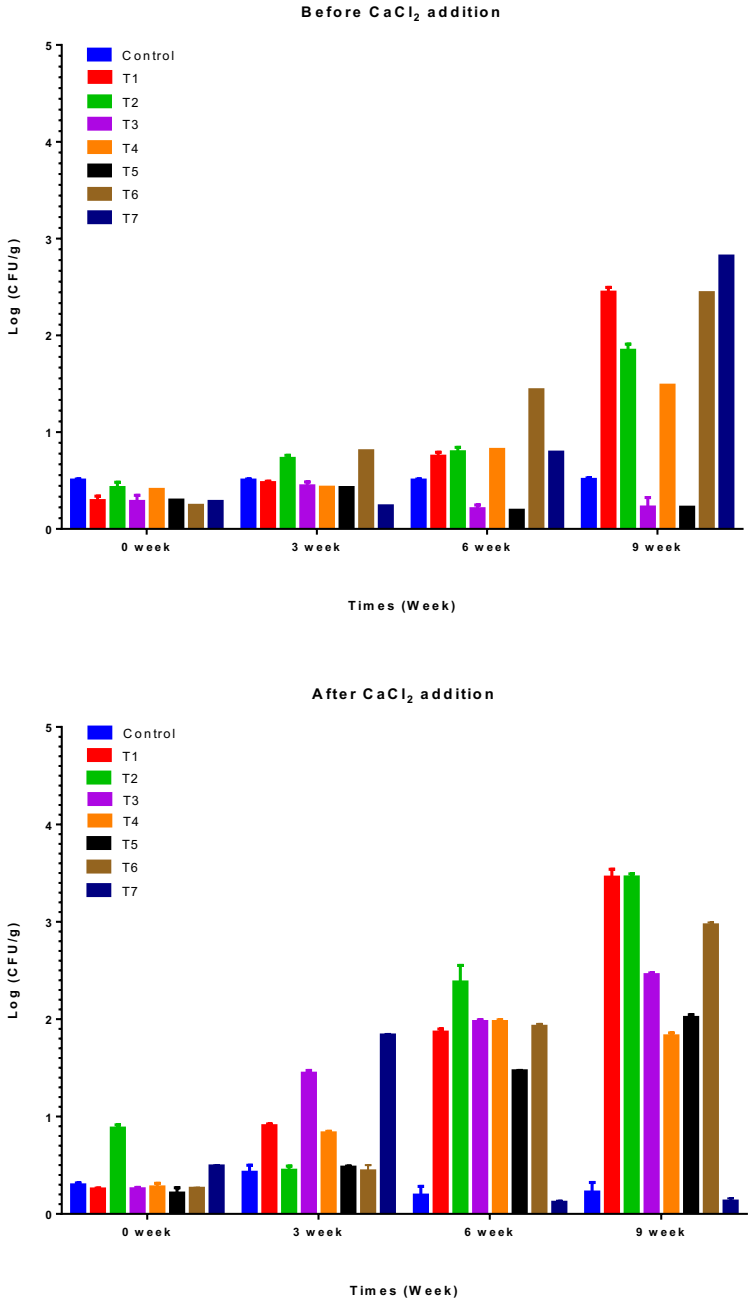

Fig. 2. Changes of yeasts and molds growth in walnuts coated with various coating formulations during storage before and after the addition of $CaCl_2$. C=control, T1=0.75% sodium alginate+1.00% glycerol; T2= 0.75% sodium alginate+1.00% sorbitol; T3=0.75% sodium alginate+0.25% sorbitol+0.50% glycerol; T4=0.75% sodium alginate+0.25% sorbitol+0.75% glycerol;T5=0.75% sodium alginate+1.00% sorbitol+0.50% glycerol; T6=0.75% sodium alginate+1.00% sorbitol+0.75% glycerol; T7=0.75% sodium alginate+0.75% sorbitol+0.75% glycerol.

Table 4. Color parameters changes of walnut kernels during storage.

