Effects of chitosan coatings incorporating with free or nano-encapsulated Satureja plant essential oil on quality characteristics of lamb meat

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A B S T R A C T

There is a growing demand for bio-based and active packaging as one of the preferred emerging technologies to improve food quality and extend shelf-life. The objectives of this study were to formulate a novel biodegradable coating embedded with nano-encapsulated Satureja khuzestanica essential oils (SKEO) and to compare effect of chitosan coatings containing free or nano-encapsulated SKEO on chemical, microbial, and sensory properties of lamb meat samples over a 20 day period at 4°C. Nanoliposomes with the average sizes of 93–96 nm were prepared using different concentrations of soy-lecithin ratios (60:0, 50:10, 40:20, and 30:30) by thin-film hydration-sonication method. Size distribution and encapsulation efficiency (EE) of nanoliposomes were calculated 0.83–0.88 and 46–69%, respectively. Nanoliposomes with the lower droplet size and maximum EE were selected for coating of meat samples. The results indicated that the coating treatments could effectively retard microbial growth and chemical spoilage, which reflected itself in lower pH and 2-thiobarbituric acid values (P<0.05). Encapsulation decelerated the release of SKEO and led to a prolonged antimicrobial and antioxidant activity and also improved sensory attributes. The study suggests that chitosan coating containing encapsulated SKEO can be a promising candidate for extending the shelf-life of lamb meat.

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1. Introduction

The growing demand for natural antimicrobial and antioxidant compounds, along with the consumer awareness concerning the use of synthetic chemical preservatives have contributed to the rise of a new trend called “green consumerism” in food industry, which is the basis for the development of alternative approaches for food preservation (Imran et al., 2012). Among natural additives, plant extracts and essential oils (EOs) owing to their antioxidant and antimicrobial traits have shown a considerable potential in a range of applications in the realm of green consumerism (Mastromatteo, Mastromatteo, Conte, & Del Nobile, 2010; Ouattara et al., 2002).

Satureja khuzestanica Jamzad is an endemic plant belonging to the Lamiaceae family that are widely distributed in South-west Iran and known for its medical uses as analgesic and anti-septic (Amanlou, Fazeli, Arvin, Amin, & Farsam, 2004). S khuzestanica EOs have been reported to have biological properties, such as antibacterial, antifungal, antioxidant, and anti-inflammatory, that have been mostly attributed to their phenolic compounds content, especially carvacrol (Hadian, Hossein Mirjalili, Reza Kanani, Salehnia, & Ganjipoor, 2011). Accordingly, SKEO can be exploited as a potential new source of natural antioxidant and antibacterial agent to replace synthetic ones.

However, there are some challenges concerning the direct incorporation of essential oils containing chemically reactive compounds such as phenolic compounds into complex food systems such as negative effects on the physical stability or integrity of the food chemistry, degradation of the biological activity of bioactive compounds (Sawale, Patil, Hussain, Singh, & Singh, 2017). Encapsulation can provide a potential approach to overcome these challenges by improving the oxidative stability of ingredients, protecting bioactive compounds from unwanted reactions, limiting their possible interaction with food components, preserving their stability during food processing and storage, and providing controlled or targeted delivery (Mozafari et al., 2006). Nanoliposomes are phospholipid-based systems with high surface area, which are
amphiphilic molecules consist of a water-soluble hydrophilic head section and a lipid-soluble hydrophobic tail section (Mozafari et al., 2006). Nanoscale encapsulation in the structure of lipid vesicles present a higher resistance to destabilization, also can increase the effectiveness and stability of encapsulated material (Lu et al., 2014).

Notwithstanding the efficacy of nano-encapsulated bioactive compounds in preservation of food and beverage products, there is a limitation concerning their utilization in solid foods such as fruits and meat products where their direct incorporation is not feasible (Donsi, Annunziata, Sessa, & Ferrari, 2011). Embedment of nanoliposomes in edible coatings can offer a new approach to circumvent the referred limitation and to overcome the challenge of immobilization of nanoliposomes on foods surface (Salvia-Trujillo, Rojas-Grau, Soliva-Fortuny, & Martin-Bellos, 2015). This strategy is effective not only in placing bioactive agents on the surface of solid products, but also in modulating the release of active compounds, mitigating the sensory impact of essential oils, and decreasing their diffusion rate, that can eventually reduce the required concentration to achieve the desired effects, prolong the activity of active packaging, and enhance the functionality of edible coatings (Donsi, Annunziata, Vincensi, & Ferrari, 2012).

