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Development of novel active coating from Sagez and Sagez-zein to increase the shelf life of sweet lemon (*Citrus limetta*)

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A B S T R A C T —

The effect of simple and composite coatings of Sagez (4,8 and12%) and zein (4,8 and 12%) on shelf life, microbial spoilage and quality attributes of sweet lemon during storage ($5\pm1^{\circ}$ C and 70 % RH) for 90 days according to a full factorial design was evaluated. Changes in weight, pH, acidity, color (L* value and hue angle), firmness, sensory score and total fungal and yeast were evaluated during the storage time. The performance of the Sagez–zein composite coatings on the sample quality protection was compared with simple and uncoated sample. The results showed that the color, firmness, weight loss (WL), microbial spoilage and sensory attributes of sweet lemon were strongly affected by the Sagez coatings during storage. It was observed that the simple zein coatings and non-coated sample could not extend the shelf life of sweet lemon. Based on results Sagez can be used as an efficient edible coating to extend the shelf life and controlling spoilage of sweet lemon. The results indicated that an increase in the amount of zein in composite coatings significantly (p < 0.05) increased the total mold and yeast, weight loss and firmness and decreased the sensory scores compared to Sagez simple coatings.

Keywords: Antimicrobial activity, Edible active coating, Sagez, Sweet lemon, Zein

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1. Introduction

Edible active coatings can extend the shelf life and improve the quality of fresh fruits and vegetables and protect them from physical, chemical, and microbial damage by controlling moisture and gas (O_2 and CO_2) transfer as well as microbial load. (Poverenov et al., 2013; Huber & Embuscado, 2009). In addition, they can improve the appearance of fruits and their marketability.

Citrus limetta (sweet lemon) is a species of citrus, commonly known as sweet lime, sweet lemon, and sweet limetta, which is widely cultivated in tropical countries. The fruit has a light yellow color and a thin rind. It is used as a folk medicine because of its antihypertensive effect (Barreca, 2010). The annual production of sweet lemon in Iran ranged between 550 and 600 thousand tons. In Iran, it is used in the form of fresh fruit or juice to treat influenza and cold because it has high ascorbic acid content. Sweet lemon is widely used in tropical regions of America, Pakistan, and India. It is used for its cooling effects in cases of jaundice or high fever (Abhishek et al., 2009).

Nowadays food industries have shown increasing interest in applying natural, safe and low-cost antimicrobial agents because of

growing demand for high-quality foods and fruits devoid of chemical preservatives (Chien et al., 2007; Maqbool et al., 2011).

Investigations on edible coatings with antimicrobial activity has been widely increased. These types of coatings can promote the shelf life and safety of food by preventing the growth of spoilage and pathogenic microorganisms (Ponce et al., 2008).

Pistacia atlantica is a species of pistachio tree from Anacardiaceae family. The genus *Pistacia* contains about 11 species that usually are native to the Mediterranean areas. Iran native species consists of *pistacia atlantica*, *vera*, and *khinjuk* (Karimi et al., 2009).

The gum mastic (Sagez) is an oleoresin exudate that can be obtained from the trunk and branches of pistachio tree (Dogan et al., 2003). The Pistacia resins are a complex mixture of different bioactive groups consisting of triterpenes and essential oils being characteristic of these resins followed by phenolic compounds (Hadjimbei et al., 2014; Sharifi & Hazell, 2009).

The essential oils of *pistacia atlantica* consist of two groups of chemical constituents, the terpene hydrocarbons (monoterpenes and sesquiterpenes) and an oxygenated compound including monoterpene and sesquiterpene alcohols (Hadjimbei et al., 2014). The essential oils composition of *Pistacia atlantica* that have identified by Haghdoost et al. (2013), are alpha-Pinene (46.57%) as

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the main constituent followed by beta-pinene (9.08%), transverbenol (6.41%), sabinene (4.49%), trans-pinocarveol (4.05%), limonene (3.6%) and camphene (2.03%). The antibacterial and antifungal activity of these essential oils has been frequently reported (Alma et al., 2004; Benabderrahmane et al., 2009).

The resins of *pistacia* have two triterpenoid fraction including acidic and neutral fraction characterized by penta and tetracyclic triterpenes. Several triterpenoid compounds were isolated from acidic fraction like masticadienonic acid, masticadienolic acid, morolic acid, oleanolic acid, ursonic acid, and natural fraction consist of tirucallol, dammaradienone, β -Amyrin, lupeol, oleanolic aldehyde, and 28-norolean-12-en-3-one. It has been reported that these fractions have significant antimicrobial and antioxidant activity (Bozorgi et al., 2013).

