The effect of clove essential oil loaded chitosan nanoparticles on the shelf life and quality of pomegranate arils

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1. Introduction

Pomegranate (Punica granatum), widely grown in Iran, Spain, India and USA, is one of the most important commercial fruit crops which is native to Iran (Meighani, Ghasemnezhad, & Bakhshi, 2015). This fruit is a source of carbohydrates, minerals, crude fibers, various biologically active compounds such as vitamin C and certain phenolic compounds such as punicalagin, ellagic acid, gallocatechin and anthocyanins, known to act as natural antioxidants (Sabokbar & Khodaiyan, 2015). Pomegranate fruit is very appreciated probably due to the presence of these biological active compounds that have valuable properties such as anti-mutagenicity, antihypertension and reduction of liver injury (Sabokbar & Khodaiyan, 2016).

The sensitivity of pomegranate fruit to cuts, sunburn, bruises, cracking and chilling injury occurring at low temperatures, make external defected pomegranates inappropriate for fresh sale and consumption, and hence they are generally destined to industrial applications or animal use (Artés, Tudela, & Villaescusa, 2000). Therefore, minimal processing can be an extremely good way to acquire a commercial benefit from external defected pomegranates with very good internal quality (López-Rubira, Conesa, Allende, & Artés, 2005). However, respiration rate and detrimental biochemical changes of processed pomegranate arils such as development of off flavors and texture breakdown are accelerated by their surface damages. Furthermore, surface microbial contamination can lead to fruit spoilage (Brasil, Gomes, Puerta-Gomez, Castell-Perez, & Moreira, 2012).

The edible coating made from biodegradable ingredients has been considered as a technology to extend the shelf life of coated products through modifying their internal atmosphere (Perdones, Sánchez-González, Chiralt, & Vargas, 2012). It slows down the respiration rate and acts as a good barrier to gas transport (Varasteh, Azrani, Barzegar, & Zamani, 2012) and hence improves visual and tactile features of coated fruits, also protects them from moisture migration and microbial growth on their surfaces (Mohammadi, Hashemi, & Hosseini, 2016). The demand for healthy and environmentally friendly production systems of fruits and vegetables has been increased during the past few years. For this purpose, essential oils (EOs), generally recognized as safe (GRAS), have been considered as promising alternatives to chemical-based preservatives due to good antimicrobial properties (Linde, Combrinck, Regnier, & Virijević, 2010; Sánchez-González et al., 2011).

Among EOs, clove essential oil (CEO) and its main component eugenol have been considered as natural preservatives due to their positive effects against fungal pathogens of fruits and nuts (Amiri, Dugas, Picot, & Bompex, 2008; Passone, Girardi, & Etcheverry, 2012). CEO has been isolated from the buds of Eugenia Caryophyllata and widely used in flavoring industry, fragrance and cosmetics. The main constituents of this oil are eugenol (4-allyl-2-methoxy phenol), the phenylnpropanoid, eugenyl acetate, the monoterpene ester and β-
The oil has been shown to have the wide spectrum properties including antibacterial, antifungal, antioxidant, insecticidal, antiviral, anti-mutagenic, anti-inflammatory and hepatoprotective effects (Chiaib et al., 2007; Sebaaly, Jrai, Fessi, Charcosset, & Greige-Gerges, 2015; Chen et al., 2017).

In order to maintain antimicrobial activity of EOs, despite their high volatility, encapsulation has been recently considered as an efficient method for their protection from environmental degradation due to the exposure to oxygen, light, moisture, pH and heat (Donsì, Annunziata, Sessa, & Ferrari, 2011). The nanometric size delivery systems or nanoencapsulation increase the passive cellular adsorption mechanisms enabling the enhancement of antimicrobial activity of EOs by lowering the doses used and thus minimizing the alteration of organoleptic properties of coated products (Donsì et al., 2011).

In recent years, chitosan (Ch), generally recognized as safe (GRAS), has received much attention in the encapsulation of bioactive compounds such as EOs due to its nontoxicity, biocompatibility, biodegradability, antimicrobial properties and ability to form gels, films and particles (Reawchaoon & Yoksan, 2011; dos Santos et al., 2012; Wang, Wu, Qin, & Meng, 2014; Feyzioglu & Tornuk, 2016). In this regard, nanochitosan-based coating loaded with Zataria multiflora EO was applied for cucumber and resulted in a reduction of respiration rate, weight loss and color change of coated fruit. At the same time, ferric reducing power and levels of DPPH-radical scavenging activity were improved. Furthermore, microbial population and fungal decay were reduced and thus the shelf life of coated cucumber was extended (Mohammadi et al., 2016).