On the other hand, edible and biodegradable coatings can further retard food deterioration and spoilage by acting as selective barriers to gas, moisture, and solute migration during processing, handling, and storage (Catarino et al., 2017). Different studies have indicated promising results for incorporating antimicrobial nanoe-mulsions into bio-based edible coatings in prolonging the shelf-life of meat-based products (C. Wu et al., 2016).

Chitosan is a natural polysaccharide with a wide range of unique applications in food industry including biopolymeric packaging owing to its polyelectrolytic nature and distinguishing features (Andishmand, Tabibiazar, Mohammadifar, & Hamishehkar, 2017; Ghelejlu, Esmaiili, & Martin-Bellos, 2015). This strategy is effective not only in placing bioactive agents on the surface of solid products, but also in modulating the release of active compounds, mitigating the sensory impact of essential oils, and decreasing their diffusion rate, that can eventually reduce the required concentration to achieve the desired effects, prolong the activity of active packaging, and enhance the functionality of edible coatings (Donsi, Annunziata, Vincensi, & Ferrari, 2012).

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2. Material and methods

2.1. Materials

Chitosan powder of medium molecular weight (MW: 190–310 kDa) with a deacetylation degree of 75–85% and glycerol (>97% purity) were purchased from Merck (Darmstadt, Germany). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was provided from Sigma–Aldrich (Germany). Folin-Ciocalteu reagent, sodium carbonate anhydrous, and acetic acid were purchased from Merck (Darmstadt, Germany). Methanol, other reagents and solvents were of analytical grade or higher available purity.

For nanoliposomes preparation, phospholipid (L-a-lecithin granular, 99%) was obtained from Across Co. (USA). Cholesterol, 95%, dichloromethane, and methanol were provided by Merck (Darmstadt, Germany).

2.1.1. Plant material and extraction

The aerial parts of Satureja khuzestanica Jamzad, was purchased from east of Iran (Lorestan) and authenticated by a pharmacognosy specialist at Tehran University of Medical Sciences. Satureja leaves were washed and air-dried at ambient temperature in the shade and hydrodistilled for 3 h, using an all-glass Clevenger-type apparatus, according to the method recommended by the British Pharmacopoeia (1988). EO sample was dried over anhydrous sodium sulphate and stored in sealed vials at 4 °C until being used.

2.2. Preparation and characterization of nanoliposomes

Nanoliposomes were prepared by means of thin-film hydration and sonication method (Kirby & Gregoriadis, 1984). Four different lecithin: cholesterol ratios (60:0, 50:10, 40:20, and 30:30) were dissolved in dichloromethane/methanol (1:1) in a 100 ml round-bottom flask. 60 mg S. khuzestanica EO was dissolved in 10 ml methanol and then mixed with the mixture. Subsequently, the organic solvent was removed using rotary evaporator at 30 °C until a thin film was formed on the walls. The lipid film was hydrated with 15 ml distilled water and the mixture was homogenized at 20,000 rpm for 10 min by an Ultra-Turrax homogenizer (Heidolph, Germany) at a temperature above the gel—liquid transition temperature (Tc) of the amphiphiles (Haghju, Beigzadeh, Almasi, & Hamishehkar, 2016). For obtaining unilamellar nanovesicles, the liposomal suspensions were exposed to a probing sonication (Sonics & Materials vibracell, England) at 70% sonication strength in ice bath to prevent heating for 6 min (6 cycles of 1 min sonication and 1 min rest intermittently to allow cooling of the sample) (Haghju et al., 2016).

