Journal of Food Safety and Food Quality 72, Heft 6 (2021), Seiten 177–214

Arch Lebensmittelhyg 72,
202–212 (2021)
DOI 10.2376/0003-925X-72-202

ISSN 0003-925X

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Effect of emulsion and nano-emulsion of *Salvia chorassanica* essential oil coatings on strawberries quality

Auswirkung von Emulsionen und Nanoemulsionen mit ätherischem Öl von Salvia chorassanica auf die Qualität von Erdbeeren

Azam Mehraban[1]), Mohsen Vazifedoost[2]), Zohreh Didar[2]), Mohammad Hossein Haddad Khodaparast[3]), Masoumeh Mehraban Sang Atash[4])

Summary

In this study, the effect of *Salvia chorassanica* essential oil emulsion and nano-emulsion coatings on the shelf-life of strawberries during cold storage were investigated. The nano-emulsion of *S. chorassanica* essential oil was prepared with ultrasound treatment and its characteristics were analyzed. The results of antifungal assay indicated that nano-emulsion of *S. chorassanica* essential oil had lower minimum inhibition concentration (MIC) (6.25 µl/ml) than its emulsion (12.5 µl/ml) and thiabendazole fungicide (12.5 µl/ml) against molds spoilage of strawberry (*A. niger, R. stolonifer* and *B. cinere*). Also, the evaluation of quality properties of strawberry samples revealed that *S. chorassanica* essential oil nano-emulsion coating remarkably delayed the loss in weight, firmness, TSS(%), mold decay, anthocyanin and ascorbic acid contents of strawberry during 12 days. Consequently, the *S. chorassanica* essential oil nano-emulsion coating was recommended as a suitable method to prolong the shelf-life of strawberry fruit.

Keywords: Salvia chorassanica, essential oil, nano-emulsion, coating, strawberry

Introduction

Strawberries are one of the most popular fruits in many countries due to their desirable aroma and taste. Strawberries contain bio-active compounds such as various minerals and vitamins (vitamin C and E) that promote health (Velde et al., 2013). Strawberries have a very short shelf-life less than one week even under ideal conditions at 4°C because of the high rate of respiration and perishability. The quality factors of strawberries such as color, texture and appearance after harvest are changed by physical damage and fungal rot (*Botrytis cinerea, Rizopus stolonifera* and *aspergilus niger*) (Treviño-Garza et al, 2015).

Many studies have confirmed that the chemical fungicide compounds caused a potential risk for the environment and human health. Therefore, natural preservatives such as essential oils have received a lot of attention as a good alternative of synthetic fungicides to improve food quality and prolong the shelf life (Li et al, 2018). Essential oils are biodegradable and eco-friendly volatile natural plant products. They are secondary metabolites formed by different parts of aromatic plants with a strong odor. Typically, their complex volatile substances are included terpenes, terpenoids and phenols. In various studies, the same essential oil type was indicated variable chemical composition because of different geographic regions or using different extraction methods (Wan et al, 2019).

Despite the potential of essential oils as natural antimicrobial agents, their applications in food have been limited due to their low solubility in water and high volatility. Production of nano-emulsions is a new strategy for improving the characterization of essential oils (Ryu et al, 2018).

Generally, nano-emulsions consist of two incompatible liquids that one of the liquids with nano-sized particles (20–200 nm) dispersed in another (Zhang et al, 2017). According to various researches, the result of the reduction in particle size and increase the total surface area of particles in nano-emulsions is an improvement in antioxidant, antibacterial and antifungal activity of essential oil (Weiss et al, 2009, Donsì et al., 2011; Wan et al., 2018). In general, high energy and low energy approaches are the main methods that have been developed to form nano-emulsions. The high-energy methods include; high-pressure homogenization, micro fluidization, sonication, method in jet disperser, high-amplitude ultrasonic methods, while the low-energy methods include; solvent displacement method, phase inversion methods, and spontaneous for producing nano-emulsions (Hassan and Mujtaba, 2019).

The sage plant, Salvia belongs to the family of Lamiaceae, which also includes the mints, many species of it widely grow in different regions of Iran and Turkey. Salvia species are used in folk medicine and as additives in food products in these countries. Essential oils and extracts of different species of Salvia have revealed anti-tumor, anti-inflammatory, cytotoxic, antioxidant and antimicrobial properties in previous researches (Firuzi et al, 2013; Mehraban et al, 2016; Yilar et al,2018). *Salvia chorassanica* is a native specie in Khorasan province, Iran. As far as we know, there is no information about the composition of *S. chorassanica* essential oil and its properties. Therefore, the aim of this study was the investigation of *S. chorassanica* essential oil compounds and the preparation of its essential oil nano-emulsion. Also, the antifungal activity of *Salvia chorassanica* essential oil emulsion and nano-emulsion against spoilage molds of strawberry were analyzed and their applications as a coating in maintaining the quality properties of strawberry fruit during cold storage were evaluated.

Material and methods

Sample collection

Aerial parts of *S. chorasanica* after the flowering stage were collected from eastern Khorasan Province in the east of Iran (36.31 N, 59.16 E) and identified in the herbal systematic laboratory and Research Center for Plant Sciences in Ferdowsi University of Mashhad. The aerial parts of plants were washed with distilled water and dried in darkness and dust-free condition for 2 weeks.

Extraction of *Salvia chorassanica* essential oil

First of all, 200 g of dried sample was introduced to the Clevenger-type apparatus in order to hydro-distillation for 5h at 60°C. N-Hexane was used as an extracting solvent for the concentration of all of the aroma constituents. After evaporation of the N-Hexane under Inert gas (N2), the essential oil was dried over anhydrous sodium sulfate, and stored in sealed vials in dark at 4 ºC for further experiments. The following equation was used for oil yield calculation.

$$\text{essential oil (\%)} = \text{Weight of essential oil (g)} / \text{Weight of dried sample (g)} \times 100 \quad (1)$$

Essential oil components analysis

The identification of constituents of the essential oil was performed by Gas-Liquid Chromatography coupled to Mass Spectrometry (GC-MS) (Model 5977A, Agilent Technologies, USA). The gas chromatograph was equipped with an HP-5MS capillary column (phenylmethyl siloxane, 30 m × 0.25 mm ID 0.25um, Agilent technologies). Helium (99.999 % purity) at a constant flow of 1 ml/min was selected as the carrier gas and Other GC conditions were the same as described above. Also, the mass spectrometer was set in EI mode at 70 eV. The interface temperature was adjusted to 280 °C and mass range between m/z 35–500 amu. The 1µl of essential oil was injected into the instrument and after crossing the GC-MS equipment, the spectrums were obtained. Finally, the spectrums of peaks compounds were compared with the database of the spectrum of known compounds stored in the GC-MS NIST 08 library and different components were identified.

Emulsion and Nano-emulsion preparation

S. chorassanica essential oil loaded nano-emulsion was manufactured using the ultrasonication. According to Lee et al (2019), *S. chorassanica* essential oil (10 wt.%), tween 80 as a surfactant (10 wt.%), and distilled water (80 wt.%) were mixed by a magnetic stirrer (5000 rpm) (Labtron, Germany) for 5 minutes at room temperature. Then Ultra-thorax homogenizer (Heidolph, Germany) was used at a rate of 10,000 rpm for 3 minutes to homogenize. In order to reduce the size of the resulting emulsion particles and nano-emulsion formation, it was subjected to ultrasound (Bandeline, Germany) for 5 minutes (nominal power of 750 watts and frequency of 20 kHz).

Particle size analysis

The mean particle diameter of the nano-emulsion droplets was measured using a particle size analyzer (Cordouan Technologies, France). In order to determine the stability of nano-emulsion during storage time, the average particle size of its droplets was measured on days 0, 3, 6, 9, 12 and 15. The Span (distribution width) was determined using the following equation (2).