To our knowledge, the effect of clove essential oil loaded chitosan nanoparticles (CEO-ChNPs) or encapsulated CEO, chitosan nanoparticles (ChNPs) and free CEO as surface coatings on extending the shelf life and improving the quality of pomegranate arils has not been reported yet. Therefore, we intended to compare the effect of CEO-ChNPs, ChNPs, CEO and Ch on improving the microbial, physicochemical and sensory qualities of ready to eat pomegranate arils.

2. Materials and methods

2.1. Materials

Medium molecular weight Ch (75–85% degree of deacetylation, CAS # 9012-76-4), TPP (CAS # 7758-29-4; technical grade) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (Germany). Tween 80, tri-Sodium citrate dehydrate, Sodium hydroxide, Sodium carbonate, Sodium acetate, Potassium chloride, Folin-Ciocalteu’s phenol reagent and Potato dextrose agar (PDA) were supplied by Merck-Chemicals Co. (Germany). Glacial acetic acid, Hydrochloric acid and Methanol (reagent grade) were acquired from Scharlab, S.L. (Spain). Clove (Eugenia Caryophyllata) EO (100% pure) mainly composed of 77.21% eugenol, 8.31% eugenyl acetate and 7.19% β-caryophyllene, was obtained from Barij Essence Pharmaceutical Co. (Iran). All chemicals were applied as received without any purification.

2.2. Methods

2.2.1. Preparation of antifungal coating dispersions of CEO-ChNPs, ChNPs, CEO and Ch

CEO-ChNPs and ChNPs were prepared according to the method described by Hasheminejad, Khodaiyan, and Safari (2019). The solution of Ch (0.3% (w/v)) was produced in aqueous acetic acid (1% (v/v)) by stirring overnight at 25°C. After adjusting the pH to 4.6 using 9 N NaOH, the prepared solution was filtered by Büchner funnel and Whatman 42 paper. Tween 80 (HLB 15.9, 1% w/v) was then added as a surfactant to the 0.3% Ch solution and stirred at 25°C for 30 min. After getting a homogeneous mixture, the oil was gradually dropped in prepared aqueous solution to obtain an oil-in-water emulsion with the mass ratio of Ch to CEO of 1:1. At the same time, the agitation was done at a speed of 700 rpm for 10 min at 25°C. The TPP solution (0.3% (w/v)) was then produced in distilled water and flush mixed with prepared emulsion. The mixture was subsequently subjected to agitation for 30 min to effect crosslinking. The final pH of the mixture should be 4.6. The same procedure without adding CEO was done for the preparation of ChNPs. The spontaneous formed nanoparticles (NPs) were collected by centrifuge (SIGMA 8 K, Germany) at 10,000g for 35 min at 4°C and washed several times with aqueous tween 80 solution 1% (v/v), then dispersed in distilled water to reach the final concentration of 0.15% (w/v). To obtain a homogeneous dispersion, NPs were treated by ultrasonic homogenizer (TOPSONICS, UP400, Iran) at 60 W for 6 min with a sequence of 3 s sonication and 7 s rest. Ch solution was then prepared at the concentration of 0.15% (w/v) and used as coating dispersion after adjusting the pH to 4.6 and filtration. The oil-in-water emulsion of CEO was finally prepared by gradually dropping the oil (0.15% (w/v)) in the stirring solution of aqueous tween 80 (1% (w/v)). The prepared coating dispersions were stored at 4°C.

2.2.2. Aril coating and storage conditions

Pomegranate (Punica granatum L. cv. Malase Saveh) fruits were obtained after maturing stage from a commercial farm in Ismaiel Abad village, Saveh. The arils of fully ripe intact and uniform pomegranates were manually extracted. Then the arils were randomly divided into 150 polyethylene boxes (150 g aril inside each box). Every 30 boxes were dipped in one of the four antifungal coating dispersions of Ch, CEO, ChNPs and CEO-ChNPs for 2 min. As control, 30 boxes were dipped in distilled water. For each group of coating and control, three replicates were used. After immersion, the excess liquid of the arils was drained by a nylon filter and then, the arils were air dried. Eventually, the boxes were sealed and stored for 60 days at 5 ± 0.5°C with 90 ± 5% relative humidity.