2.2.1. Nanoliposomes size measurement

The mean diameter and particle size distribution of liposomes were assessed using dynamic light scattering (DLS) technique employing a Shimadzu particle size analyzer (SALD 2101, Japan) according to the method of Haghju, Beigzadeh, Almasi and Hamishehkar (Haghju et al., 2016). Prior to size measurement, the samples were diluted (1:100) with distilled water to avoid multiple laser dispersion induced by accumulation of particles. All measurements were carried out at 25 °C and three replicates were considered for each formulation. Results are presented as an average diameter of the liposome suspension (z-average mean) with the polydispersity index (PDI) which evaluates the size distribution width. Following Volume mean diameter (VMD) or De Broukere mean (D 4, 3) equation was used for calculation of average particle size:

$$D = \sqrt[3]{\frac{\sum n_i d_i^3}{\sum n_i d_i^2}}$$

Where $D$ is the volume mean diameter, $n_i$ is the number of particles, and $d_i$ is the mean particle diameter.
2.2. Zeta potential

Zeta potential determines the surface charge at the interface of the droplets dispersed in the aqueous solution and indicates the stability of colloidal suspensions or emulsions; the higher zeta potential values, the more stable suspensions (J. Wu et al., 2015). Electrophoretic mobility or Zeta potential of nanoliposomes was determined using a Malvern Zeta sizer Nano ZS (Malvern Instruments, Worcestershire, UK) at 25 °C, pH: 7.4, and 149 W. All samples were diluted (50 folds) using distilled water and were repeated triples.

2.2.3. Encapsulation efficiency (EE %)

The percentage of the total compound entrapped within the liposome determines the encapsulation efficiency which is an important parameter in liposomal characterization. The amount of phenolic compounds of the encapsulated SKEO was examined for determination of optimal proportion of lecithin to cholesterol with some modifications (Haghju et al., 2016). Liposomes were isolated through Amicon filter and then deliberately disrupted by adding 0.025% nonionic Triton X-100, and total phenols were quantified by Folin–Ciocalteau method. One milliliter sodium carbonate 2% and 200 μL Folin–Ciocalteau reagent were added to 10 ml of the samples and centrifuged at 1200 rpm for 5 min. 30 min after storage of samples at room temperature, the spectrophotometric measurements were carried out at 750 nm using a UV spectrophotometer (pharmacia biotech ultraspec 2000, UK). Encapsulation efficiency was expressed as a percentage according to the following equation:

\[
EE\% = \frac{C_{\text{lysed}}}{C_{\text{total}}} \times 100
\]

Where \(C_{\text{lysed}}\) is the total phenolic content of liposomes containing SKEO after isolation with Amicon filter and treatment with the surfactant Triton X-100 and \(C_{\text{total}}\) is the concentration of total polyphenols treated with Triton X-100.

2.2.4. Scanning electron microscopy

The morphology and structure of NPs were visualized, using scanning electron microscope (SEM). Freeze-dried samples were mounted on aluminum stubs and, subsequently, gold-coated in vacuum by a sputter (DST1, Nanostructured coating co., Tehran, Iran). The samples were observed by a scanning electron microscope (KYKY-EM3200; KYKYTechnology Development Ltd.,Beijing, China) at excitation voltage of 25 KV.

2.3. Preparation of coating solution

Coating solutions were prepared according to the method reported by Ojagh, Rezaei (Ojagh, Rezaei, Razavi, & Hosseini, 2010) with some modifications. A film-forming dispersion was prepared by dissolving 2% (w/v) of chitosan in 1% (v/v) acetic acid solution using a magnetic stirrer for 2 h at 95 °C. The resultant chitosan solution was filtered through a Whatman No. 3 filter paper to remove any undissolved particles. Glycerol was added to the film forming solution as plasticizer at the concentration of 25% (w/w) of chitosan powder. The solution was returned to the magnetic stirrer/ hot plate and heated at 60 °C for another 30 min. The solution was cooled to room temperature, then at a level of 1% v/v, proper amounts of free EO and SKEO-loaded nanoliposomes with the highest EE% (at proportion of lecithin to cholesterol of 50:10) were added into chitosan solution and stirred at 300 rpm for 1 h. The obtained solution was used to prepare the edible coating.

2.4. Samples preparation

Legs of lambs were obtained from the abattoir 48 h post-slaughter. 96 steaks of about 60 g weight (6 × 6 cm and 2-cm-thick) were aseptically cut from the central part of the leg, using sterile cutting boards and knives. Each steak was placed into a polystyrene tray and stored in the refrigerator until being coated by the immersion method. After preparing the solutions, meat samples were taken out of the refrigerator and were randomly divided into four groups (i) uncoated (Control); (ii) coated with solely chitosan solution (Ch); (iii) coated with chitosan solution containing SKEO (Ch + EO); (iv) coated with chitosan solution containing SKEO-loaded nanoliposomes (Ch + Nano) and samples in each group were immersed in the corresponding solutions for 1 min. Then, the pieces were allowed to drip excess solution. The coated samples were dried at 25 °C for 15 min. The samples of each groups were placed individually into sterile plastic Petri dishes and were sealed hermetically and refrigerated at 4 ± 1 °C for up to 20 days. Three samples from each four treatments were analyzed on days 0, 1, 4, 7, 10, 13, 16, and 20. All analyses were performed using both Semimembranosus and Biceps femoris muscles.