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$$\text{Span} = (d_{90} - d_{10}) / d_{50} \quad (2)$$

where d_{10}, d_{50} and d_{90} are particle sizes corresponding to 10, 50 and 90% intensity on a relative cumulative particle size distribution curve.

Antifungal activity

Determination of Minimum Inhibitory Concentration

Minimum Inhibitory Concentration (MIC) of the emulsion and nano-emulsion of *S. chorassanica* essential oil against *Botrytis cinerea, Rizopus stolonifera,* and *Aspergilus niger* as the decay factors of strawberry fruits were investigated the microdilution method. All tested fungi were cultivated for a week on PDA medium at 25°C. The spore suspension was prepared in sterile distilled water containing 0.05% of Tween 80 and the spore concentration was adjusted by a hemocytometer (10^5spore/ml). The emulsion and nano-emulsion of *S. chorassanica* essential oil were serially diluted in a 96 well microtiter plate containing potato dextrose broth medium (PDB) (Merck, Germany) to produce a concentration range of 1.56–50 µl/ml. Then 20 µl of spore suspension of each fungus was inoculated in per well and incubated for 3 days at 25 °C. In this assay, the wells containing emulsion or nano-emulsion of *S. chorassanica* essential oil without inoculum served as a negative control. Thus the lowest concentration of emulsion and nano-emulsion of *S. chorassanica* essential oil with no macroscopic growth of fungi was considered as the MIC and lack of growth on PDA (Merck, Germany) medium after 3 days was confirmed the MFC (Minimum fungicidal concentration).

Coating application

The strawberries were bought from the local market of Mashhad, Iran. All the fruits were selected based on color, uniform size, as well as the absence of physical damage and fungal infection. Two treatments were used for strawberries coating including emulsion and nano-emulsion of *S. chorassanica* essential oil. The fruits were immersed in 0.1% hypochlorite sodium for 30 s to remove surface contamination. Then, strawberries were completely washed with distilled water and dipped for the 60s in spore suspensions of *Botrytis cinerea, Rizopus stolonifera* and *Aspergillus niger* (10^4 spore/ml). The emulsion or nano-emulsion of S. chorassanica essential oil at MIC and MFC concentration that determined in the previous section sprayed on strawberries surface and dried for 4 h at room temperature. Another strawberry samples were sprayed with distilled water as a control treatment. Afterward, 50 strawberries of each treatment were distributed in 5 polyethylene trays and stored at refrigerated for 12 days (Naserzadeh et al, 2019).

Mold decay

The mold decay of strawberries samples was evaluated by mold count during the cold storage. 10 g from each treatment group was added to 90 ml of sterile physiological saline solution (8.5 g/1000 ml distilled water) and homogenized using a homogenizer (Heidolph, Germany). The serial dilutions of the homogenates were prepared and inoculated (100 µl) on the surface of PDA plates and incubated at 25°C for 5 days. The results of counting were expressed as log CFU/g after incubation time (Robledo et al, 2018).

Weight loss

Weight loss of different strawberry groups was evaluated by monitoring their mass changing during cold storage compared to their initial weight and expressed as a percentage (Chu et al, 2020).

$$\text{Weight loss (\%)} = W_1 - W_2 / W_1 \times 100 \quad (3)$$

pH and titrable acidity

The strawberries cut into small pieces were homogenized for 1 min at high speed using a blender (Parskhazar, Iran). The pH of strawberry puree was measured with a calibrated pH meter (Metrohm, Swiss). Acidity was examined according to the AOAC 942.15 method (AOAC, 1995), and was expressed as g of citric acid per 100 g of fruit.

Total soluble solids (TSS)

The digital hand refractometer (Atago, Japan) was used to determine the soluble solids content in the juice of blended strawberries at 20 °C and expressed as a percentage. All measurements were performed in triplicate (Velickova et al, 2013).

Surface color

The surface color changes of control and treated strawberries during cold storage (0, 3, 6, 9 and 12 day) was measured by using a colorimeter (Loviband Tintometer, England) to obtain L*(lightness), a*(redness) and b*(yellowness) value as the mean of 10 readings. The total color difference DE, as well as the chroma value and hue angle were calculated using the following equations:

$$\Delta E = \sqrt{(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})} \quad (4)$$

$$\text{Chroma} = \sqrt{(a^{*2} + b^{*2})} \quad (5)$$

$$\text{hue angle} = \tan^{-1}(b^*/a^*) \quad (6)$$

Firmness

The firmness of the control and coated strawberries was evaluated using a texture analyzer (TAXT Plus Stable Micro System, England) with 5 kg load cell according to Velickova et al method (2013). Briefly, each strawberry divided in two-part was placed on a flat platform and a probe (5 mm diameter) with a speed of 1 mm /s penetrated into the strawberry tissue and the penetration depth was adjusted on 3 mm. Finally, the firmness of strawberry samples was expressed in Newton.

Anthocyanin contain

Total anthocyanins of strawberry samples were estimated by a pH-differential method during storage time. Two dilutions of strawberries juices in potassium chloride buffer (pH 1) and sodium acetate buffer (pH 4.5) were prepared. Afterward, the absorbance of each sample was measured at 510 and 700 nm. The total anthocyanins content was represented in mg of cyaniding-3-o-glucoside equivalents per l of fruit juice by using the following equations: (Jakobek et al, 2007)

$$A = [(A_{510} - A_{700})_{pH1} - (A_{510} - A_{700})_{pH4.5}] \quad (7)$$

$$\text{Anthocyanin Pigment (mg/L)} = (A \times MW \times DF \times 1000) / (\epsilon \times 1) \quad (8)$$

Molar extinction coefficient (ϵ) of cyanidin-3-O-glucoside = 26900 L/mol cm, molar weight (MW)= 449 g/mol.

Ascorbic acid contain

Ascorbic acid content was detected by using 2,6-dichlorophenol indophenols titration method as described by AOAC (2000).

Sensory analysis

The effect of coating with essential oil and nano-emulsion of *S. chorassanica* on sensory characteristics of strawberry fruit, including flavor, color, aroma, texture, general acceptance was investigated. The sensory parameters were evaluated by 10 trained panelists. The 5-point hedonic method was used to analyze the results.

Statistical analysis

In this study, each experiment was repeated three times and the results were reported as means±SD. Differences among the average of treatments were analyzed by Duncan's test using SPSS (Version 23, SPSS Inc., Chicago, IL) software. Significant differences between treatments were concluded when p was <0.05.

Results and discussion

Extraction and identification of the *S. chorassanica* essential oil constituents

Extraction of *S. chorassanica* essential oil after the flowering stage was performed with hydrodistillation Clevenger type of apparatus. The yield of essential oil was 0.35 % (w/w) by using 200 g of dried leaves of *S. chorassanica*. The chemical compositions of the *S. chorassanica* essential oil and the retention time are depicted in Table 1. The GC–MS analysis of *S. chorassanica* essential oil indicated the presence of twenty compounds representing 97.56% of the total oil. The main compounds of *S. chorassanica* essential oil were such as Caryophyllene (37.6 %), Germacrene D (15.63 %), Bicyclogermacrene (11.81 %), Caryophyllene oxide (7.88 %), Spathulenol (7.57 %) and Terpinolene (3.49 %). Similarly, the major composition contents of salvia xanthocheila essential oil which was collected from Damavand region of Iran were reported by Salehi et al., (2005) included Germacrene D (44 %) and α-copaene (11.9 %) and ß-caryophyllene (6.7 %). In contrast, the main compounds of other species of salvia that were reported in some researches were different from the current study (Delamare et al., 2007; Yousefzadi et al., 2007; Khedher et al., 2017). Generally, the chemical compositions of the plant's essential oils depend on geographical and climate conditions, the method of essential oil extraction and also the plant growth phase (Shahbazi et al., 2016).