2.2.3. Microbial analysis

Microbial count was performed at the beginning day (day 0) and then every 6 days during 54-day storage at 5°C as described by López-Rubira et al. (2005) with some modifications. From the day 18 of storage, counting for the samples with early signs of fungal decay was not performed. To enumerate viable yeasts and molds, aril juice was extracted by a garlic press. Then, the prepared juice and/or diluted juice of each treatment were uniformly spread-plated (0.1 mL) on chloramphenicol potato dextrose agar (pH 5.6) and incubated at 25°C for 3–5 days. For dilution, 1 mL of aril juice was mixed with 9 mL of physiological serum and then more dilutions were prepared from initial dilution. All experiments were done in triplicate. Microbial count was shown as logarithm of colony forming units per mL of juice (log CFU/mL).

2.2.4. Weight loss (%)

The percentage of weight loss in control and coated pomegranate arils was measured during 54-day storage at 5°C using an electronic balance (GES12, Sartorius, Germany). From the day 18 of storage, the weight of the samples with early signs of fungal decay was not measured. For control and coated treatments, all measurements were done in triplicate. The percentage of weight loss for each treatment was calculated according to the method of Fawole and Opara (2013) using formula 1 (Fawole & Opara, 2013):

\[
\text{Weight loss} = \frac{W_1 - W_2}{W_1} \times 100
\]

where \(W_1\) and \(W_2\) are the weight (g) of pomegranate arils at the beginning day (day 0) and every 6 days, respectively.

2.2.5. Chemical analysis

For each step of chemical analysis, sampling was performed on the beginning day (day 0) and then every 6 days until 54 days during storage.

N. Hasheminejad and F. Khodaiyan

Food Chemistry 309 (2020) 125520
storage at 5 °C. From the day 18 of storage, chemical analysis for the samples with early signs of fungal decay was not performed. Aril juice of each treatment was extracted by a garlic press and then clarified by centrifuge to use for chemical analysis including total soluble solids (TSS), titratable acidity (TA), pH, antioxidant activity (AA), total phenolic content (TPC) and total anthocyanins content (TAC).

2.2.5.1. TSS, TA and pH. To evaluate TSS, TA and pH, the method described by Ghasemnezhad, Zareh, Shiri, and Javdani (2015) was used. TSS in control and coated treatments were calculated by a refractometer (Refractometer Abbe, Bellingham & Stanley Ltd, UK) and data were expressed as °Brix. A pH meter (GLP 22, CRISON, Spain) was used for measuring pH and TA. The calculation of TA was performed by titrating 5 mL of juice to reach the endpoint of pH 8.2 with 0.1 N NaOH and recording the titration volume. The resulting data were expressed as citric acid percentage. All measurements were done in triplicate.

2.2.5.2. TPC evaluation. The TPC of control and coated treatments was measured according to the method described by Çam and Hsql (2010) with some modifications. To determine TPC, 0.5 mL of 10-fold diluted aril juice of each treatment was mixed with 2.5 mL of 10-fold diluted Folin-Ciocalteu’s phenol reagent. After 1 min incubation, 2 mL of 7.5% sodium carbonate was added to the whole mixture and the final mixture was allowed to stand in darkness for 30 min. The blank sample was prepared in the same manner using distilled water. The absorbance of control and coated samples versus blank was then measured at 760 nm using a UV–vis spectrophotometer (SP-UV 500DB spectrophotometer, Spectrum instruments, Canada). TPC of control and coated treatments was analyzed in triplicate and the results were expressed as mg gallic acid equivalents per 1 L of juice (mg GAE/L).

2.2.5.3. TAC evaluation. TAC of control and coated treatments was calculated spectrophotometrically by pH differential method as described by Lako et al. (2007) with some modifications. Briefly, 0.4 mL of aril juice of each treatment was mixed with potassium chloride buffer with pH 1.0 and sodium acetate buffer with pH 4.5, separately. The absorbance of the resulting mixtures was then measured at 510 and 700 nm against buffer systems as blanks. All experiments were performed in triplicate and the results were expressed as mg cyanidin-3-glucoside per 1 L of juice. To determine TAC, the absorbance (A) was first calculated through Eq. (2) (Lako et al., 2007):

\[
A = (A_{510} - A_{710})/\varepsilon_{510} - (A_{510} - A_{700})/\varepsilon_{700}
\]

Then, TAC based on the concentration of cyanidin-3-glucoside, a monomeric anthocyanin pigment, was calculated using formula 3 (Lako et al., 2007):

\[
\text{TAC (mg/L)} = \frac{A \times \text{MW} \times \text{DF} \times 1,000}{\varepsilon \times 1}
\]

where A is the absorbance, MW is the molecular weight of cyanidin-3-glucoside (449.2), DF is the dilution factor (10) and ε is the molar absorptive coefficient of cyanidin-3-glucoside (26,900).