2.5. Microbiological analysis

At each sampling day, meat samples (25 g) were weighed aseptically, transferred to a stomacher bag (Seward Medical, London, UK), containing 225 ml of sterile quarter-strength Ringer's solution, and homogenized using a stomacher (Lab Blender 400, Seward Medical, London, UK) for 60 s at room temperature. For microbial enumeration, 0.1 ml samples of serial dilutions (1:10 diluent, and quarter-strength Ringer's solution) of lamb homogenates were spread on the surface of dry media. Total viable counts (TVC) were determined using plate count agar (PCA, Merck code 1.05463, Darmstadt, Germany), after incubation for 3 days at 30 °C (APHA, 1984). Lactic acid bacteria (LAB) were enumerated using the medium of Man, Rogosa and Sharpe (MRS) agar (Merck, 1.10660), pH 5.7, and incubated at 32 °C for 48 h. Pseudomonads were determined on cetrimide fusidin cephaloridine agar (Oxoid code CM 0559, supplemented with selective supplement SR 0103, Basingstoke, UK) after incubation at 42 °C for 18 h (APHA, 1984). Three replicates of at least three appropriate dilutions depending on the sampling day were enumerated. Microbiological data were transformed into logarithms of the number of colony forming units (cfu/g).

2.6. Determination of pH

To determine pH value, a mixture of 10 g of meat in 100 mL of distilled water was prepared and digital pH meter was used (Aktaş, Aksu, & Kaya, 2003).

2.7. Determination of thiobarbituric acid reactive substances (TBARS)

The thiobarbituric acid index was determined based on the method described by Ouattara, Giroux with modifications (Ouattara et al., 2002). It is based on reaction between thiobarbituric acid (TBA) with secondary lipid oxidation products such as aldehydes and ketones. 10 g meat sample was homogenized with 30 mL 4% perchloric acid and 1 mL Butylated hydroxytoluene (BHT) (dissolved in ethanol) at 4000 rpm for 2 min. The mixture was filtered through a Whatman No. 4 filter. 5 mL of the resulting solution was mixed with 5 mL 0.02 M TBA in a stoppered test tube. The
2.9. Statistical analysis

Scores for “discoloration” referred to degree of discolored surface: 1 = none, 2 = slight, 3 = small, 4 = moderate, and 5 = extreme. Reduction in the recorded scores indicates the increase of brown-ness. Scores for “off-odor” referred with the intensity of odours associated to lipid oxidation: 1 = none; 2 = slight; 3 = small; 4 = moderate; and 5 = extreme. The attribute ‘Red color’ was scored in the red non-discolored part of the steak, using also an intensity 5-point scale using a questionnaire according to method pre-

2.8. Sensory evaluation

Samples were evaluated by six semi-trained panellists according to the method of (Cross, Moen, & Stanfield, 1978). All of the panellists had a background in meat evaluation and previously participated in another study conducted by (Noori, Zeynali, & Almasi, 2018) at department of food science of Urmia University. They were selected based on their sensitivity and limit detection of taste and smell of very low concentrations of SKEO. The attributes studied were: ‘red color’, ‘discoloration’ and ‘off-odor’. The attributes “discoloration” and “off-odor” were rated using a 5-point descriptive scale using a questionnaire according to method presented by (Djenane, Sánchez-Escalante, Beltrán, & Roncales, 2001).