TABLE 1: *The chemical composition of S. chorassanica essential oil identified by GC-MS.*

No.	Compound	RT*	Concentration (%)
1	Terpinolene	7.29	3.49
2	Linalool	7.62	1.05
3	p-Cymen-8-ol	9.88	0.48
4	β-Cyclocitral	11.22	0.38
5	Elemene isomer	13.68	1.31
6	Copaene	14.81	1.05
7	(-)-β-Bourbonene	15.02	0.95
8	Isogermacrene D	15.15	1.56
9	Caryophyllene	15.93	37.6
10	Humulene	16.80	1.42
11	Germacrene D	17.43	15.63
12	Bicyclogermacrene	17.79	11.81
13	β -Cadinene	18.32	0.98
14	Dihydroagarofuran	18.44	0.71
15	Spathulenol	19.70	7.57
16	Caryophyllene oxide	19.82	7.88
17	Epiglobulol	20.12	0.66
18	(-)-Spathulenol	20.98	0.9
19	β-Eudesmol	21.49	2.01
20	Isoaromadendrene epoxide	21.83	0.52
	Total		97.56

Characterization of the nano-emulsion

Droplet size analysis

The small particle size of the nano-emulsion is an important factor in the stability of its colloidal system and improving its efficiency by increasing the area to volume ratio. The final particle size of the nano-emulsion is affected by droplet cleavage and recoalescence when the system is exposed to high shear during ultrasonication. The emulsifier also stabilizes the nano-emulsion while preventing the breakup of droplets (Oh et al., 2011). The droplet size and distribution of the nano-emulsions were determined during the storage using a DLS particle size analyzer (Table 2). The mean particle size of the nano-emulsion produced in this study on the first day was 91.83 ± 8.31 nm. The results showed that the storage time was effective in the stability of the nano-emulsion. The particle size of nano-emulsion of *S. chorassanica* essential oil increased with increasing storage time in the refrigerator. The average particle size of nano-emulsion increased to 191.61 ± 6.51 nm after two weeks. Similarly, increasing the mean of droplet size was observed in previous studies during the storage time (Sundararajan et al., 2018; Gahruie et al., 2017).

Moreover, the homogeneity of tested nano-emulsion was evaluated by the distribution width (span) index which is the major parameter for the determination of nano-emulsion stability. The composition of the oil phase or type of essential oil effect on nano-emulsion droplet size and size distribution (Surh et al., 2017). A higher span describes a non-uniformity and a wide range of droplet size dispersion (Tang et al., 2012). The distribution width (span) index of *S. chorassanica* essential oil nano-emulsion varied between

TABLE 2: *Droplet size and distribution width (Span) of S. chorassanica essential oil nano-emulsions.*

	Days					
	0	3	6	9	12	15
Mean diameter (nm)	91.83 ± 8.81	108.39 ± 8.06	131.66 ± 9.47	143.26 ± 8.8	174.19 ± 9.3	193.61 ± 9.75
span	1.08 ± 0.43	1.23 ± 0.36	1.58 ± 0.32	2.11 ± 0.3	2.21 ± 0.21	2.28 ± 0.36

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TABLE 3: *The minimum inhibition concentration (MIC) and minimum fungicidal concentration (MFC) of S. chorassanica emulsion and nano-emulsion essential oil and thiabendazole fungicide against strawberry spoilage molds.*

Mold	Essential oil emulsion (µl/ml)		Essential oil nano-emulsion (µl/ml)		Thiabendazole fungicide (µl/ml)	
	MIC	MFC	MIC	MFC	MIC	MFC
A. niger	12.5	25	6.25	25	12.5	25
R. stolonifer	12.5	25	6.25	12.5	12.5	25
B.cinere	12.5	25	6.25	12.5	12.5	25

1.08 ± 0.43 and 2.28 ± 0.36 during storage. Accordingly, the lower homogeneity of droplet size was observed on the 15th day of storage.

In vitro antifungal activity of *S. chorassanica* essential oil emulsion and nano-emulsion

The results of the antifungal activity of emulsion and nano-emulsion of *S. chorassanica* essential oil were shown in Table 3. According to the microdilution test, antifungal properties against the three spoilage molds of strawberry increased by reducing the particle size of *S. chorassanica* essential oil in nano-emulsion. The minimum inhibition concentration (MIC) and minimum fungicidal concentration (MFC) of essential oil nano-emulsion were reduced by half compared to the essential oil emulsion. On the other hand, the MIC of thiabendazole fungicide was higher than *S. chorassanica* essential oil nano-emulsion. The MIC of emulsion and nano-emulsion of *S. chorassanica* essential oil were achieved 12.5 and 6.25 µl/ml, respectively. There was no difference between the sensitivity of three molds to essential oils. The highest MFC of nano-emulsion was observed for *A. niger*.

As far as we know, no information has been published about the antifungal activity of *S. chorassanica* essential oil. The different antimicrobial activity of some Salvia species essential oils was exhibited in Yousefzadi., et al (2007) research, due to various components of them. It has been accepted that phenolic components in essential oils are responsible for their antifungal and antibacterial activities (Burt, 2004; Farzaneh et al., 2015). The hydrophobic properties of essential oils have an important role in the penetration into fungus cell membranes, disruption of cellular structure and increasing cellular permeability. These events cause leakage of cell contents, resulting in the fungal cell death (dos Santos et al., 2012). Moreover, disturbance in ATP synthesis in the mitochondria and the accumulation of ROS in the presence of essential oils are other inhibiting mechanisms against fungi strains (Kubo et al., 2003).

TABLE 4: *The fungal growth (log CFU/g) in coated and uncoated strawberries during cold storage.*

Mold	day	Essential oil emulsion (µl/ml)			Essential oil nano-emulsion (µl/ml)			Thiabendazole fungicide (µl/ml)			control
		6.25	12.5	25	6.25	12.5	25	6.25	12.5	25	
A. niger	3rd	2.24 (0.07)	1.81 (0.05)	0	1.91 (0.07)	0	0	2.45 (0.1)	1.81 (0.05)	0	4.24 (0.3)
	6th	2.65 (0.03)	2.15 (0.21)	0	2.35 (0.07)	0	0	2.65 (0.1)	2.15 (0.35)	0	5.8 (0.2)
	9th	2.95 (0.21)	2.45 (0.21)	0	2.58 (0.15)	2.15 (0.21)	0	2.97 (0.2)	2.46 (0.15)	0	7.38 (0.15)
	12th	2.99 (0.07)	2.61 (0.03)	1.01 (0.1)	2.65 (0.07)	2.31 (0.07)	0	3.77 (0.2)	2.81 (0.12)	0	7.87 (0.2)
R. stolonifer	3rd	2.5 (0.07)	1.15 (0.21)	0	1.72 (0.05)	0	0	2.48 (0.2)	1.15 (0.35)	0	4.65 (0.3)
	6th	2.38 (0.12)	1.76 (0.1)	0	2.15 (0.21)	0	0	2.5 (0.3)	1.76 (0.12)	0	5.15 (0.1)
	9th	2.58 (0.17)	1.97 (0.03)	0	2.15 (0.21)	0	0	2.65 (0.2)	1.97 (0.26)	0	6.24 (0.2)
	12th	2.95 (0.04)	2.8 (0.21)	0	2.38 (0.12)	0.85 (0.21)	0	2.98 (0.1)	2.48 (0.15)	0	7.48 (0.2)
B.cinere	3rd	2.15 (0.21)	1.64 (0.06)	0	1.8 (0.05)	0	0	2.15 (0.1)	1.64 (0.21)	0	4.89 (0.1)
	6th	2.45 (0.21)	1.97 (0.03)	0	1.95 (0.07)	0	0	2.45 (0.2)	1.97 (0.2)	0	5.62 (0.2)
	9th	2.75 (0.05)	2.23 (0.12)	0	2.38 (0.12)	0	0	2.74 (0.2)	2.24 (0.2)	0	6.48 (0.15)
	12th	2.95 (0.03)	2.38 (0.03)	0.95 (0.2)	2.52 (0.09)	1.15 (0.05)	0	2.92 (0.1)	2.38 (0.15)	0	7.65 (0.3)

Data are presented as mean (SD) of three repetitions.