			Sample							
			$C^{\dagger}$	T1	T2	T3	T4	T5	T6	T7
L*	1st week	+ $CaCl_2$	42^{d*}	47^{a}	48^{a}	46^{b}	46^{b}	48^{a}	47^{a}	48^{a}
		- Ca		44^{c}	43^{c}	43^{c}	42^{d}	41^{d}	42^{d}	42^{d}
	3rd week	+ Ca	42^{d}	46^{b}	48^{a}	45^{b}	46^{b}	48^{a}	47^{a}	46^{b}
		- Ca		44^{c}	42^{d}	42^{d}	42^{d}	41^{d}	41^{d}	41^{d}
	6th week	+ Ca	42^{d}	46^{b}	47^{a}	46^{b}	46^{b}	48^{a}	46^{b}	46^{b}
		- Ca		43^{c}	42^{d}	42^{d}	42^{d}	41^{d}	41^{d}	40^{e}
	9th week	+ Ca	40^{d}	45^{b}	47^{a}	46^{a}	45^{b}	46^{a}	45^{b}	46^{a}
		- Ca		46^{a}	45^{b}	42^{c}	41^{c}	41^{c}	41^{c}	40^{d}
b*	1st week	+ Ca	29^{c}	31^{b}	32^{a}	31^{b}	31^{b}	32^{a}	30^{b}	31^{b}
		- Ca		30^{b}	29^{c}	29^{c}	29^{c}	28^{c}	28^{c}	28^{c}
	3rd week	+ Ca	28^{b}	30^{a}	31^{a}	28^{b}	31^{a}	31^{a}	28^{b}	29^{b}
		- Ca		29^{b}	29^{b}	29^{b}	28^{b}	27^{c}	27^{c}	27^{c}
	6th week	+ Ca	26^{c}	28^{b}	30^{a}	29^{a}	30^{a}	29^{a}	29^{a}	28^{b}
		- Ca		27^{c}	28^{b}	28^{b}	26^{c}	26^{c}	26^{c}	27^{c}
	9th week	+ Ca	24^{d}	28^{b}	30^{a}	27^{b}	29^{a}	29^{a}	29^{a}	28^{b}

†C=control, T1=0.75 % sodium alginate+1.0 % glycerol; T2= 0.75 % sodium alginate+1.0% sorbitol; T3=0.75 % sodium alginate+0.25 % sorbitol+0.50 % glycerol; T4=0.75% sodium alginate+0.25 % sorbitol+0.75 % glycerol;T5=0.75 % sodium alginate+1.0 % sorbitol+0.50 % glycerol; T6=0.75 % sodium alginate+1.0 % sorbitol+0.75 % glycerol; T7=0.75 % sodium alginate+0.75 % sorbitol+0.75 % glycerol; * Mean value ± SD; Same letters indicate no significant difference in each week ($p < 0.05$).

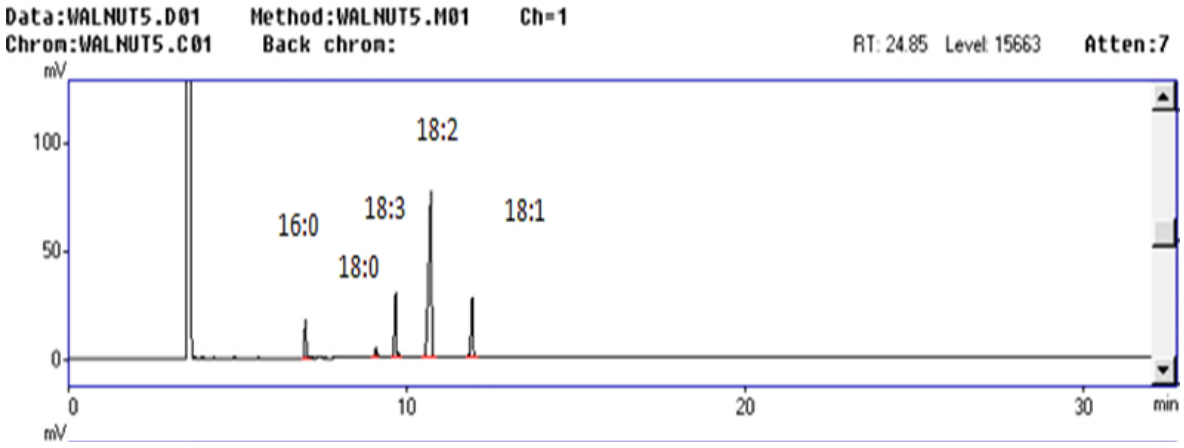

Fig. 3. Chromatogram of GC analysis of free fatty acids contents.

Table 5. Major fatty acids content of control walnut kernel oil.