3. Results and discussion

3.1. Characterization of nanoliposomes

3.1.1. Size of nanoliposomes

The size of nanoliposomes is an important factor in their stability and potential to release the entrapped compounds in the liposome core. The particle sizes and polydispersity index of the prepared nanoliposomes are presented in Table 1. Mean size and size distribution of the different nanoliposome formulations were in the range of 93–96 nm and 0.83 to 0.88, respectively. The DLS analysis and polydispersity index confirmed a bimodal distribution in the particle size. Results showed that z-average diameter and polydispersity index of loaded nanoliposomes were not affected significantly (p > 0.05) with different amounts of cholesterol in four formulations. Similar results were reported in other studies in the case of vitamin loaded nanoliposomes (Mohammadi, Ghanbarzadeh, & Hamishehkar, 2014) and Ibuprofen loaded liposomes (Mohammed, Weston, Coombes, Fitzgerald, & Perrie, 2004). However, there is a discrepancy between the reported effects of cholesterol on particle size in the literature which can be attributed to different preparation method of liposome (Alexander, Lopez, Fang, & Corredig, 2012), type of phospholipids, material viscosity, and applied sonication parameters (amplitude and time) (Alexander et al., 2012).

3.1.2. Zeta potential

Zeta potential is a key factor in characterizing the behavior of colloidal systems, measurement of the surface electrical charge of the particles, and assessment of nanoliposomes stability (da Silva Malheiros, Sant’Anna, de Souza Barbosa, Brandelli, & de Melo Franco, 2012). The higher absolute value of the zeta potential, the more physical and chemical stability of the colloidal suspension due to large repulsive forces reducing the rate of aggregation and fusion (J. Wu et al., 2015). Table 1 demonstrates the variation of the zeta potential of nanoliposomes depending on the ratio of phosphatidylcholine (PC) (lecithin) and cholesterol in the formulation. Negative values of the zeta potential in the present study have been mainly attributed to the presence of terminal groups on the lipids, which are in good agreement with the reported values of other studies (Da Silva Malheiros et al., 2012).

Moreover, the results indicated that as the proportion of cholesterol increased, the absolute value of the negative charge of liposomes grew significantly. The addition of cholesterol in liposome membranes might have stabilized the lipid chain by filling in the molecular cavities of vesicles and increasing the packing densities of phospholipids molecules which have been attributed to the ability of cholesterol to complex with phospholipids (da Silva Malheiros et al., 2012). The inclusion of cholesterol within the nanoliposome formulations increased the zeta potential value approximately by 70% (P < 0.05). The zeta potential values are comparable with the reported values in other studies (Haghju et al., 2016).

3.1.3. Encapsulation efficiency (EE %)

EE is mainly dependent on the type, ratio of the used lipid, and the inner volume of the vesicles (Fan, Xu, Xia, & Zhang, 2008). Table 1 represents the EEs of nanoliposomes consisting of different mass ratios of lecithin and cholesterol. The entrapment efficiency increased by 8% when the ratio of lecithin to cholesterol changed from 60:00 to 50:10 (P < 0.05). But when the ratio of cholesterol further increased, the values of EE dropped off from 69.05 to 59.75% and 46.88% (P < 0.05). The EE value was found to be higher at the low level of cholesterol. The increase of EE with the inclusion of small ratio of cholesterol could be ascribed to the ability of cholesterol to rigidify and reduce the permeability of liposomal membrane by filling the molecular cavities or leaking spaces in the bilayer membranes (Fan et al., 2008). An appropriate proportion of

<table>
<thead>
<tr>
<th>Code</th>
<th>Lecithin: Cholesterol</th>
<th>z-average diameter (nm)</th>
<th>Polydispersity Index</th>
<th>Zeta potential (mV)</th>
<th>Encapsulation Efficiency%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60:00</td>
<td>96.00 ± 1.15</td>
<td>0.85 ± 0.01</td>
<td>−13.51 ± 0.29a</td>
<td>61.06 ± 1.45c</td>
</tr>
<tr>
<td>2</td>
<td>50:10</td>
<td>93.01 ± 0.57</td>
<td>0.83 ± 0.01</td>
<td>−15.30 ± 0.38a</td>
<td>69.05 ± 0.81h</td>
</tr>
<tr>
<td>3</td>
<td>40:20</td>
<td>94.04 ± 0.56</td>
<td>0.87 ± 0.02</td>
<td>−19.50 ± 0.33b</td>
<td>59.75 ± 3.14d</td>
</tr>
<tr>
<td>4</td>
<td>30:30</td>
<td>93.00 ± 1.73</td>
<td>0.88 ± 0.04</td>
<td>−23.03 ± 0.60a</td>
<td>46.88 ± 0.74e</td>
</tr>
</tbody>
</table>

Data are mean values ± SD.