2.2.5.4. AA evaluation. AA of uncoated and coated samples were measured using DPPH (2,2-diphenyl-1-picrylhydrazyl) method as described by Çam and Hsql (2010) and Meighani et al. (2015) with some modifications. To determine AA, 0.1 mL of 10-fold diluted aril juice of each sample was mixed with 5 mL of 0.1 mM methanolic solution of DPPH. The final mixture was then kept in darkness at 25 °C for 20 min. The control solution was made in the similar manner using distilled water. All experiments were done in triplicate and the absorbance of the resulting mixtures was measured at 517 nm using a UV–vis spectrophotometer. The results were expressed as the percentage of decrease in absorbance relative to the control solution, corresponding to the percentage of free-radical (DPPH) scavenged (% DPPHsc) by each sample. AA was calculated through formula 4 (Meighani et al., 2015):

\[
\text{AA (}% \text{DPPHsc}) = A_{\text{control}} - \frac{A_{\text{sample}}}{A_{\text{control}}} \times 100
\]

where Acontrol and Asample are the absorbance of control solution and sample, respectively.

2.2.6. Sensory analysis
Sensory key attributes (flavour, colour and texture), as well as, sensory acceptability of control and coated pomegranate arils were evaluated according to the scale described by Xing et al. (2011). Samples were coded with random numbers and presented in a random order to 30 panelists who had previously received several training sessions. The nine point hedonic scale (9: excellent, 7: very good, 5: good, 3: fair and 1: poor) was used to rate the samples. Sensory analysis was performed at the beginning day (day 0) and then every 6 days until day 54 during storage at 5 °C. From the day 18 of storage, the samples with early signs of fungal decay were eliminated and the sensory test continued with non-contaminated remaining samples.

2.2.7. Statistical analysis
The one-way analysis of variance (ANOVA) was done through IBM SPSS Statistics version 23 (IBM Corporation, USA). To determine the statistical differences among the mean values of various treatments with 95% significance level, Duncan’s multiple range tests were used.

3. Results and discussion

3.1. Preparation of antifungal coating dispersions of CEO-ChNPs, ChNPs, CEO and Ch
First of all, it is better to mention that all necessary analyses like DLS and FE-SEM for size and morphology determination of NPs, as well as FTIR, EE and LC for the confirmation of the successful encapsulation of CEO were carried out and all data have been included in our previous publication in Food Chemistry (doi: https://doi.org/10.1016/j.foodchem.2018.09.085). As presented in Fig. 4 of the previous publication, at 1.5 mg/mL, CEO-ChNPs, prepared using initial mass ratio of Ch to TPP to CEO of 1:1:1, could completely inhibit the in vitro growth of Aspergillus niger MF540907, isolated from spoiled pomegranate (Hasheminejad et al., 2019), therefore, the antifungal dispersions of CEO-ChNPs, ChNPs, CEO and Ch with the concentrations of 1.5 mg/mL were prepared for pomegranate aril coating in this research.