Zein is an alcohol-soluble protein in corn that is known as a hydrophobic protein due to its high content of nonpolar amino acid (Ghanbarzadeh et al., 2007). The good barrier properties of zein make it suitable for food packaging applications (Tihminlioglu et al., 2010).

The objective of this research was to study the potential effect of different concentration of simple and composite coatings of Sagez and zein for preserving the quality of sweet lemon and to investigate the antimicrobial effect of Sages.

2. Material and Methods

2.1. Materials

Sweet lemon fruits were harvested in November at commercially mature stage from a commercial orchard in Bam, in Kerman Province, Iran. They were sorted for uniformity in size, weight (120–150g), shape and color. Fruits with spots of mechanical damage, sunburn, blemishes, disease and pest damage were discarded. Selected fruit samples were randomly distributed into groups of 10 fruit and for each treatment, three replicates were used.

The Mastic gum of *Pistacia atlantica* was collected from the Zagros Mountains of Lorestan province in Iran. Zein (90%) was obtained from Sigma Chemical and defatted by washing with hexane.

2.2. Development of coatings

For quality control, Pistacia atlantica resin (50g) was achieved by steam distillation with water (with 1.2 Lwater) in a Clevengertype apparatus for 10 hours according to the method described by Sharifi and Hazell (2009). Pale yellow essential oil from the resin was obtained (8% v/w). The solvent used to dissolve the zein and Sagez was 70% isopropanol (100%) and 30% water solution. Propylene glycol (6%) was added as a plasticizer to all coatings. A series of 0, 4, 8, and 12% of zein with 0, 4, 8 and 12% of Sagez coating solution was generated, the sample with no coating (0,0) was mentioned as control sample (Table 1). All samples stored in cold condition (5°C) for 90 days and their physicochemical properties were evaluated periodically.

2.3. Application of coating and storage condition

Samples were dipped in the aforementioned solutions for 1 min, allowed to dry at room temperature for 1 h and then stored on PET trays at 5° C and 75% relative humidity.

2.4. Weight Loss

The weight loss (WL) of whole sweet lemons were measured using a digital balance (Mettler Toledo, PB602-S, FACT, Switzerland) and was reported as percentage loss in weight based on the original mass.

2.5. Ascorbic Acid, pH, and Total Soluble Solid

Ascorbic acid content of the samples was determined using the 2, 6- dichlorophenol-indophenol titration method (AOAC, 1996). The titration end point was detected visually and all analyzes were conducted in Triplicate. The results were expressed as a mg of Ascorbic Acid per 100 ml of fresh juice. Measurement of pH was conducted by a pH meter (Metrohm, Herisau, Switzerland). Total soluble solids were measured by a hand-held refractometer (MC, Korea) at 25°C.

2.6. Firmness

The firmness of the peel was evaluated using a texture analyzer (Hounsfield Test Equipment, UK) with a 500N load cell and 3mm-diameter cylindrical probe. The skin of the sweet lemon samples was punched at 3 mm/s probe speed. Measurements were carried out on five fruits for control and coated samples.

2.7. Sensory Evaluation

Organoleptic quality of samples was evaluated by 10 untrained panelists, at the end of storage periods. They were rated on a 9point hedonic scale ranging from "very strong dislike" to "very strong like" for visual appearance, color, taste and overall acceptance (including the taste and texture). The average scores of all the 10 panelists was computed for different characteristics.

2.8. Microbiological analysis

The microbiological tests were performed in triplicate. Each fruit was washed in a bag containing 90 mL of sterile saline solution (0.9 % NaCl) with gentle shaking. Serial dilutions were carried out and 1 mL was added to potato dextrose agar supplemented with 100 ppm chloramphenicol for controlling bacterial growth. The plates were incubated at 25°C for 5 days. The number of colony-forming units (CFU) per gram of surface of the fruit was calculated.

2.9. Color measurements

Sweet lemon color was determined with a hand-held Minolta ® Chroma Meter CR300 (Minolta Camera Co., Ltd., Osaka, Japan) set to D65 illuminant/10° observer. The CIELAB color space was used to determine the parameters: L (lightness), a (greenness-redness) and b (blueness - yellowness). The five replications were measured and average value recorded for each specimen. Hue angle (h°) was calculated by the following equation:

$$h^{\circ} = tan^{-1}(\frac{b}{a})$$

2.10. Statistical Analysis

All analyzes were conducted in triplicate and were expressed as means \pm standard deviations. The experiment was conducted with three replicates. Effect of simple and composite coatings on quality attributes of sweet lemons in comparison with uncoated fruits was analyzed by one way ANOVA (SPSS version 16, SPSS Inc., Chicago, USA). Differences between means were determined by the Duncan a significance level of 0.05.