Mean values with different letters within a column indicate significant differences (p < 0.05).
cholesterol to lecithin could be indispensable to the maximization of EE. A similar dependency of Encapsulation efficiency on cholesterol content has been observed in various studies (Mohammed et al., 2004; J.; Wu et al., 2015).

3.1.4. Scanning electron microscopy

SEM was carried out to investigate the morphology of SKEO-loaded nanoliposomes with the lower droplet size and maximum encapsulation efficiency (50:10 lecithin:cholesterol molar ratio) according to the data obtained by previous experiments. As shown in SEM images, semispherical nanoliposome particles were produced (Fig. 1). The mean diameter was in agreement with the data obtained by the particle size analyzer device.

3.2. Microbial stability

Microbiological evaluation along with chemical indices have been used extensively to assess the quality and shelf-life of meat. Total viable count (TVC), Pseudomonads (PBC), and LAB counts of control and coated meat samples during 20 days of refrigerated storage were evaluated, and the mean counts (as log CFU g⁻¹) were presented in Fig. 2, which indicates a significant impact of the treatments (Ch, Ch + EO, Ch + Nano) along with the storage time on TVC (P < 0.05). Regarding the upper acceptability limit recommended by (ICMFS, 1986) for total viable count in fresh meat (7 log CFU g⁻¹ fresh), it could be observed in Fig. 2A that control samples exceed the limit after 9 days of storage, comparing to 12 days noted for chitosan coated samples (CH), and 20 days for Ch + EO samples, whereas Ch + Nano samples contained TVC (4.85 log CFU g⁻¹ below the critical value after a storage period of 20 days. The obtained results indicate that all chitosan coatings have slowed down the microbial growth in comparison with control samples. The mechanism of microbial growth inhibition by the chitosan molecules has been ascribed to polycationic nature of the polysaccharide, which interrupts the cell membrane (Helander, Nurmiaho-Lassila, Ahvenainen, Rhoades, & Roller, 2001) or a film-forming ability around the bacterial cell (Zheng & Zhu, 2003). Antimicrobial effect of chitosan coating has been reported by other researchers (Kanatt, Rao, Chawla, & Sharma, 2013).

Compared to the control samples, a microbiological shelf-life extension of 10 or 15 days was achieved for meat samples coated with chitosan solution containing free or encapsulated SKEO. The extended shelf-life for these treatments could be attributed to the antimicrobial action of phenolic compounds content of SKEO, especially Carvacrol which has been reported to have propitious antimicrobial and antioxidant activity due to its lipophilic character and phenolichydroxyl group (Zengin & Baysal, 2014).

Fig. 2 also revealed that the encapsulation of the active compounds in nanoliposomes led to a slightly lower antimicrobial activity of Ch + Nano coating at the initial time compared to Ch + EO (P < 0.05). Free SKEO possibly controlled the initial bacterial growth burst and eventually, SKEO release from nanoliposomes interactions with bacterial cells could have improved the antimicrobial potential. As shown by the (Fig. 2A), Ch + Nano coatings exhibited a prolonged and consistent antimicrobial activity or retardation of microbial growth on meat pieces during storage time in comparison with coating containing free EO. The interaction of liposome with target cells can occur by adsorption onto the cell surface, fusion with the cell membrane, and release of bioactive compounds (Torchilin, 2005). Similar results were reported in other

![Fig. 1. SEM image of SKEO loaded nanoliposomes.](image)
studies in the case of gelatin film incorporated with cinnamon essential oil loaded nanoliposomes (J. Wu et al., 2015). Some other studies have reported that encapsulation of EOs in liposomes produced more antimicrobial and antioxidant activity than the same EOs in pure form because it could reduce evaporation of EOs and facilitate its delivery to the bacterial cell wall (Ghaderi-Ghahfarokhi, Barzegar, Sahari, Gavilghi, & Gardini, 2017; Rai et al., 2017).