Mold decay of strawberry

Strawberry is a perishable fruit due to high moisture content and nutrients richness that provide an excellent growth medium for fungal development. Essential oils are suitable food preservatives with antimicrobial properties that are known as GRAS (Generally Regarded As Safe) by FDA (Food and Drug Administration) and can consume in relatively high concentrations without side effects (Burt, 2004). The in-vivo evaluation of the antifungal activity of emulsion and nano-emulsion of *S. chorassanica* essential oil was illustrated in Table 4. Generally, an increase in the concentration of the emulsion and nano-emulsion of *S. chorassanica* essential oil led to increased inhibition of spore germination. The highest antifungal activity was achieved at the concentration of 25 µl/ml for nano-emulsion. Nano-emulsion was more effective in preventing mold decay of strawberry during storage than emulsion essential oil and the thiabendazole fungicide. The *A. niger* showed the least sensitivity to nano-emulsion and the first signs of its growth on the strawberry surface appeared on day 9. The use of *S. chorassanica* essential oil nano-emulsion and thiabendazole fungicide coating at the concentration of 25 µl/ml on the strawberry surface was able to delay the growth of all molds for more than 12 days. These results were agreement with another study that has reported greater antifungal activity of nano-emulsion in comparison to emulsion essential oil and thiabendazole fungicide in controlling mold decay of strawberry (Naserzadeh et al., 2019). The application of different coating containing emulsion and nano-emulsion essential oil on strawberry surface significantly decreased the fungal infection compared to the control sample (Asghari et al., 2009; Nabigol et al., 2011; Oliveira et al., 2019; Chu et al., 2020).

Weight loss

The weight loss of strawberry fruit during storage is increased due to the evaporation of cell moisture due to respiration, which depends on the temperature and humidity of the storage place. The weight loss changes of three different groups of strawberry treatments were depicted in Figure 1. Accordingly, all strawberries indicated weight loss as storage time increased. The highest weight loss of the strawberry samples included control (10.39%), emulsion (5.36%) and nano-emulsion coating (4.31%) were observed at the end of the 12th day. Generally, the application of the coating on the fruit surface could decrease the weight loss by reduction of fruit respiration and water loss. The lowest weight loss was obtained for the strawberry sample coated with *S. chorassanica* essential oil nano-emulsion. This can be due to the hydrophobic nature of essential oil and reduction of the diameter of pores of the fruit surface after the use of essential oils nano-emulsion coating.

In similar, the effect of using nano chitosan coating on the strawberry surface by delaying the weight loss was reported by Eshghi et al. (2013). The weight loss of coated strawberries depended on essential oil type and their concentration during cold storage. The rate of weight loss of strawberries decreased with increasing the concentration of essential oil coating (Mohammadi, 2014).

The results of various studies revealed that the use of hydrophilic polysaccharide coatings such as pure chitosan and alginate could not be effective in preventing the surface evaporation of fruit moisture and the addition of emulsion or nano-emulsions of essential oils could improve their efficiency to decrease the mass loss of fruit (Predons et al., 2012; Guerrero et al., 2015). The study of Chu et al. (2020) showed that the addition of cinnamon essential oil nano-emulsion to pullulan coating reduced the weight loss of coated strawberries (25%) compared to control (38%). The lowest rate of weight loss observed in the present study in comparison with other researches indicated the better performance of *S. chorasanica* nano-emulsion.

pH and Titratable Acidity

The titratable acidity in strawberry fruit decreases naturally during storage at low temperatures due to increased cell metabolism and the use of organic acids in the respiratory process (Gol et al., 2013). As shown in Fig 2, the lowest titratable acidity was finding in uncoated strawberries at the end of storage time. The nano-emulsion and emulsion of essential oil coatings probably reduced the rate of respiration and kept the titratable acidity constant. Also, a slight increase in titratable acidity was observed in the 12th days, which may be related to the fruit ripening process. This results was accordance with other previous studies included Dong and wang (2017) and Chu et al., (2020) that considered that adding essential oil or nano-emulsion in poly-

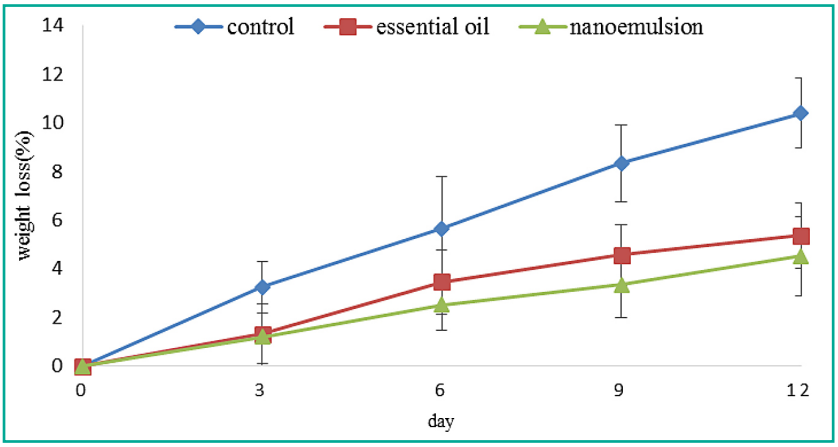

FIGURE 1: *Weight loss of coated and uncoated (control) strawberries during cold storage.*

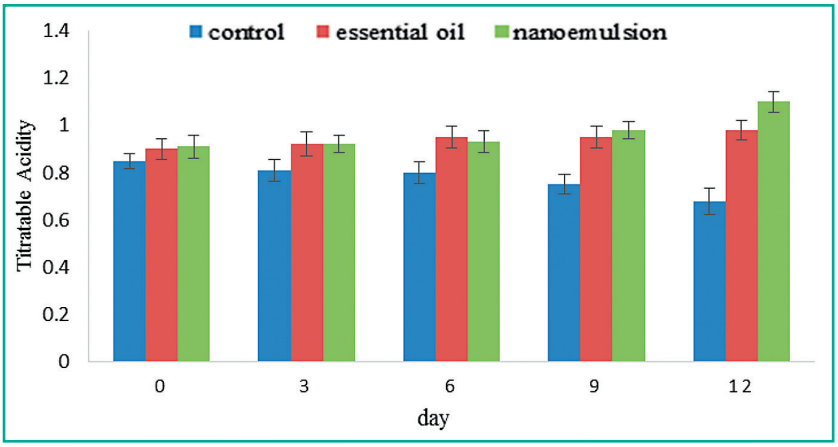

FIGURE 2: *Titratable acidity of coated and uncoated (control) strawberries during cold storage.*

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saccharides coatings delayed the titratable acidity drop in fruit during the cold storage. In addition, the pH of all treatments slightly decreased at the end of 12 days (Fig 3) consistent with Asghari et al. (2009) study. In this study, uncoated strawberry samples exhibited the lowest acidity and the highest pH during storage time. It should be noted that during the fruit ripening process, organic acids increase to a constant range and then decrease due to the use of organic acids in the respiration process (Chu et al., 2020).