3. Results and Discussion

3.1. Weight loss

The One of the most important aspects of a coating is its ability to control the weight loss. This is more vital for non-climacteric fruit such as citrus that reduce in weight loss rate is important because the increase in transpiration rate, makes increasing accelerates senescence and reducing their postharvest life (Chavez et al., 1993; Bosquez-Molina et al., 2003). As can be seen from Fig. 1, compared to control samples, all types of coatings could reduce WL significantly. The value of WL for control sample was 35.37% after 3 months storage at 5°C. The results given in Table 1 indicate that WL of coated samples reduced when the concentration of zein and Sagez in the coating composition increased. However no significant effect on weight loss was observed when zein coating was used at the concentrations over 8%. The results show that decrease in weight loss is mainly influenced by the concentration of Sagez in the coating mixture.

The WL of sweet lemon well controlled when Sagez was used alone at a concentration of 12%. The single component application of zein also decreased WL but was not significant in comparison with Sagez. One can comprehend from Fig. 1 that loose adhesion of zein coating to the surface of sweet lemon proves its defects on preventing WL.

Weight loss in fresh fruits and vegetables is mainly due to the loss of water caused by transpiration and respiration (Sara et al., 2014). Results indicate that when the mixture of zein and Sages is used as composite coating, the higher concentrations of zein in the solution led to higher water loss (Table 1). These phenomena might be due the effect of coating material on the properties of fruit surface (Elena et al., 2014) or lack of adherence of zein-Sagez coating to the surface of sweet lemon (Fig. 1). Another reason for this observation could be related to excessive thickness of the coating due to higher viscosity of the solution which entirely covered the surface of fruit (Ali et al, 2014; Maqbool et al., 2011; Asgar et al., 2010). This can also be due to raising heat inside the fruit thus increasing anaerobic fermentation (Weichmann, 1987; Asgar et al., 2014., Maqbool et al., 2011). Similar result reported by park et al. (1994) who observed that tomato fruit coated with too much thick layer of zein film showed too- low O2 concentration and too-high CO₂ concentration that caused to ethanol production.

3.2. Total soluble solid (TSS), pH and ascorbic acid

The measured TSS for fresh sample was 7.13%. Table 1 shows the gradual increase of TSS in the samples during storage. In fact, this phenomenon is due to weight loss (water removal) and hydrolysis of polysaccharides (Bhullar, 1993; Verma & Dashora, 2000). The highest value of TSS was 9.8% in control fruit after 3 months of storage. The minimum level of TSS was measured in samples coated by Sagez 12% and zein 12%. Table 1 shows the lower TSS content of Sagez coated samples compared to the control ones. The TSS content of samples reduced as the concentration of Sagez increased in the coating solution. On the other hand, a higher concentration of zein in composite coatings led to increase TSS. This could be due to the best semi-permeable properties of the single component Sagez coating on the surface of fruit and atmosphere modification inside the fruit by decreasing O₂, increasing CO2, which reduced production of ethylene (Ali et al., 2010)

The initial value of pH was 5.62 ± 0.09 . As indicated in Table 1, the pH value of all coated and control samples moved up under storage time but at a slower rate in samples, which coated by higher concentration of Sagez. The higher value of pH can be due to the utilization of acids with respiration during storage (Togrul et al., 2004; Medeiros et al., 2012). The maximum and minimum change of pH value was observed in the control sample and coated samples by a single component solution of Sagez at concentration of 12% respectively. It can be concluded that the Sagez coatings (12%) could reduce the rate of respiration and delayed the consumption of acids consequently. Similar results were reported by Togrul and Arslan (2004) in the case of the coating of mandarin.