3.2. Microbial analysis
In both coated and uncoated arils, the total yeast and molds increased significantly (P < 0.05) during storage at 5 °C, however the total count in coated samples was significantly (P < 0.05) lower than control at the end of cold storage (Fig. 1(A)). Except the arils coated with ChNPs and CEO-ChNPs, the total yeast and molds increased significantly (P < 0.05) from day 0 to 12 in control and other coated arils (Fig. 1(A)). The incidence of fungal decay occurred in control arils at the day 18 of cold storage (Fig. 1(B)) however, it was delayed in the arils coated with Ch, CEO, ChNPs and CEO-ChNPs until day 30, 30, 42 and 60 of storage at cold, respectively (Fig. 1(B)). As seen in Figs. 1–6, microbial, physicochemical and sensory analyses were not carried out for the unusable samples with early signs of fungal decay. Considering the results, at the end of storage, yeast and molds population was significantly (P < 0.05) lower in the arils coated with CEO-ChNPs compared to the arils coated with other coating dispersions, therefore, the dispersion of CEO-ChNPs was the most effective coating for microbial shelf life extension of ready to eat pomegranate arils during 54-day
storage at cold (Fig. 1(A)). The superior performance of CEO-ChNPs (encapsulated CEO) over other coatings might be due to the controlled release of the preserved volatile oil from ChNPs during the experiment, leading to better inhibitory effect, as well as the inhibitory effect of ChNPs itself (Hasheminejad et al., 2019). EOs and their lipophilic phenolic compounds can alter the cell permeability of yeasts and fungi through interaction with ergosterol of cell membrane and its biosynthesis (de Lira Mota, de Oliveira Pereira, de Oliveira, Lima, & de Oliveira Lima, 2012). Previous reports have shown the fungicidal activity of CEO on several food-borne fungal species and Aspergillus niger. A cellular deformity, observed by SEM micrographs in Saccharomyces cerevisiae cells, is another example of the disruptive function of CEO on cytoplasmic membrane (Chaieb et al., 2007). Moreover, positively charged amino groups of Ch can interact with negatively charged residues of macromolecules present in fungal cells to alter the membrane permeability thus Ch can act as a barrier against pathogen infection on fruit surfaces through its film-forming and anti-fungal properties (Xing et al., 2011; Rocalasalbas et al., 2013). Recent study showed that the formulation of Ch into ChNPs can lead to a significant enhanced antifungal activity. Ultra-structural studies through SEM analysis on mycelium growth inhibition of Fusarium oxysporum showed that ChNPs can increase membrane permeability through inducing cell wall damage (Dananjaya et al., 2017). Improved microbial quality is in agreement with previous reports on grape fruit coated with the combination of Ch and bergamot EO (Sánchez-González et al., 2011), as well as, fresh-cut papaya treated with the polysaccharide-based multilayered antimicrobial coating made of Ch and pectin in combination with the complex of microencapsulated beta-cyclodextrin and trans-cinnamaldehyde (Brasil et al., 2012).

### 3.3. Weight loss (%)

The percentage of weight loss in both uncoated and coated pomegranate arils has been shown in Fig. 2 during their 54-day storage at 5 °C. The weight loss of both control and coated arils increased significantly (P < 0.05) during cold storage, however all coated treatments could significantly (P < 0.05) decrease the weight loss compared to the control at the end of their storage at 5 °C (Fig. 2). Fruit weight loss might be related to the moisture migration from the fruit surfaces and increased metabolic activities associated with fruit senescence and cell destruction as a result of higher respiration rate. The decrease in weight loss by surface coatings might be due to the

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**Fig. 1.** Effect of Ch, CEO, ChNPs and CEO-ChNPs on (A) yeast and molds population of pomegranate arils from day 0 to 54 during storage at 5 °C (Results were reported as mean ± SD) and (B) delay in incidence of fungal decay during 60 days of storage at 5 °C.
formation of a gas and water vapor barrier, resulting in reduced metabolic activities and reduced moisture migration from the fruit surfaces in coated fruits (Sánchez-González et al., 2011; Brasil et al., 2012). The highest percentage of weight loss (14.34 ± 0.52%) was recorded in control arils after 12 days of storage while the lowest value (8.33 ± 0.52%) was recorded in the arils coated with CEO-ChNPs after 54 days (Fig. 2). According to the results, the incorporation of CEO into ChNPs could provide the most effective treatment (P < 0.05) on reducing the weight loss of pomegranate arils (Fig. 2) since the hydrophilic nature of EO could enhance the ability of polysaccharide-based coating to provide an efficient water vapor barrier against moisture migration. In addition, the possible interaction of EO compounds with charged Ch groups might increase the resistance of fruit surfaces to the gas permeability and thereby slowing down fruit respiration and reducing the metabolic activities associated with fruit senescence and cell destruction (Sánchez-González et al., 2011; Brasil et al., 2012). The reduced weight loss was in accordance with previous observations in pomegranate coated with the combination of putrescine and carnauba wax (Barman, Asrey, Pal, Kaur, & Jha, 2014), grape coated with Ch-bergamot EO (Sánchez-González et al., 2011), fresh-cut papaya treated with the multilayered anti-microbial coating (Brasil et al., 2012), pomegranate coated with Ch, resin and carnauba wax (Meighani et al., 2015), as well as, fresh-cut lettuce coated with CEO (Chen et al., 2017).