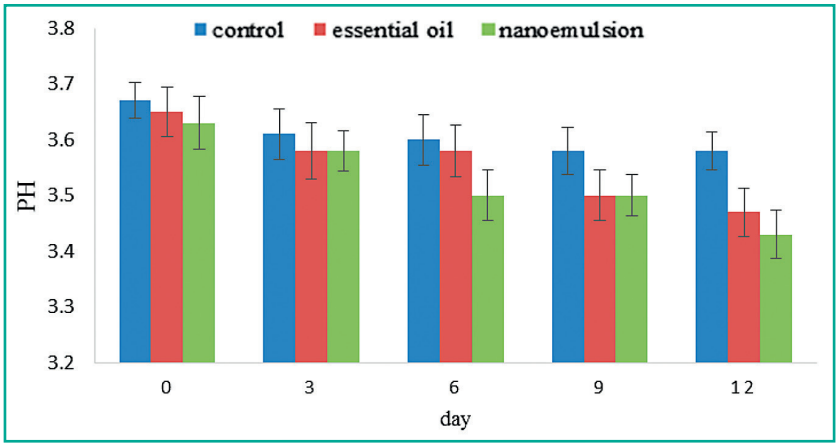

FIGURE 3: *pH of coated and uncoated (control) strawberries during cold storage.*

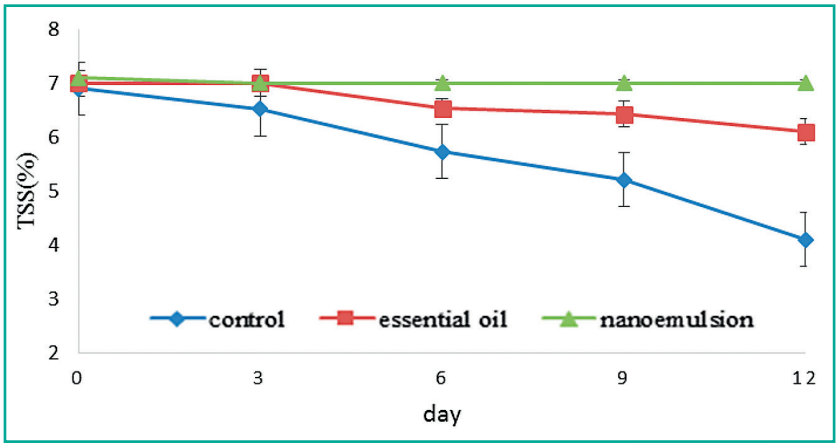

FIGURE 4: *Total soluble solids (TSS) of coated and uncoated (control) strawberries during cold storage.*

Total soluble solids (TSS)

The TSS of coated and uncoated strawberries was shown in Fig 4. The changes of TSS in the nano-emulsion coating was almost constant from the initial to the end of cold storage. On the other hand, the highest drop in TSS was exhibited in the uncoated strawberry. The reason for TSS decreasing significantly was more respiration and sugars break up in control samples compared to other treatments. The TSS contain of fruit depend on several factors such as fruit sugar, acidity and soluble pectin in the fruit (Asghari et al., 2008). The results were agreement with previous studies. Chu et al. (2020) reported that pullulan-cinnamon nano-emulsion coating was more effective in preventing the loss of strawberry TSS during 6 days compared to pullulan coating and control samples. There were no difference among TSS of strawberries coated with seven types of essential oils in research of Mohammadi (2014). Moreover, Asghari et al. (2008) observed an increase in TSS of coated strawberry samples after increasing the concentration of basil essential oil from 60 to 1000 µl /L.

Color surface

The surface color of strawberry fruit is an important factor in the evaluation of quality and marketability. Figure 5 shows luminosity (L*), hue and chroma changes of different strawberry treatments during 12 days of storage at refrigerator temperature. The luminosity (L*) of strawberry surface decreased in all coated and control samples, which results in the loss of fruit moisture. Moreover, the control sample had a lower luminosity than coated samples at the end of the 12th day. In this regard, Perdones et al., (2012) stated that the addition of lemon essential oil to the chitosan coating increased the L* value of strawberry samples compared to control and chitosan-coated samples. The hue value of samples remained approximately constant throughout the storage time and slightly decreased on the 12th day. The highest hue was observed for the control sample at the end of storage. As shown in Fig 5, the chroma value first increased and then decreased. Also, the coated and uncoated samples showed no significance in chroma value. Briefly, no adverse effect was observed in the color of strawberries after coating with emulsion, and nano-emulsion of essential oil and coated samples looked redder and brighter. Generally, the color changes of strawberry samples in storage time probably related surface moisture evaporation, chemical and enzymatic reactions and changes in the anthocyanins contents that depending on storage conditions (Koh and Melton, 2002).

Firmness

The fruit firmness is an appropriate parameter for the determination of its quality and shelf life. The firmness of different treatments of strawberry fruit during cold storage was illustrated in Fig 6. The results showed that the firmness of all treatment was reduced during 12 days of storage in the refrigerator. The lowest and highest rate of softening at the end of storage occurred in nano-emulsion coated (16 %) and uncoated (control)(60 %) strawberries, respectively. However, there was no significant difference between the coated samples ($p > 0.05$). The major reason for firmness loss of fruit during the storage is related to respiration, degradation of fruit cell wall and damage of tissue structure due to mold decay (Fan et al., 2009). The emulsion and nano-emulsion of *S. chorassanica* essential oil significantly prevented fruit softening by reduction of respiration and fungi infection in comparison with the control sample. Chu et al., (2019) reported that adding cinnamon nano-emulsion in pullulan coating was able to improve the maintenance of strawberries tissue firmness. The investigation of various concentrations of basil essential oil on quality characteristics of strawberry during cold storage was demonstrated that increasing essential oil concentration to 500 µl/ ml could reduce firmness loss in fruit. However, a high concentration of essential oil (1000 µl /L) due to increased chemical interactions and damage to fruit surface cells led to a decrease in fruit firmness (Asghari et al., 2008).