The effect of coating treatment on ascorbic acid content is listed in Table 2. The initial concentration of ascorbic acid in the all samples was 60.23±1.87 mg/100 ml of fresh juice. The results indicate that the content of ascorbic acid increased in control and coated fruits with zein after one-month storage. Nevertheless, the vitamin C content appeared to decrease in the second and third months of storage. This phenomenon can be related to higher weight loss (water loss) of control sweet lemons after one month of storage. It is evident from the Table 2 that ascorbic acid contents of fruits coated with the zein was not significantly decreased compared to control samples (Table 2). The results showed that sweet lemons coated with Sagez, especially at a concentration of 12% have maximum ascorbic acid content (53.2 and 51.33 mg/100 mL juice) after 60 and 90 days of storage which was significantly higher than control and all other samples (Table 2). The lower reduction of ascorbic acid content in Sagez coated samples proves that it can intelligently control respiration rate and ripening of fruit during storage. Ascorbic acid is a water-soluble vitamin that is easily oxidized to dehydro ascorbic acid in the presence of Ascorbinase enzyme in over ripening (Bisen et al., 2012; Togrul & Arsalan, 2004). Ascorbic acid can be oxidized by several factors such as exposure to oxygen, metals, light, heat, and alkaline pH (Ball, 1997). Edible coatings can reduce the autoxidation of ascorbic acid in sweet lemon by controlling the permeability of O2 and CO2. Similar results have been reported by other researchers in the case of citrus fruits (Bisen et al., 2012; Togrul & Arsalan, 2004).

Measured property Coating composition (%)		WL (%)			рН			TS (%)			
		Storage time (day)									
		30	60	90	30	60	90	30	60	90	
Sagez	Zein										
	0	22.6±1.2ª	$28.8{\pm}1.0^{a}$	35.1±0.8ª	$5.74{\pm}0.05^{a}$	6.01±0.01 ^{ab}	6.19±0.02 ^a	8.56±0.15 ^a	9.16±0.15 ^a	9.8±0.1ª	
0	4	$20.0{\pm}1.5^{b}$	22.5 ± 0.7^{a}	$34.0{\pm}1.0^{b}$	$5.74{\pm}0.06^{a}$	6.03 ± 0.03^{a}	6.18 ± 005^{a}	8.5 ± 0.1^{ab}	9.1 ± 0.1^{ab}	9.7 ± 0.2^{a}	
0	8	18.3±1.0°	25.0±1.1 ^b	$31.0\pm1.0^{\circ}$	$5.72{\pm}0.06^{a}$	6.01 ± 0.01^{ab}	6.17 ± 0.02^{a}	$8.46{\pm}0.15^{ab}$	8.9 ± 0.1^{bc}	9.73±0.1ª	
	12	17.0 ± 0.7^{d}	$24.8{\pm}1.0^{b}$	31.0±1.0°	$5.73{\pm}0.08^{a}$	6.02 ± 0.03^{ab}	6.18±0.01 ^a	8.3±0.1 ^b	8.73±0.15 ^{cd}	9.6±0.1ª	
	0	$10.8{\pm}0.5^{\mathrm{hi}}$	20.3 ± 0.9^{d}	$26.4{\pm}0.8^{\rm f}$	$5.79{\pm}0.08^{\mathrm{a}}$	5.96 ± 0.05^{abc}	$6.14{\pm}0.03^{ab}$	$8.06 \pm 0.2^{\circ}$	8.73±0.2 ^{cd}	9.3±0.1 ^b	
4	4	$12.0{\pm}0.8^{\rm fg}$	$16.8 \pm 1.4^{\circ}$	$28.5\pm0.5d^{e}$	$5.71{\pm}0.08^{a}$	5.97 ± 0.03^{ab}	6.12 ± 0.02^{ab}	7.96±0.15°	8.6±0.1 ^{de}	9.2±0.1 ^b	
4	8	13.3±0.7°	24.0±0.6 ^b	$29.6{\pm}1.0^{d}$	$5.7{\pm}0.08^{\mathrm{a}}$	$5.97{\pm}0.03^{ab}$	6.13 ± 0.02^{ab}	$7.93 \pm 0.06^{\circ}$	8.4 ± 0.1^{ef}	9.1 ± 0.15^{bc}	
	12	$11.5\pm0.5^{\text{gh}}$	23.0±1.2°	$28.5{\pm}0.5^{\text{de}}$	$5.75{\pm}0.05^{a}$	6.01 ± 0.02^{ab}	6.13±0.02 ^{ab}	8.03±0.2°	$8.33{\pm}0.05 f^g$	8.93±0.05°	
	0	7.7 ± 0.4^{1}	16.2 ± 0.8^{f}	$21.8{\pm}1.0^{\rm h}$	$5.76{\pm}0.02^{a}$	5.95 ± 0.02^{abc}	6.06 ± 0.05^{bc}	7.6 ± 0.1^{d}	$8.2\pm0.1f^g$	8.56±0.15 ^d	
8	4	10 ± 0.5^{ij}	19.6 ± 0.8^{d}	$24.0{\pm}1.0^{g}$	$5.79{\pm}0.02^{a}$	6.01 ± 0.04^{ab}	6.10±0.03 ^{abc}	7.66 ± 0.2^{d}	8.23 ± 0.05^{fg}	8.56±0.15 ^d	
8	8	$14.2{\pm}0.8^{\rm ef}$	22.0±1.2°	29.2 ± 0.7^{d}	$5.70{\pm}0.05^{a}$	6.02 ± 0.0^{2a}	6.12 ± 0.03^{ab}	7.7 ± 0.1^{d}	8.1 ± 0.1^{g}	$8.4{\pm}0.1^{d}$	
	12	$10.8{\pm}1.0^{\rm hi}$	22.5±0.6°	28.01±0.9e	5.77 ± 0.05^{a}	6.0 ± 0.01^{abc}	$6.12{\pm}0.02^{ab}$	7.66 ± 0.2^{d}	8.13±0.15 ^g	8.43 ± 0.05^{d}	
	0	$5.5\pm0.6^{\mathrm{m}}$	$9.3{\pm}0.8^{h}$	$13.7{\pm}0.8^{j}$	5.78±0.09 ^a	5.76±0.06°	5.92±0.01°	7.26±0.15 ^e	7.73 ± 0.1^{h}	8.06±0.1e	
12	4	8.1 ± 0.8^{kl}	14.6±0.3 ^d	$15.0{\pm}1.0^{h}$	5.77±0.02ª	5.92 ± 0.04^{bc}	6.0 ± 0.02^{bc}	7.26±0.15 ^e	7.7 ± 0.1^{h}	8.03±0.1e	
	8	9.1±0.3 ^{jk}	13.4±0.6 ^e	23.2±0.8g	5.78±0.09 ^a	5.95±0.04 ^{abc}	6.09±0.04 ^{abc}	7.36±0.2 ^e	$7.73{\pm}0.15^{h}$	8.1±0.1e	
	12	8.8 ± 0.4^{kl}	$15.8{\pm}1.1^{\mathrm{fg}}$	21.0 ± 0.7^{hi}	5.79±0.08 ^a	5.97 ± 0.0^{3ab}	6.10v0.01 ^{abc}	7.3±0.2 ^e	7.6 ± 0.2^{h}	8.03±0.15 ^e	