3.4. Chemical analysis

3.4.1. TSS, TA and pH

Chemical quality parameters of coated and uncoated pomegranate arils (TSS, TA and pH) have been shown in Fig. 3(A)-(C) from day 0 to 54 during storage at 5 °C. As seen in Fig. 3(A) and (B), except the arils coated with CEO-ChNPs, all the other coated and uncoated arils showed a significant (P < 0.05) decrease in their TSS and TA amounts during storage at 5 °C. The observed decrease in TSS and TA values could be a result of sugar degradation and consumption of organic acids as the main respiratory substrates during fruit storage (Fawole & Opara, 2013; Sayyari et al., 2011). Based on the results, the arils coated with CEO-ChNPs could significantly (P < 0.05) maintain higher amounts of TSS and TA compared to the control arils at the end of their storage at 5 °C (Fig. 3(A) and (B)). The lowest levels of TSS (16.00 ± 0.14) and TA (0.63 ± 0.02) were recorded in control arils after 12 days of cold storage while the highest levels of TSS (17 ± 0.14) and TA (0.83 ± 0.02) were recorded in the arils coated with CEO-ChNPs after 54 days of storage at 5 °C, therefore, the dispersion of CEO-ChNPs was the most effective coating (Fig. 3(A) and (B)). In the other words, the enrichment of ChNPs with CEO could maintain (P < 0.05) higher amounts of TSS and TA through creating a modified internal atmosphere and delaying fruit respiration, along with the possible effect of EO on fruit metabolic reactions (Brasil et al., 2012). The main role of TSS and TA can be related to the fruit flavour, hence the delay in reducing the amounts of TSS and TA seems to be strongly linked to the fulfillment of consumer expectations (Fawole & Opara, 2013; Song et al., 2016). The higher amounts of both TSS and TA compared to the control have been previously reported on coated fresh-cut papaya (Brasil et al., 2012), pomegranate fruit (Meighani et al., 2015) and also loquat fruit (Song et al., 2016) during storage at low temperatures. The juice pH with the value of 3.59 ± 0.01 at the day 0 significantly (P < 0.05) increased in both control and coated arils and reached to the maximum value of 3.70 ± 0.005 in control at day 12 and minimum value of 3.64 ± 0.005 in pomegranate arils coated with CEO-ChNPs at day 54 during cold storage (Fig. 3(C)). The results showed that although there was a significant (P < 0.05) difference among control, ChNPs and CEO-ChNPs treatments in lowering the increase of pH at the end of storage, the most effective coating (P < 0.05) was CEO-ChNPs (Fig. 3(C)). The pH changes might be associated with the effect of applied treatment on the rate of respiration and metabolic activities of the fruit (Jitareerat, Paumchai, Kanlayanarat, & Sangchote, 2007). Lowering the increase of pH by CEO-ChNPs might be related to the lower respiration rate and as a result, lower metabolic activities leading to fruit senescence and cell destruction. Moreover, loading of CEO into ChNPs could probably modify fruit respiration pattern through possible interaction of EO compounds with cell membranes affecting on fruit metabolic pattern and senescence (Sánchez-González et al., 2011; Perdones et al., 2012). The lower pH changes has been previously reported on strawberries coated with Ch-lemon EO (Perdones et al., 2012), as well as, pomegranates coated with Ch, resin and carnauba wax (Meighani et al., 2015).

3.4.2. TPC evaluation

According to the results, at the end of cold storage, TPC decrease was observed in control and coated pomegranate arils, however this
Fig. 3. Changes of chemical quality parameters: (A) TSS, (B) TA and (C) pH in uncoated and coated pomegranate arils from day 0 to 54 during storage at 5 °C. Results were reported as mean ± SD.
decrease was not significant \((P < 0.05)\) in the arils coated with CEO-ChNPs (Fig. 4(A)). The lowest TPC amount \((2046.41 \pm 121.42 \text{ mg GAE/L})\) was recorded in control arils after 12 days of cold storage, while the highest amount \((2381.05 \pm 110.63 \text{ mg GAE/L})\) was measured in pomegranate arils coated with CEO-ChNPs after 54 days of storage at \(5^\circ\text{C}\) (Fig. 4(A)). The loss of TPC might be due to the higher rate of respiration and consequently higher degradation of certain phenolic compounds (Ali, Maqbool, Alderson, & Zahid, 2013). The degradation of these compounds due to the activity of the browning enzymes such as polyphenol oxidase (PPO) and phenolalanine ammonia-lyase (PAL) can lead to the enzymatic browning. PPO is a copper \((\text{Cu}^{+2})\) containing enzyme involved in oxidizing phenolic compounds into quinones which eventually constitute brown pigments while PAL is involved in the biosynthesis of phenolic substrates for PPO (Chen et al., 2017). The results showed that the dispersion of CEO-ChNPs was the most effective coating in preventing \((P < 0.05)\) the loss of TPC in the arils coated with this dispersion (Fig. 4(A)). Preventing the loss of TPC might be related to the inhibition of PPO and PAL enzymes by Ch and CEO (Lai, Yang, Chen, & Hsiao, 2006; Chen et al., 2017). Recent study has shown that CEO and eugenol can inhibit enzymatic browning effectively in fresh-cut vegetables. Eugenol can interact with the active site of PPO and PAL, the substrate binding site of these enzymes, through its aromatic ring in a competitive manner (Chen et al., 2017). The activity of these browning enzymes can also be inhibited by Ch through removing the metal ions present in their active sites (Lai et al., 2006). Recent study on jujube fruit has shown that the highest amount of TPC has been found in fruits coated with Ch-cinnamon EO and then cinnamon EO followed by Ch coated fruits (Xing et al., 2015). The higher amounts of TPC compared to the control has been previously found in tomato fruits coated with gum arabic (Ali et al., 2013) and aloe gel (Mirdehghan & Valero, 2017), as well as strawberries coated with 0.5% Ch (Wang & Gao, 2013).