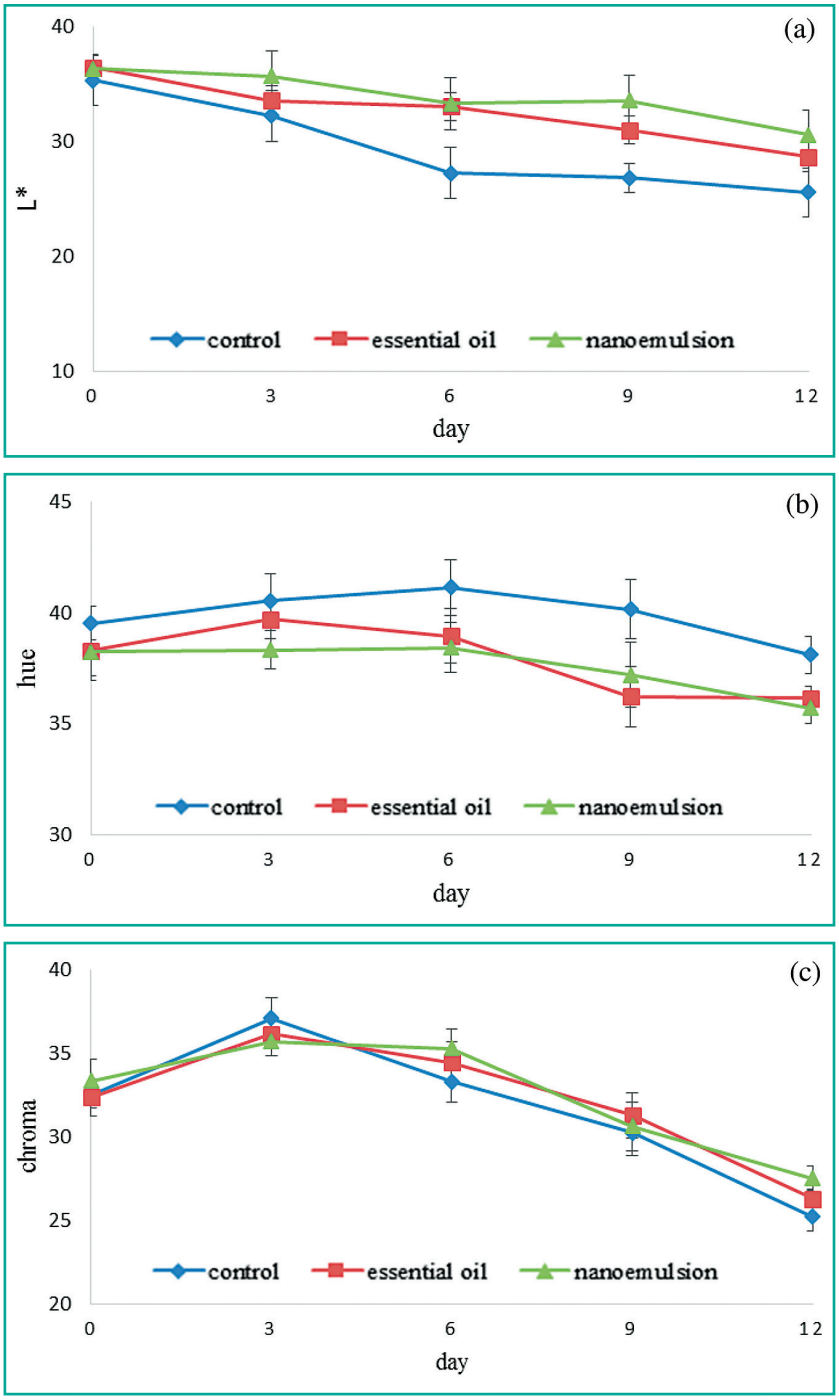

FIGURE 5: *Luminosity (a), hue (b) and chroma (c) of coated and uncoated strawberries during cold storage.*

Anthocyanin contents

Anthocyanins are water-soluble pigments that are involved in the red or blue color of fruits and vegetables. As the fruits ripen, the amount of anthocyanins and the intensity of the color increase. The amount of anthocyanin content varies in different types of strawberries (Zhang et al., 2017). The acidity increases during fruit ripening, which leads to enhance polyphenol oxidase activity and the destruction of anthocyanin pigments. The changes in anthocyanin contents are often considered a measure of fruit aging (Karaaslan and Yaman., 2017).

The changes in anthocyanin contents in strawberry samples were shown in Figure 7. Accordingly, the anthocyanin contents in all strawberry treatments significantly decreased throughout 12 days ($p < 0.05$). The application of coatings reduced the anthocyanin loss in strawberry samples. The highest amount of anthocyanin at the end of storage time respectively belonged to the treatment of nano-emulsion (32.29 mg / 100g), essential oil (18.32 mg /100g), and uncoated control sample (10.35 mg / 100g). In the same way, Vargas et al., (2006) observed a reduction of anthocyanin contents in chitosan-coated and uncoated strawberry samples. In contrast, the addition of thymol to gluten and soy protein coatings could increase anthocyanin contents in strawberry samples at the end of two weeks of storage at 4 °C (Amal et al., 2010).

Ascorbic acid contents

The ascorbic acid contents of coated and uncoated strawberry samples during cold storage were exhibited in Figure 8. A significant difference was observed among ascorbic acid contents of nano-emulsion coated strawberry and other samples at the end of storage time ($p < 0.05$). Similarly, some studies have indicated a decrease of ascorbic acid contents in strawberry fruit during storage. In addition, the use of various coatings on the surface of this fruit was considered effective in preventing the loss of vitamin C (Asghari et al., 2009; Amal et al., 2010; Mohammadi et al., 2014). The reduction of fruit vitamin C is occurred during storage time because of its oxidation by the enzyme ascorbate oxidase and reaction with air oxygen. The use of essential oils as fruit coatings reduces the activity of this enzyme and maintains the ascorbic acid contents (Amal et al., 2010).

Sensory analysis

The results of the sensory analysis of coated and uncoated strawberries were plotted in Fig 9 (a and b). The coated samples with emulsion and nano-emulsion of *S. choras-*

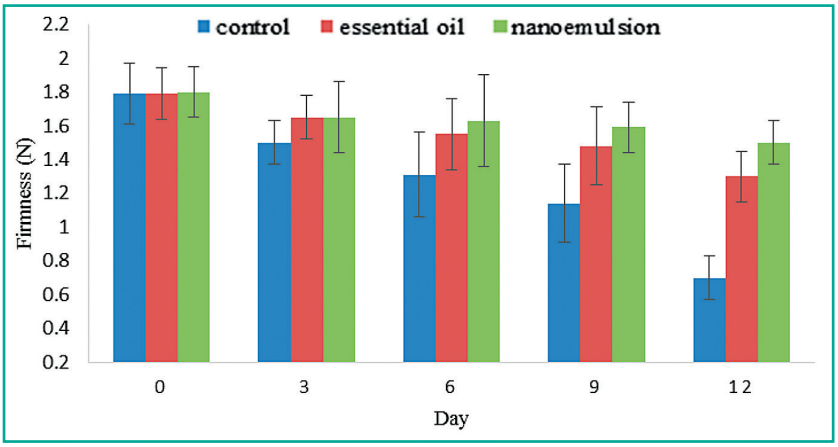

FIGURE 6: *Firmness of coated and uncoated (control) strawberries during cold storage.*

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sanica essential oil earned lower aroma and flavor scores in the initial day probably due to the intensity effect of essential oil aroma compounds, while no difference observed among color and texture of samples. In the study of Perdones et al. (2012), chitosan- lemon essential oil coated strawberries were indicated lower preference than chitosan-coated and control samples. Due to the appearance of signs of mold growth on the surface of control strawberries, the sensory evaluation test was performed until day 6. The changes in aroma and flavor of coated samples after one week altered the preference of panelists. The coated strawberry treatments were achieved the highest score for all their sensory properties (flavor, color, aroma, texture and general acceptance) in comparison with the control sample after 6 days. The greatest effect of coating was associated with preserving the color and texture of the strawberry fruit during cold storage.

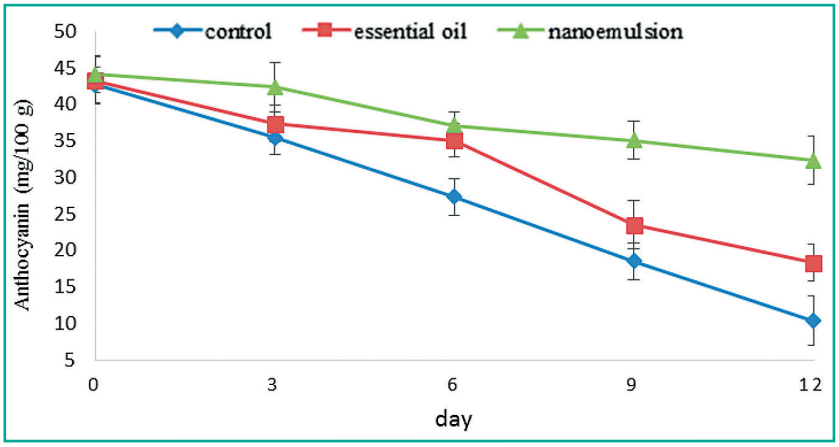

FIGURE 7: *Anthocyanin contents of coated and uncoated (control) strawberries during cold storage.*