Fatty acid	Content (%)
Palmitic acid (16:0)	6.10
Stearic acid (18:0)	3.60
Oleic acid (18:1)	13.10
Linoleic acid (18:2)	62.10
Linolenic acid (18:3)	15.10

3.6 Free fatty acid (FFA) content

The FAs composition of control walnut kernel oil was analyzed, and the chromatogram and results are presented in Table 5 and Fig. 3. Linoleic acid (18:2) was the most abundant (62.10%) FA of walnut oil, and the amounts of saturated and unsaturated FAs were 9.70 and 90.3 %, respectively. According to the literature, walnut oil is rich in unsaturated FAs (oleic, linoleic, and linolenic acids) (Maguire, O'sullivan, Galvin, O'connor, & O'brien, 2004).

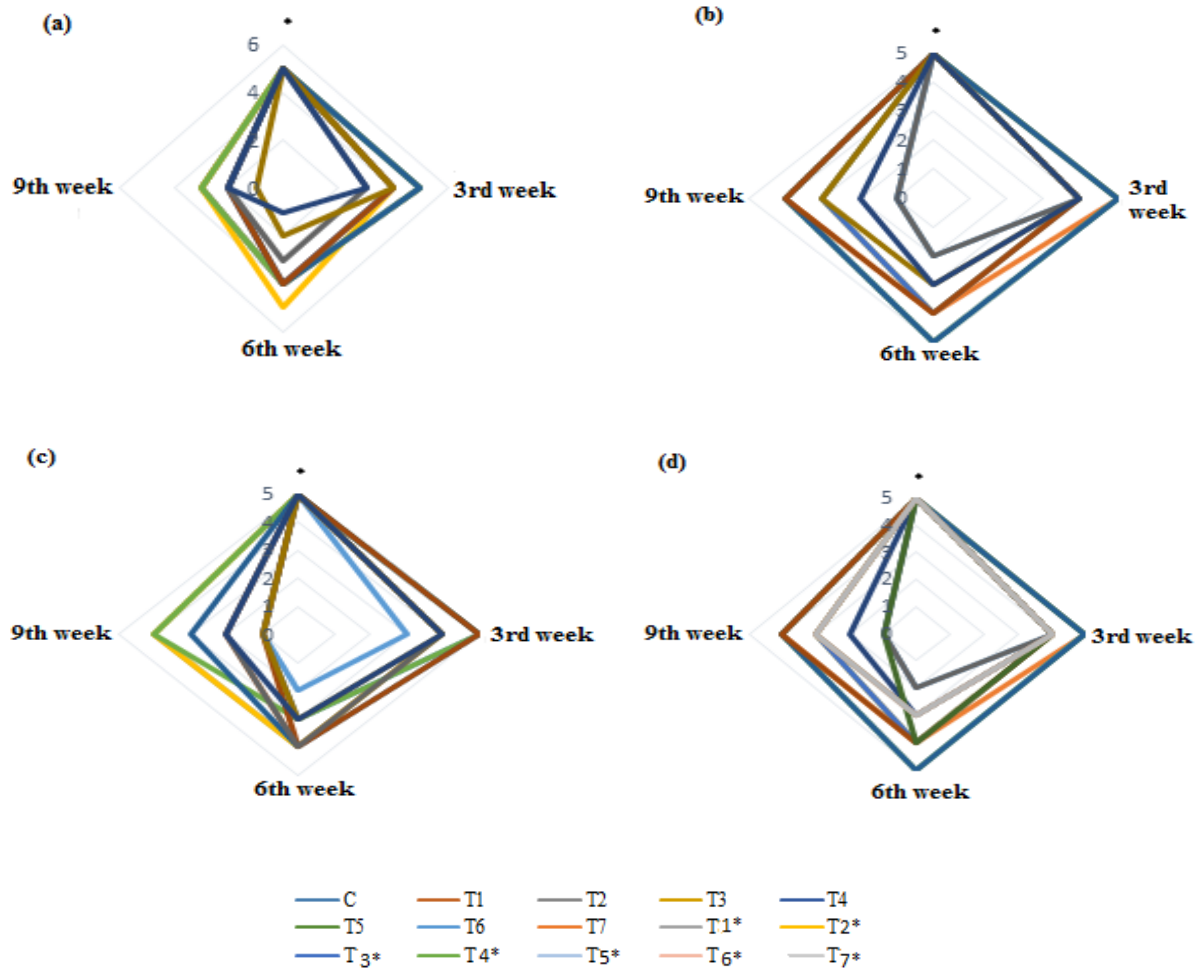

Fig. 4. Sensory evaluation of (a) texture; (b) rancidity taste; (c) flavor; and (d) overall acceptability. C=control, T1=0.75% sodium alginate+1.00% glycerol; T2= 0.75% sodium alginate+1.00% sorbitol; T3=0.75% sodium alginate+0.25% sorbitol+0.50% glycerol; T4=0.75% sodium alginate+0.25% sorbitol+0.75% glycerol;T5=0.75% sodium alginate+1.00% sorbitol+0.50% glycerol; T6=0.75% sodium alginate+1.00% sorbitol+0.75% glycerol; T7=0.75% sodium alginate+0.75% sorbitol+0.75% glycerol; T1*=T1+ $CaCl_2$ treatment; T2*=T2+ $CaCl_2$ treatment; T3*=T3+ $CaCl_2$ treatment; T4*=T4+ $CaCl_2$ treatment; T5*=T5+ $CaCl_2$ treatment; T6*=T6+ $CaCl_2$ treatment; T7*=T7+ $CaCl_2$ treatment.

Also, according to another study, the significant FAs of African walnut oil samples were palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3), and arachidic (20:0) acids (Nkwonta, Alamar, Landahl, & Terry, 2016). Some reports exhibited 64.4% linoleic, 15.2% linolenic, 13.2% oleic, 6.5% palmitic, and 2.7% stearic acids for walnut kernels oil from Bavanat gardens, Iran (Bastani *et al*., 2010).

3.7 Sensorial properties

Sensory evaluation was performed every three weeks during the storage period (Fig. 4). Flavor scores of uncoated samples decreased during storage, and they were the lowest in all weeks compared with flavor scores of coated walnut kernels. The texture of coated samples was not significantly different ($P<0.05$) from the texture of uncoated walnut kernel from the point of panelists' view. Coated walnut with the formulation of 0.75% SA + 1.00% S (T2) along with $CaCl_2$ treatment indicated significantly ($P<0.05$) more desired texture as compared to coated walnut with 0.75% SA+1.00% S (T2), but $CaCl_2$ treatment did not show significant ($P<0.05$) difference in texture parameter of other coating formulations. The maximum score of not feeling rancidity taste was related to coated walnut with T4 coating formulation (0.75% SA + 0.25% S + 0.75% G) in all weeks and also coated walnut with T1 coating formulation (0.75% SA+1.00% G), and they were significantly different

with other coating formulations. All coated walnut kernels showed less rancidity taste than an uncoated sample. This was in agreement with the results of (Baldwin & Wood, 2006), who reported the most rancid flavor for control pacan samples, reducing off-flavor using coatings and no significant (P<0.05) difference between the texture of coated and uncoated kernels. The differences between coated walnuts and uncoated samples were significant (P<0.05) for overall acceptability evaluation. Coated walnuts had more acceptability than uncoated walnut. Coating walnuts and pine nuts with whey protein isolate, pea starch, and their combination with carnauba wax improved the samples' sensorial attributes compared to uncoated nuts (Mehyar *et al*., 2012). Coated fish fillets with 3% alginate-Ca^{2+} coating had higher scores in sensorial attributes than untreated samples (López-Fernández *et al*., 2021; F. Lu *et al*., 2009).

Conclusions

The effects of SA coating and $CaCl_2$ treating were investigated on oxidative stability, fungal growth, and sensorial properties of walnut kernels. Moisture loss, PV, and AV of samples reduced significantly (P < 0.05) as a result of SA coating along with $CaCl_2$ treatment. It showed that coatings made an excellent barrier against water vapor and oxygen permeability. The fungal growth rate decreased significantly (P<0.05) in coated kernels compared to the uncoated sample. The color parameters (L* and b*) values of coated and $CaCl_2$ treated walnuts were significantly (P<0.05) higher than uncoated and no cross-linked walnuts. Moreover, SA coating did not create an undesirable effect on sensorial attributes of walnut kernels, and consumers' acceptances for coated walnuts were higher than in the control sample. According to obtained results for walnut kernels, coating with SA solution and $CaCl_2$ treatment can provide an excellent protective method for preserving different nuts.

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