Table 1. Weight loss (WL), pH and total soluble solids of sweet lemons during storage at 5°C.

Table 2. Changes in ascorbic acid content, Hue angle and lightness of sweet lemons during storage at 5°C.

Measured property Coating composition (%)		Vit. C content (mg/100ml)			Hue angle			L value		
					Storage time (day					
		30	60	90	30	60	90	30	60	90
Sagez	Zein									
0	0	62.83 ± 2^{a}	50.66±1 ^{ab}	42.33±0.5 ^g	45±1 ^e	42.66±0.5 ^h	39.66±1.5 ^e	61.33±3 ^b	51.66±5 ^d	44.66±4.5 ^f
	4	62.33±1.5 ^a	53.01±1ª	43.33±1.5 ^{fg}	45.66±0.5 ^e	42.66±1.5 ^h	40 ± 1^{de}	64 ± 4.5^{b}	51.33 ± 2.5^{d}	47.33±3.5 ^{ef}
	8	61.66 ± 2^{a}	52.06±1 ^{ab}	44.66±1.5 ^{efg}	47.66±0.5 ^d	43.66±0.5 ^h	41 ± 1^{cde}	63.33±3.5 ^b	53.66±3 ^d	48.33±1.5 ^{et}
	12	61.46 ± 2^{a}	33.53 ± 2^{a}	$45.05 \pm 1.5 c^{def}$	48 ± 1^{d}	44.6±1.5 ^{gh}	41.33±1 ^{cde}	64.33±3 ^b	61±3.5°	52 ± 4^{de}
	0	53.36±1°	33.52 ± 2^{ab}	47.03±2 ^{bcde}	52±1 ^{abc}	47.6±1.5 ^{cde}	53±1°	79.66±4 ^a	$65\pm4^{\circ}$	$55 \pm .2^{d}$
4	4	56.12±1.5 ^{bc}	52.66 ± 2^{a}	46.03±1 ^{cdef}	52 ± 1^{abc}	45.66±1 ^{fg}	42 ± 1^{cd}	79.66±2 ^a	63.66±4°	56.33±2.5°
4	8	57.85±1.5 ^b	53.02±1ª	47.66±2.5 ^{bcde}	51.33±1.5 ^{bc}	45.33±1.5 ^g	41.33±1 ^{cde}	78.33±0.5 ^a	55.66 ± 2^{d}	53±5 ^{de}
	12	56.33±2bc	53.02±1.5 ^a	45.66±1 ^{cdef}	51±1°	46 ± 2^{efg}	42.33±0.5°	77.33 ± 2.5^{a}	62.33±1°	52.66±4 ^{de}
8	0	55.04±0.5 ^{bc}	52.33 ± 1.5^{ab}	50.04 ± 1.5^{ab}	52.66±0.5 ^{abc}	49.66±1 ^{bc}	47±1 ^b	80.33 ± 4^{a}	77 ± 5.5^{ab}	66.33±3 ^b
	4	54.66±2.5 ^{bc}	51.01 ± 1.5^{ab}	48.05±1 ^{bcd}	53.33±0.5 ^{ab}	48.33±1.5 ^{cd}	46±1 ^b	79.33±3ª	$66.66 \pm 8^{(c)}$	61±2.5 ^{bc}
0	8	56/06±1bc	49.33±1.5 ^b	46.33±1 ^{cdef}	52±1 ^{abc}	47.33±1 ^{def}	42.33±1°	77.66 ± 2.5^{a}	$65\pm4^{\circ}$	55 ± 2.5^{d}
	12	55.66±1.5 ^{bc}	50.33±1 ^{ab}	46.04±1.5 ^{cdef}	53±1 ^{abc}	48 ± 1^{cd}	42.66±0.5°	78 ± 2.5^{a}	66±4°	55.33±1.5 ^d