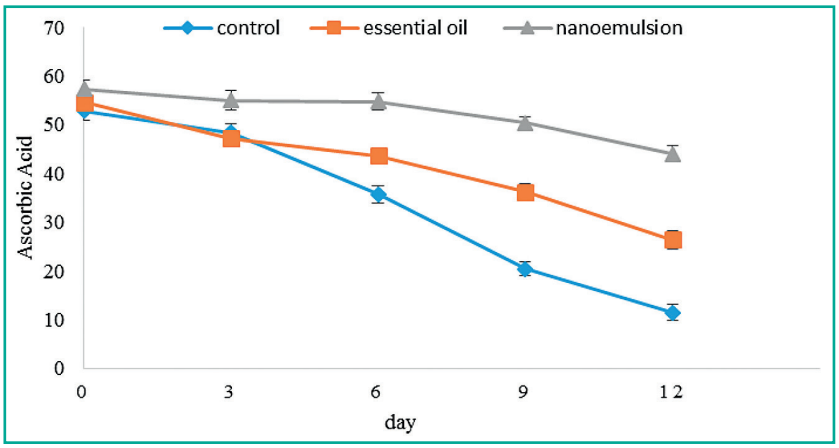

FIGURE 8: *Ascorbic acid contents of coated and uncoated (control) strawberries during cold storage.*

Conclusion

The coating of strawberries with emulsion and nano-emulsion of *S. chorassanica* essential oil promoted their shelf-life throughout cold storage. It seemed that coatings with decreasing the metabolic activities such as respiration could prevent loss of fruit quality characteristics. The coated strawberry samples showed better color, texture and protection against fungal decay at the end of storage time. On the other hand, nano-emulsion coating with smaller particle sizes was more effective than emulsion essential oil on the delaying of mass loss, reduction of ascorbic acid and anthocyanin contents and fungal growth. According to these results, essential oil and can be a suitable alternative to chemical toxins to extend strawberry shelf-life.

Conflict of interest

The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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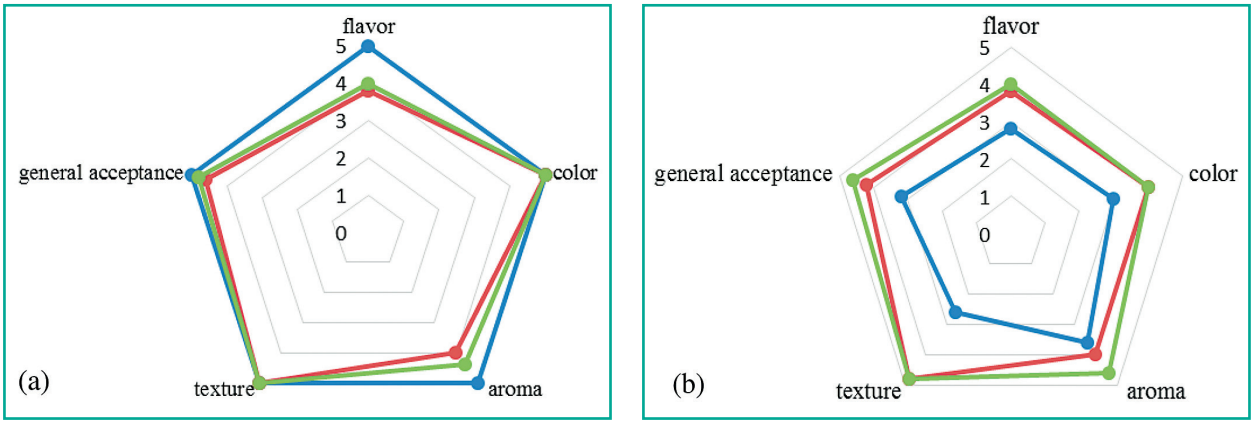

FIGURE 9: *Sensory profile of coated and uncoated strawberry at 0 (a) and 6th (b) day of cold storage.*

References

Abdou ES, Galhoum GF, Mohamed EN (2018): Curcumin loaded nano-emulsions/pectin coatings for refrigerated chicken fillets. Food Hydrocolloids: 83, 445–453.

Amal SHA, El-Mogy MM, Aboul-Anean HE, Alsanius BW (2010): Improving strawberry fruit storability by edible coating as a carrier of thymol or calcium chloride. J of Horticultural Sci & Ornamental Plants: 2(3), 88–97.

AOAC (2000): Official methods of analysis (17th ed.). Washington DC, USA: Association of Official Analytical Chemists.

Asghari MA, Mostoufi Y, Shoeybi S, Fatahi M (2009): Effect of cumin essential oil on postharvest decay and some quality factors of strawberry. J of Medicinal Plants: 31(8), 25–43.

Burt S (2004): Essential oils: their antibacterial properties and potential applications in foods a review. Int J of food microbiol: 94(3), 223–253.

Chu Y, Gao C, Liu X, Zhang N, Xu T, Feng X, Tang X (2020): Improvement of storage quality of strawberries by pullulan coatings incorporated with cinnamon essential oil nano-emulsion. LWT: 122, 109054.

Delamare APL, Moschen-Pistorello IT, Artico L, Atti-Serafini L, Echeverrigaray S (2007): Antibacterial activity of the essential oils of *Salvia officinalis* L. and *Salvia triloba* L. cultivated in South Brazil. Food chem: 100(2), 603–608.

Dong F, Wang X (2017): Effects of carboxymethyl cellulose incorporated with garlic essential oil composite coatings for improving quality of strawberries. Int J of Biological Macromolecules: 104, 821–826.

Donsì F, Annunziata M, Sessa M, Ferrari G (2011): Nanoencapsulation of essential oils to enhance their antimicrobial activity in foods. LWT-Food Sci and Technol: 44(9), 1908–1914.

dos Santos NST, Aguiar AJAA, de Oliveira CEV, de Sales CV, e Silva SDM, da Silva RS, de Souza EL (2012): Efficacy of the application of a coating composed of chitosan and *Origanum vulgare* L. essential oil to control *Rhizopus stolonifer* and *Aspergillus niger* in grapes (*Vitis labrusca* L.). Food Microbiol: 32(2), 345–353.

Eshghi S, Hashemi M, Mohammadi A, Badie F, Hosseini ZM, Ahmadi K, Ghanati K (2013): Effect of nano-emulsion coating containing chitosan on storability and qualitative characteristics of strawberries after picking. Iranian J of Nutrition Sci & Food Technol: 8(2), 9–19.

Fan Y, Xu Y, Wang D, Zhang L, Sun J, Sun L, Zhang B (2009): Effect of alginate coating combined with yeast antagonist on strawberry (Fragaria × ananassa) preservation quality. Postharvest Biol and Technol: 53(1–2), 84–90.

Farzaneh M, Kiani H, Sharifi R, Reisi M, Hadian J (2015): Chemical composition and antifungal effects of three species of Satureja (*S. hortensis, S. spicigera* and *S. khuzistanica*) essential oils on the main pathogens of strawberry fruit. Postharvest Biol and Technol: 109, 145–151.

Firuzi O, Miri R, Asadollahi M, Eslami S, Jassbi AR (2013): Cytotoxic, antioxidant and antimicrobial activities and phenolic contents of eleven Salvia species from Iran. Iranian J of pharmaceutical research: IJPR, 12(4), 801.

Gahruie HH, Ziaee E, Eskandari MH, Hosseini SMH (2017): Characterization of basil seed gum-based edible films incorporated with *Zataria multiflora* essential oil nano-emulsion. Carbohydrate polymers: 166, 93–103.

Gol NB, Patel PR, Rao, TR (2013): Improvement of quality and shelf-life of strawberries with edible coatings enriched with chitosan. Postharvest Biol and Technol: 85, 185–195.

Guerreiro AC, Gago CM, Faleiro ML, Miguel MG, Antunes MD (2015): The effect of alginate-based edible coatings enriched with essential oils constituents on *Arbutus unedo* L. fresh fruit storage. Postharvest Biol and Technol: 100, 226–233.

Hassan KA, Mujtaba MA (2019): Antibacterial efficacy of garlic oil nano-emulsion. AIMS Agriculture and Food: 4(1), 194-205.