All edible coatings exerted a similar antimicrobial effect against Pseudomonads bacteria which forms a significant part of the spoilage microflora of meat stored under refrigeration. PBC in all treatments was significantly lower than control (P < 0.05). Overall antimicrobial effect was reliant upon microbial group susceptibility and sample storage time. As the Fig. 2B represents, the Ch + Nano was the most effective treatments for the inhibition of Pseudomonas spp. that sustained the PBC under 3 log cfu/g until day 20 of storage. Encapsulation of bioactive compounds into liposomes offers several benefits such as increased availability and interaction of the active compounds with microorganisms, protection of active agents from degradation, as well as lower sensorial impact and required concentration of active agents by liposomes to reduce same or higher number of CFU (Donsì et al., 2011). It has been reported that the encapsulation of bioactive compounds in nano-liposomes or nanoemulsion formulations owing to their larger surface area and higher affinity of nano-droplets with bacterial cells results in a quantum-size effect and increases the antimicrobial activity of Eos (Noori et al., 2018). Hence the Ch + Nano coating could be an ideal choice to increase the shelf-life of lamb meat.

The initial LAB counts (Fig. 2C) were around 2.39 log cfu/g and increased progressively with the time. Ch + EO and Ch + Nano treatments resulted in a reduction in LAB counts by 3.36 and 4.27 log cfu/g (P < 0.05) respectively on the 13th day of storage. The results are in good agreement with the reported values of other studies using different EOs (Ghaderi-Ghahfarokhi et al., 2017).

3.3. pH

Fig. 3 presents the pH values of the different chitosan coated and uncoated control samples during 20 days storage at 4 °C. The initial pH of all sample was found to be 6.2. The application of all chitosan coatings onto the surface of meat samples resulted in the decrease of pH values (P < 0.05), likely because of the low pH of the chitosan coating solution containing acetic acid in the formulation. This lower pH in coated samples was maintained throughout the storage period (P < 0.05) which can be attributed to the antimicrobial effect of chitosan. Similar decrease in pH values as result of chitosan coating treatment was reported in other studies in the case of tilapia fillets (Chen et al., 2011) and shrimp meat (Simpson, Gagne, Ashie, & Norozi, 1997).

pH of control meat samples increased 1.25 units after 20 days whereas this value for coated samples with Ch and Ch + EO increased much less (0.46 and 0.19). There were no significant differences in pH values (p > 0.05) among the samples with chitosan coatings containing free and encapsulated SKEO which showed a preservative impact by sustaining the natural pH of meat during 20 days of the storage. In general, the increase of pH values attributed to the formation of alkaline autoxidation compounds (nitrogenous compounds) and production of bacterial metabolites in the muscle as the results of protein autolysis and microbial growth (Kılıç, Şimşek, Claus, & Atülgan, 2014). The almost constant pH behavior of coated samples during the storage could be associated to their protective activity against substrate decomposition, lower microbial growth compared to the control.

3.4. Lipid oxidation

The TBARS assay measures the secondary oxidation products responsible for oxidative rancidity which is one of the main factors limiting the shelf-life of muscle foods (Gray et al., 1996). The effects of the edible coatings on oxidative stability of the meat samples were measured in terms of thiobarbituric reactive substances (TBARS) throughout storage and results are shown in Fig. 4.

Results illustrated that initial TBARS values for all treatments were about 0.33 mg MDA/kg meat. TBARS values equal to or greater than 5 mg MDA/kg meat comprise the threshold for detecting off-odours and off-taste for humans (Insaurist et al., 2001). However, the TBARS values increased gradually during storage in all samples, particularly in the control sample which showed the highest increase (p < 0.05). Antioxidant activity of just chitosan coating was only significant within the earlier period of the storage as compared with control. TBARS values of chitosan coated samples revealed that chitosan coating inhibited lipid oxidation and showed antioxidant properties. The mechanism of antioxidant activity of chitosan could be attributed to residual amino groups of chitosan forming a stable fluorosphere with volatile aldehydes derived from breakdown of fats during oxidation such as malondialdehyde (Kanatt et al., 2013). On the other hand, it is possible that the

![Fig. 3](image36x118to280x261) The pH value of meat samples during 20 days storage at 4 °C (Control: uncoated; Ch: coated with solely chitosan solution; Ch + EO: coated with chitosan solution containing SKEO-loaded nanoliposomes). Data are means ± SD. Mean values with different letters in each day indicate significant differences (p < 0.05).