### 3.4.3. TAC evaluation

Different levels of anthocyanins have been reported for several Iranian cultivars. The amount of anthocyanins found in the present study was in the range reported for TAC amounts by previous researchers (Mousavinejad, Emam-Djomeh, Rezaei, & Khodaparast, 2009; Varasteh et al., 2012; Ghasemnezhad et al., 2015). The development of internal cell destruction and aril browning or pigment degradation are serious problems during pomegranate storage at cold since anthocyanins are the antioxidant components and the key color of pomegranate...
phenolics such as ellagic acid derivatives and polyphenols such as flavonoids and anthocyanins as a result of senescence and decay (Fawole & Opara, 2013; Mohammadi et al., 2016). These bioactive compounds are responsible for AA of pomegranate fruit, however anthocyanins could play a principle role in exhibiting AA (Fawole & Opara, 2013; Ghaseemnejad et al., 2015). Delay in reducing AA values of pomegranate arils coated with CEO-ChNPs could be related to the delay in the biochemical and physiological changes of fruit during storage or delay in ripening process of fruit and consequently delay in the loss of anthocyanins, flavonoids, individual phenolics and polyphenols by this coating treatment (Ali et al., 2013; Meighani et al., 2015). In addition, delay in AA reduction of these arils might be related to the possible induction of antioxidant enzymes and consequently the decrease in cell destruction by this coating, as mentioned earlier (Song et al., 2016). Previous studies on Iranian cultivars of pomegranate fruit have shown a positive correlation between the total phenolic content and the AA of pomegranate fruit (Mousavinejad et al., 2009; Fawole & Opara, 2013). The study on sweet pepper has shown that the enrichment of Ch coating with cinnamon EO can increase the activity of scavenger antioxidant enzymes and subsequently decrease the permeability of cell membranes. Thus, the higher amounts of AA are maintained by this combination coating compared to the Ch or pure cinnamon EO (Xing et al., 2011). The higher values of AA have been previously observed in tomato fruit coated with gum arabic (Ali et al., 2013), cold-stored pomegranate fruit coated with Ch, resin and carnauba wax (Meighani et al., 2015), as well as, strawberry fruit coated with 0.5% Ch (Wang & Gao, 2013).

3.5. Sensory analysis

The evaluation of sensory key attributes such as flavour, colour and texture, also sensory acceptability showed no significant (P < 0.05) differences among control and coated arils at the beginning day; this indicated that there were no undesirable changes as a result of the coating application (Fig. 6(A)–(D)). Although, the mean scores of sensory key attributes (flavour, colour and texture), also sensory acceptability decreased significantly (P < 0.05) in both coated and uncoated arils at the end of cold storage, they were significantly (P < 0.05) higher in coated arils compared to control arils (Fig. 6(A)–(D)). The highest scores (> 5) belonged to the arils coated with CEO-ChNPs at

Fig. 5. Changes of AA (%DPPHsc) in uncoated and coated pomegranate arils from day 0 to 54 during storage at 5 °C. Results were reported as mean ± SD.