12	0	57.46±1 ^{bc}	53.02 ± 2^{a}	51.33±2.5 ^a	53.66±1.5 ^a	53±1ª	$51.33{\pm}1.5^{a}$	80.66 ± 2^{a}	79±2.3ª	79.66±1.5 ^a
	4	56.09±2.5 ^{bc}	53.03 ± 2^{a}	48.33±2bc	53.66±1.5 ^a	51±1 ^b	$47.33{\pm}1.5^{a}$	78 ± 3^{a}	$78.66{\pm}1.5^{a}$	65.33±4.5 ^b
	8	56.03±1 ^{bc}	50.25 ± 08^{ab}	48.33±2.5 ^{bc}	52.66±0.5 ^{abc}	49.33±0.5 ^{bcd}	45.33±1.5 ^b	77.66±1.5 ^a	66.33±7°	62 ± 2.5^{b}
	12	55.02 ± 2^{bc}	51.66±1 ^{ab}	47.33±1,5 ^{bcde}	53.33±0.5 ^{ab}	49.66±1.5 ^{bc}	46±1 ^b	78 ± 3^{a}	73±2 ^b	66.66±1 ^b

Measured property			Firmness (N))	Total mold and yeast (log cfu/g)						
Coating				S	torage time (da	y)					
composition											
(%)		30	60	90	0	30	60	90			
Sagez	Zein										
	0	19.8±0.8	23.8±1	28.2±1.2	1.8±0.2	6±0.35	7.05±0.22	7.9±0.35			
0	4	20±1	22.8±0.5	27.5±1	1.45 ± 0.25	5.8±0.3	6.5±0.31	7.7±0.2			
0	8	20.1±1.8	23±1.5	27.5±1.1	1.4±0.3	5.5±0.1	6.4±0.21	7.4 ± 0.1			
	12	19±0.9	23.6±0.7	27.3±1.4	1.2±0.19	5.2 ± 0.11	6.2±0.23	7.4±0.2			
	0	18 ± 1	19.4 ± 0.6	23.8±0.9	1.25 ± 0.4	3±0.4	4.1±0.17	5.17 ± 0.1			
4	4	17.9 ± 1.1	20.5±1.5	23.7±1.3	1.5±0.35	3.4±0.18	4.75±0.15	6.1±0.1			
4	8	20±0.9	$21.4{\pm}1.5$	24±1	1.4±0.3	3.5±0.25	4.8±0.44	6.2 ± 0.18			
	12	18 ± 1.2	22.3±0.7	24.1±1.1	1.6±0.3	3.4±0.22	4.9±0.5	6.22±0.3			
	0	18.2 ± 1	20±1	22±0.7	1.18 ± 0.15	2.6±0.2	3.7±0.12	4.2±0.12			
8	4	19±0.8	20.5 ± 1.2	22±1	1.22 ± 0.2	3.2±0.2	4.18 ± 0.11	4.84 ± 0.24			
0	8	20.5±0.6	23.3±0.6	25±1.1	1.5±0.25	2.9±0.2	4.4±0.32	4.9±0.1			
	12	20±0.5	22±0.1	22.5±0.5	1.4 ± 0.15	3.24±0.15	4.55±0.13	5.1±0.17			
12	0	16.5±0.4	17±0.4	17.8 ± 1	1.09 ± 0.05	2.2±0.13	2.6±0.1	3.1±0.08			
	4	17.3±1.5	17.4±1	$18.4{\pm}11$	1±0.05	2.45 ± 0.1	3.2±0.12	3.8±0.05			
	8	16.9±1	18±1	19±1	1.22±0.15	2.8±0.4	4.08±0.13	4.65±0.5			
	12	17.4±0.5	17±0.3	18±0.9	1.25 ± 0.15	2.77±0.2	4±0.1	4.77±0.22			