Jakobek L, Šeruga M, Medvidović-Kosanović M, Novak I (2007): Anthocyanin content and antioxidant activity of various red fruit juices. Deutsche Lebensmittel-Rundshau: 103(2), 58–64.

Karaaslan NM, Yaman M (2017): Anthocyanin profile of strawberry fruit as affected by extraction conditions. Int J of food properties: 20(sup3), S2313–S2322.

Khedher MRB, Khedher SB, Chaieb I, Tounsi S, Hammami M (2017): Chemical composition and biological activities of *Salvia officinalis* essential oil from Tunisia. EXCLI journal: 16, 160.

Koh TH, Melton LD (2002): Ripening-related changes in cell wall polysaccharides of strawberry cortical and pith tissues. Postharvest Biol and Technol: 26: 23–33.

Kubo I, Fujita KI, Kubo A, Nihei KI, Lunde CS (2003): Modes of antifungal action of (2 E)-alkenals against *Saccharomyces cerevisiae.* J of agri and food chem: 51(14), 3951–3957.

Lee JY, Garcia CV, Shin GH, Kim JT (2019): Antibacterial and antioxidant properties of hydroxypropyl methylcellulose-based active composite films incorporating oregano essential oil nanoemulsions. Lwt: 106, 164–171.

Li ZH, Cai M, Liu YS, Sun PL (2018): Development of finger citron (*Citrus medica* L. var. *sarcodactylis*) essential oil loaded nano-emulsion and its antimicrobial activity. Food Control: 94, 317–323.

Mehraban A, Haddad Khodaparast MH, Mehraban Sang Atash M (2016): Evaluation of Inhibitory and Lethal effects of aqueous, ethanolic and hydroalcoholic extracts of aerial parts of *salvia chorassanica* against some gram-negative and gram-positive bacteria in vitro. Qom University of Medical Sci J:10(2), 2–11.

Mohammadi S, Aroiee H, Aminifard MH, Tehranifar A, Jahanbakhsh V (2014): Effects of Essential Oils to Control *Rhizopus stolonifer* in vitro and in vivo on Strawberry. J Biol Environ Sci: 8, 1603–1610.

Nabigol A, Morshedi H (2011): Evaluation of the antifungal activity of the Iranian thyme essential oils on the postharvest pathogens of strawberry fruits. African J of Biotech: 10(48), 9864–9869.

Naserzadeh Y, Mahmoudi N, Pakina E (2019): Antipathogenic effects of emulsion and nano-emulsion of cinnamon essential oil against Rhizopus rot and grey mold on strawberry fruits. Foods and Raw materials: 7(1).

Oh DH, Balakrishnan P, Oh YK, Kim DD, Yong CS, Choi HG (2011): Effect of process parameters on nano-emulsion droplet size and distribution in SPG membrane emulsification. Int J of pharmaceutics: 404(1–2), 191–197.

Oliveira J, Parisi MCM, Baggio JS, Silva PPM, Paviani B, Spoto MHF, Gloria EM (2019): Control of Rhizopus stolonifer in strawberries by the combination of essential oil with carboxymethylcellulose. Int J of food microbial: 292, 150–158.

Perdones A, Sánchez-González L, Chiralt A, Vargas M (2012): Effect of chitosan-lemon essential oil coatings on storage-keeping quality of strawberry. Postharvest biol and technol: 70, 32–41.

Robledo N, Vera P, López L, Yazdani-Pedram M, Tapia C, Abugoch L (2018): Thymol nano-emulsions incorporated in quinoa protein/chitosan edible films; antifungal effect in cherry tomatoes. Food Chem: 246, 211–219.

Ryu V, McClements DJ, Corradini MG, McLandsborough L (2018): Effect of ripening inhibitor type on formation, stability, and antimicrobial activity of thyme oil nano-emulsion. Food chem: 245, 104–111.

Salehi P, Tolami LB, Sefidkon F (2005): Essential oil composition of *Salvia xanthocheila* Boiss. ex Benth. from Iran. J of Essential Oil Research: 17(4), 442–443.

Shahbazi Y, Shavisi N, Mohebi E (2016): Potential application of *Ziziphora clinopodioides* essential oil and nisin as natural preservatives against *Bacillus cereus* and *Escherichia coli* O157: H7 in commercial barley soup. J of Food Safety: 36(4), 435–441.

Sundararajan B, Moola AK, Vivek K, Kumari BR (2018): Formulation of nano-emulsion from leaves essential oil of *Ocimum basilicum* L. and its antibacterial, antioxidant and larvicidal activities *(Culex quinquefasciatus).* Microbial pathogenesis: 125, 475–485.

Surh J, Decker EA, McClements DJ (2017): Utilisation of spontaneous emulsification to fabricate lutein-loaded nano-emulsion-based delivery systems: factors influencing particle size and colour. Int J of Food Sci & Technol: 52(6), 1408–1416.

Tahmasebi M, Golmohammadi A, Nematollahzadeh A, Davari M, Chamani E (2019): Control of nectarine fruits postharvest fungal rots caused by *Botrytis Cinerea* and *Rhizopus Stolonifer* via some essential oils. J of Food Sci and Technol: 1–9.

Tang SY, Manickam S, Wei TK, Nashiru B (2012): Formulation development and optimization of a novel Cremophore EL-based nano-emulsion using ultrasound cavitation. Ultrasonics sonochemistry: 19(2), 330-345.

Treviño-Garza MZ, García S, del Socorro Flores-González M, Arévalo-Niño K (2015): Edible active coatings based on pectin, pullulan, and chitosan increase quality and shelf life of strawberries *(Fragaria ananassa).* J of food sci: 80(8), 1823–1830.

Vargas M, Albors A, Chiralt A, González-Martínez C (2006): Quality of cold-stored strawberries as affected by chitosan-oleic acid edible coatings. Postharvest biol and technol: 41(2), 164–171.

Velde FV, Tarola AM, Güemes D, Pirovani ME (2013): Bioactive compounds and antioxidant capacity of *Camarosa* and *Selva* strawberries (*Fragaria x ananassa* Duch.). Foods: 2(2), 120.

Velickova E, Winkelhausen E, Kuzmanova S, Alves VD, Moldão-Martins M (2013): Impact of chitosan-beeswax edible coatings on the quality of fresh strawberries (*Fragaria* ananassa cv *Camarosa*) under commercial storage conditions. LWT-Food Sci and Technol: 52(2), 80–92.

Wan J, Zhong S, Schwarz P, Chen B, Rao J (2018): Influence of oil phase composition on the antifungal and mycotoxin inhibitory activity of clove oil nano-emulsions. Food & function: 9(5), 2872–2882.

Wan J, Zhong S, Schwarz P, Chen B, Rao J (2019): Physical properties, antifungal and mycotoxin inhibitory activities of five essential oil nano-emulsions: impact of oil compositions and processing parameters. Food chem: 291, 199–206.

Weiss J, Gaysinsky S, Davidson M, McClements J (2009): Nanostructured encapsulation systems: food antimicrobials. In Global issues in food sci and technol. Academic Press, USA, 425–479.

Yilar M, Kadioglu I, Telci I (2018): Chemical composition and antifungal activity of *Salvia officinalis* (L.), *S. cryptantha* (Montbret et Aucher ex Benth.), *S. tomentosa* (Mill.) plant essential oils and extracts. Fresen. Environ. Bull: 27, 1695–1706.

Yousefzadi M, Sonboli A, Karimi F, Ebrahimi SN, Asghari B, Zeinali A (2007): Antimicrobial activity of some *Salvia* species essential oils from Iran. Zeitschrift für Naturforschung C: 62(7–8), 514–518.

Zhang S, Zhang M, Fang Z, Liu Y (2017): Preparation and characterization of blended cloves/cinnamon essential oil nano-emulsions. LWT-Food Sci and Technol: 75, 316–322.

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