![Fig. 4](image305x110to549x248) The TBA value of meat samples during storage at 4 °C (Control: uncoated; Ch: coated with solely chitosan solution; Ch + EO: coated with chitosan solution containing SKEO-coated nanoliposomes). Data are means ± SD. Mean values with different letters in each day indicate significant differences (p < 0.05).
chitosan coating could lower the diffusion of oxygen to the meat surface and consequently lipid oxidation by acting as a barrier between meat and its surroundings when used directly on the surface of meat samples. Our results are in good agreement with the reported values of other studies (Aşık & Candogan, 2014; Kılıç et al., 2014). Incorporating SKEO to chitosan protected lamb meat from oxidation, keeping TBA scores lower than 4 mg MDA/kg until day 16 of storage. It can be due to antioxidant properties of SKEO phenolic components especially carvacrol which could act as a quencher of free radicals by donating hydrogen atoms or electrons, retarding lipid oxidation (Sánchez-Escalante et al., 2003; Choe and Min, 2006). In this regard (Hashemi, Amininezhad, Shirzadinezhad, Farahani, & Yousefabad, 2016) reported that Satureja khuzestanica EOs enhanced oxidative stability of sun flower oil.

Ch + Nano treatment effectively protected the product from the beginning of storage, keeping TBA scores lower than 1.5 mg MDA/kg for the first 10 days of storage and below 2.5 mg/kg until day 20 of storage. It could be concluded that encapsulation has the potential to preserve EOs from evaporation and decomposition during storage. Nano-size formation of liposomes causes to increase specificity of surface of SKEO and thus a fast and efficient antioxidant activity would be achieved (Noori et al., 2018). Improved antioxidant capacity have also been reported for pork fillet and fish meat coated with encapsulated horseradish extract (Jung et al., 2009). The intensified antimicrobial and antioxidant activities of the nanoliposomes-loaded EOs over the free EOs, as well as their enhanced stability would allow reducing the concentration to be incorporated in foods, active coating, or packaging.

3.5. Sensory evaluation

Results of sensory analysis of lamb meat samples are shown in Table 2. All sensory attributes of samples were significantly influenced by the storage time (p < 0.05). The control lamb meat samples assumed unacceptable color after day 10. The higher acceptability score of 3 was reached for Ch coated samples after 13 days. The Ch + EO gave rise to a score of 3 at day 16 of storage. Ch + Nano delayed red color fading significantly in lamb meat. Those samples did not reach a score of 3 even at the end of the storage time. Consequently, they had an acceptable red color after 20 days storage in the refrigerator.

Discoloration and red color fading scores were highly correlated to each other. The control, chitosan, and chitosan + SKEO coated meat samples reached scores of 3 or above, representative of an unacceptable faded color according to (Djenane, Sánchez-Escalante, Beltrán, & Roncalés, 2002), at day 10 (Control), 10–13 (Ch), and 13 (Ch + EO), while (Ch + Nano) did not reach a score of 3 even at the end of storage time. Ch + Nano was the most effective in protecting lamb from color changes.

Control and Chitosan coated meat samples were given off-odor scores of 3 at day 10 of storage, corresponding to TBARS values well above 2. Greene & Cumuze, 1982 reported that a TBA range of 0.6–2.0 was required for panelists to detect oxidized flavors. Ch + EO delayed off-odor formation until day 13. Ch + Nano delayed off-odor development very effectively by giving a score of 1, revealing the absence of any perceptible off odor until 20 days. Our results are in good agreement with similar studies (Hu, Wang, Xiao, & Bi, 2015).

4. Conclusions

The results of microbiological and chemical analyses indicated that chitosan coating containing nano-encapsulated SKEO on lamb meat can lead to retention of the good quality characteristics, improvement of microbiological safety, and extension of shelf-life during chilled storage. All coated samples diminished microbial counts significantly compared to uncoated samples. Overall, encapsulation of SKEO enabled control release of the antimicrobial agents on samples' surface that resulted in an extended antimicrobial activity during 20 days of storage compared to free EO. Regarding the antioxidant activity of SKEO and the oxygen barrier properties of the coating, chitosan coating with SKEO-loaded nanoliposomes showed the best effect on oxidative stability of meat samples. These results suggest that chitosan coatings containing SKEO-loaded nanoliposomes can be employed as an active packaging in the meat industry.

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References

Cross, H., Moen, R., & Stanfield, M. S. (1978). Training and testing of judges for...
sensory analysis of meat quality [Textural properties]. Food Technology.