(Barman et al., 2014). As shown in Fig. 4(B), at the end of storage at 5 °C, TAC amounts decreased in both coated and uncoated arils, however the decrease was not significant (P < 0.05) in those coated with CEO-ChNPs. TAC reduction in minimally processed pomegranate arils could be a result of damage to pomegranate arils during peeling which can cause juice leakage through wounding of arils, as well as, oxidative processes during senescence (Ghasemnejad et al., 2015). The results showed that compared to the control, CEO-ChNPs significantly (P < 0.05) maintained higher TAC amount (935.76 ± 19.93 mg/L) during 54-day storage at cold (Fig. 4(B)); therefore, this coating dispersion could decrease (P < 0.05) anthocyanin degradation and subsequently maintained higher quality and marketability of the arils. Preventing the loss of TAC might be associated with the reduction in oxygen supply by this coating and consequently lower enzymatic oxidation and higher retention of anthocyanins through creating a protective barrier to delay ripening and senescence of the arils (Barman et al., 2014; Varasteh et al., 2012; Wang & Gao, 2013; Song et al., 2016). On the other hand, this coating could probably induce higher activity of antioxidant enzymes in the arils which can lead to a reduction in the production of reactive oxygen species (ROS) such as O$_2^−$ and H$_2$O$_2$. Reduced production of oxyradicals can subsequently lower the activity of lipooxygenase enzyme, involved in cell membrane destruction. Decreased activity of this enzyme can result in lower cell destruction (Song et al., 2016). Wang and Gao (2013) showed that the application of Ch coating on strawberry can maintain higher amounts of TPC, anthocyanins and AA in this fruit and prevent its quality deterioration through inhibition of browning enzymes and induction of antioxidant enzymes. The results of this study are in agreement with previous reports on the cold-stored pomegranates coated with putrescine-carnauba wax (Barman et al., 2014), Ch (Varasteh et al., 2012), as well as, Ch, resin and carnauba wax (Meighani et al., 2015).

3.4.4. AA evaluation

Fig. 5 shows that at the end of cold storage, compared to the control and other coated pomegranate arils, the decrease in AA values was delayed significantly (P < 0.05) in arils coated with CEO-ChNPs. Based on the results, the highest AA value (30.58 ± 1.30 %DPPHsc) was recorded in pomegranate arils coated with CEO-ChNPs after 54 days while the lowest value (23.86 ± 1.09 %DPPHsc) was observed in control arils after 12 days of storage at 5 °C (Fig. 5). The decrease in AA of pomegranate arils might be due to the reduction in individual phenolics such as ellagic acid derivatives and polyphenols such as hydrolysable tannins, flavonoids and anthocyanins as a result of senescence and decay (Fawole & Opara, 2013; Mohammadi et al., 2016).
of pomegranate arils. Recent study on sweet pepper has shown that the combination of Ch and cinnamon EO is a more successful coating than Ch or pure EO in delaying the incidence of fungal decay and subsequently maintaining the sensory quality due to the synergistic effect between Ch and EO (Xing et al., 2011). The higher scores for sensory analysis were also reported for fresh-cut papaya treated with polysaccharide-based multilayered antimicrobial coating (Brasil et al., 2012) and pomegranate fruit coated with putrescine-carnauba wax (Barman et al., 2014).

4. Conclusions

According to the results of this study, the microbial, physicochemical and sensory qualities of minimally processed (ready to eat) pomegranate arils could be affected by tested coating dispersions including Ch, CEO, ChNPs and CEO-ChNPs. The incidence of fungal decay occurred at the day 18 of storage at 5 °C in uncoated pomegranate arils while it was delayed in the arils coated with Ch, CEO, ChNPs and CEO-ChNPs until day 30, 30, 42 and 60 of storage at cold, respectively. At the end of cold storage, the coating dispersion of CEO-ChNPs could significantly (P < 0.05) maintain microbial quality, weight, TSS, TA, pH, TPC, TAC, AA and sensory quality in pomegranate arils coated with these NPs compared to the uncoated arils while only some of these quality parameters maintained significantly (P < 0.05) in the arils coated with other coating dispersions. Among tested coating dispersions, the dispersion of CEO-ChNPs was the most effective coating which extended the shelf life of pomegranate arils for 54 days and protected them against undesirable microbial, physicochemical and sensory changes. The superior performance of CEO-ChNPs (encapsulated CEO) in extending the microbial shelf life of minimally processed pomegranate arils might be due to the controlled release of the preserved volatile oil from ChNPs during the experiment, leading to better inhibitory effect, as well as, the inhibitory effect of ChNPs itself. Considering these results, CEO-ChNPs can be considered as a promising preservative coating for extending the shelf life of fresh-cut fruits and vegetables.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References