Table 3. Firmness and microbial properties of sweet lemons during storage at 5°C.

3.3. Color

Color plays an important role in the acceptability of foods. Coated samples with Sagez were able to maintain their original natural light yellow color up to 3 months of storage (Table 2). The hue angle of all samples dropped significantly during storage, however changes of hue angle values were inhibited for coated samples with high amount of Sagez and at a lower level of zein. The hue angle of fresh sample skin was 54±1.5. In general, results did not show significant changes in the hue angle of samples coated with Sagez 12% during storage. It may be due to a delay in the maturation/ripening, decreased enzymatic reaction rate and consequently low degradation in the pigment of the fruit. The uncoated samples became more red and darker along with storage time. In that case, Hue angle value decreased from 54 to 39.66 after 3 months storage at 5°C while Hue angle value of coated fruit with 12% Sagez and 0% zein changed from 54 to 51.33 in the same period of time. On the other hand, it can be assumed that the hue angle decreased during storage as a consequence of surface moisture loss, senescence process, enzymatic reaction activities and degradation in the color pigment which could be responsible for the observed darker color (Bisen et al., 2012; Perdones et al., 2012).

L value shows the visually perceptible changes during storage. Table 2 shows the L values of the uncoated and coated sweet lemons. Uncoated fruits were darkened and all the coated samples had higher L value in comparison with the control throughout storage. Decreasing L value was controlled during storage by Sagez; however, this decrease was less marked in samples coated with zein.

3.4. Antimicrobial activity

Antimicrobial activity is one of the most required and desired contributions of all coatings to food safety and quality (Campos et al., 2011). The effect of the treatments on the fungal populations is presented in Table 3. The total fungal counts in the control samples increased from the initial level of 1.6 to over 7.6 log₁₀ CFU/g at the end of the cold storage. The antifungal effectiveness of coatings

was depended on the effect of antimicrobial compounds and their concentration, so that coatings with Sagez dramatically improved the microbiological quality of fruits. The counts of fungus of the fruits with 12% Sagez increased just from the initial counts level of 1 to 3.2 log CFU/g at the end of storage time, so the Sagez treatments were able to slow down the growth of fungus. This result indicated its ability to enhance the microbiological quality of sweet lemon and potentially extend the shelf-life of the product. Such observation was also happened for the coatings of 12% zein; however, when zein was combined with Sagez, they showed an antagonistic effect on fungus. It can be explained by the poor adhesion of zein to the surface of sweet lemon during storage. This phenomenon may be due to trapping the active antimicrobial compounds of Sagez in the matrix polymer of zein and controlling the release of them (Mastromatteo et al., 2009). As a result, the effect of Sagez was declined by adding of zein. According to the literature the essential oil of the resin proved to be active against microorganisms and fungi (Benabderrahman et al., 2009; Alma et al., 2004; Magiatis et al., 1999; Ghalem and Mohamed, 2009).

3.5. Firmness of skin

Firmness is one of the main quality attributes of fruit, determining storability. Fruit softening is conducted by biochemical processes during postharvest storage and involving the biochemical and physical changes which are associated with the increase of fruit susceptibility to fungal attack (Cantu et al., 2009). In general, the firmness of coated and uncoated sweet lemons was similar and the same on the first day of the storage period (Table 3). When coated samples were dried after coating treatment, the skin firmness was measured and it was about 16.7 N for all samples. Therefore, the skin firmness of coated fruits has not been affected significantly by coating material. It can be seen from Table 3 that firmness was increased during storage. The same results were achieved by Castillo et al. (2014). Increased skin firmness of samples can be considered as a result of the water loss especially in their skin; as a result, it can be seen that the firmness values were increased because of the forming of hard dried skin. The effect of coating on the fruit firmness may be related to its ability to reduce water loss. However, the best results were achieved at a low-level concentration of zein. It means that the fruits skin firmness showed lower change with increasing Sagez concentration. The firmness of the uncoated control was enhanced from 16.7 to 28.3 N after 3 months storing in 5°C (69.4%) while firmness of the coated fruit with 12% Sagez and 0% zein was changed from 16.71 to 18.76N

(12.27%) at the same storage period and conditions. Generally, the effect of Sagez on firmness was decreased when it used in combination with zein. As mentioned before, poor adhesion properties of zein to the surface of sweet lemon and peel off that during storage until 3-month cause to a higher level of water loss of sweet lemon as well as increased values of the firmness in comparison with the coated fruits by Sagez solutions without zein.

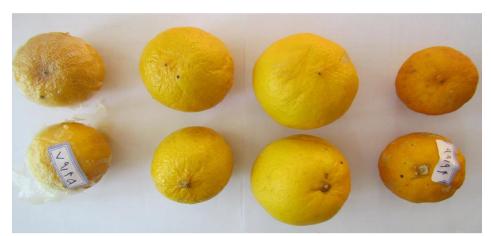


Fig. 1. The visual appearance of sweet lemons after 90 days storage at 5 °C followed by 4 days under shelf-life conditions at 20 °C. Left to right (sagez0 and zein12, sagez12 and zein 12, sagez12 and zein0, and control).

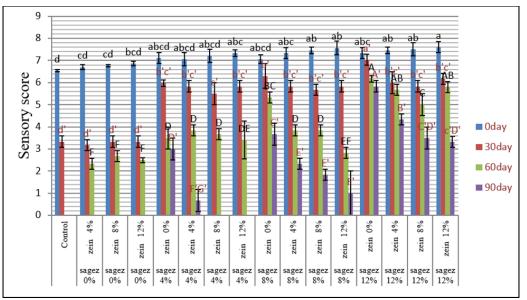


Fig. 2. Average of sensory score of sweet lemons after 90 days storage at 5°C.

3.6. Sensory Evaluation

Results of the sensory evaluation of uncoated and coated samples with Sagez and zein have been shown in Fig. 2. At the first day, all coated sweet lemons had the sensory values as high as uncoated fruit. Sensory evaluation of coated and uncoated fruit at the end of the storage period revealed significant differences in visual appearance, color, taste, texture and overall acceptability. After storage at 5°C, the sensory value of the coated fruit with 12% of Sagez was higher than the other coated samples. Coatings with Sagez dramatically improved the sensory quality of fruits due to the

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greater protection of visual appearances, reduction of water loss, and inhibition of spoilage; as a result, sensory values were increased with higher Sagez concentration. The sensory value of the uncoated control was decreased from 6.5 to 3.3 at 1-month storage at 5°C while that of the coated fruit with 12% Sagez and 0% zein was changed from 7.28 to 5.83 at the end of storage. Under storage conditions at 5°C, the glossy appearance of the sweet lemons coated with zein faded with storage time in all treatments. Thus, the reduced zein formulations might be suggested when prolonged storage is needed, because it induces higher withered during the storage and the panel did not give a high score in appearance compared to high Sagez formulations. These results were in line with previous works (Eshghi & Hashemi, 2014; Arnon et al., 2014).

4. Conclusions

The results of this research indicated that zein coatings cannot provide prolonged shelf life of sweet lemon compared to untreated samples during storage time at $5\pm1^{\circ}$ C with 70% relative humidity. The results also prove that fruits coated with Sagez benefits from longer shelf life. The more the concentration of Sagez leads to the longer shelf life of the sweet lemons. The single component coating made of 12% Sages was the best coating for maintaining postharvest quality, controlling fungi spoilage compared to other coating treatments. The results indicated that increase in the concentration of zein in composite coating unexpectedly led to considerable weight loss, total mold and yeast counts and firmness and decreased sensory scores of the samples compared to Sagez single component coatings. It can be concluded from this study that Sagez can be used as a very good novel commercial edible coating to extend the shelf life and controlling spoilage of sweet lemon.

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