Nano-ZnO/carboxymethyl cellulose-based active coating impact on ready-to-use pomegranate during cold storage

Mahmoud Koushesh Saba *, Rasoul Amini
Department of Horticultural Science, University of Kurdistan, P.O. Box 416, Sanandaj, Iran

A R T I C L E   I N F O
Article history:
Received 27 November 2016
Received in revised form 12 April 2017
Accepted 12 April 2017
Available online 13 April 2017

Keywords:
Edible coating
Microbial load
Fruit weight loss
Minimally processed

A B S T R A C T
Minimally processed pomegranate rapidly loses its overall quality because of high water loss and microbial contamination. Nano-ZnO in combination with carboxymethyl cellulose (CMC) coating was used on pomegranate arils. Arils were dipped for 4 min in distilled water (control), 0.1 or 0.2% (w/v) nano-ZnO suspension and then ZnO treated arils were coated with 0.5% (w/v) CMC and stored for 12 days at 4 °C. Coatings decreased total yeast + mold during 12 days of storage while total mesophilic bacteria was decreased during 6 days of storage. Coatings decreased weight loss and also the greatest juice percent was in coated arils. Soluble solids content decreased during storage with no significant difference between treatments. CMC + 0.2% nano-ZnO suppressed total phenol changes. Total anthocyanin, vitamin C, and antioxidant capacity were higher in coated arils. These findings suggest that nano-ZnO + CMC coating has the potential to extend minimally processed pomegranate storage life.

© 2017 Elsevier Ltd. All rights reserved.

1. Introduction

Pomegranate ( Punica granatum L.) is a highly valuable fruit due to its special sensory, nutrition properties, and bioactive constituents in term of phenolic compounds, anthocyanin content, and antioxidant activity. Pomegranate is also used in a number of systems of medicine for a variety of ailments (Howell & D’Souza, 2013; Shuid & Mohamed, 2013). Several studies have been focused on anti-carcinogenic, anti-inflammatory and antioxidant properties of pomegranate constituents during last decades (Jurenka, 2008; Medjakovic & Jungbauer, 2013). However, pomegranate consumption, especially in public area is limited because of difficulties in peeling to extract the arils, which lead its consumption to be a time consuming process (O’Grady, Sigge, Caleb, & Opara, 2014a), and phenolic metabolites irritates, and stain the fingers (Caleb, Opara, & Witthuhn, 2012).

‘Ready-to-use’ or minimally processed form can be a possible alternative method to encourage pomegranate consumption (Gil, Martinez, & Artés, 1996). Also, recently there has been an increasing market demand for minimally processed fruit and vegetables. However, minimally processed pomegranate become more perishable owing to processing results in quality deterioration associated with water loss and microbial contamination (Caleb et al., 2013; Ghasemnezhad, Zareh, Rassa, & Sajedi, 2012; Hussein, Caleb, Jacobs, Manley, & Opara, 2015; López-Rubira, Conesa, Allende, & Artés, 2005). Therefore, it is important to find out methods to keep aril quality in a satisfactory level to meet consumer demands.

Storage temperature management (O’Grady et al., 2014a), modified atmosphere packaging (Caleb et al., 2013; Caleb et al., 2012), perforated modified atmosphere packaging (Hussein et al., 2015), and coating treatments (Ghasemnezhad et al., 2012) have been used to reduce the deleterious effects brought about by minimal processing and extend pomegranate arils storage life. Coating provides a barrier that reduces gas exchange, respiration, moisture, and solute migration (Rojas-Grau, Soliva-Fortuny, & Martín-Belloso, 2009). One such product is the carboxymethyl cellulose (CMC). CMC is a linear, long-chain, water-soluble, and an anionic polysaccharide that can be used as a fruit coating material. CMC coating could successfully maintain quality of fruit and extend apple fresh-cut (Koushesh Saba & Sogvar, 2016), pear (Hussain, Meena, Dar, & Wani, 2010), and strawberry (Gol, Patel, & Rao, 2013) shelf life. CMC solely has no anti-microbial properties but can also serve as carriers of food additives (Koushesh Saba & Sogvar, 2016) and also antimicrobial agents. The combination of anti-microbial compound with edible coatings is emerging as a promising new technology (Santiago-Silva et al., 2009) that has a potential to reduce perishable crop losses.

Zinc oxide nanoparticles (nano-ZnO), as one of the multifunctional inorganic nanoparticles, are known to inhibit microbial growth (Jones, Ray, Ranjit, & Manna, 2008; Sogvar, Koushesh Saba, Emamifar, & Hallaj, 2016). Since Zinc oxide has strong
antimicrobial effect (Jones et al., 2008), it widely used in drug industry, medicinal devices, and cosmetics (Yan, Salley, & Ng, 2009). Furthermore, it has been listed as GRAS by U.S FDA and has been used in fortification of cereal-based foods (21CFR182.8991; Xie, He, Irwin, Jin, & Shi, 2011), although there is still some reservation regarding its use as a food additive. Current advances in nanotechnology provide opportunities to create new products with a vast range of applications including nanomedicine and compounds with antimicrobial properties (Oberdorster, Oberdorster, & Oberdorster, 2005). The greater surface area per mass compared with larger-sized particles of the same chemistry renders nano-sized particles more active biologically.

Nano-ZnO recently added to coating films, active packaging, to improve postharvest life of processed or minimally processed products (Emamifar, Kadivar, Shahedi, & Soleimanian-Zad, 2010; Li et al., 2011) but rarely used directly on fruit (Sogvar et al., 2016). According the importance of minimally processed pomegranate as a unique product, current study was carried out to investigate the combined effect of nano-ZnO and CMC coating as an active coating on pomegranate aril quality during storage life.

2. Materials and methods

2.1. Fruit preparation

Pomegranates (Punica granatum L.), cv ‘Malas’ were harvested from commercial orchard located in Sarvabad rural district, Marivan city, west of Sanandaj in the Kurdistan province (35°13’36” N, 46°19’32” E), Iran. 80 fruit were harvested according to commercial practice when fully matured from about 20 trees on October 19, 2014 and immediately transported to laboratory on the same day with ventilated car and stored at 4 °C with 90% relative humidity before processing. Fruit were selected for uniformity in shape, color and free from any defects (sunburn, cracking and bruising) then, washed in tap water containing 0.02% NaClO for 5 min. Fruit husk were carefully cut at equatorial zone with a sterile sharp knife in a disinfected room. Arils were manually extracted (handlers wore gloves) and those arils with pale color were discarded. Undamaged same color arils were washed in sterile distilled water at 5 °C for 2 min. After rinsing, arils were held in sieve tray for about 30 min to remove excess water. About 450 g of arils were sampled, divided to 3 of 150 g as three replications, for immediate analysis to monitor fruit characteristics at harvest before application of treatments (day 0). Remaining arils were randomly distributed into 3 groups and each treated as described below.

2.2. Aril coating

Nano-compound concentration and particle size could affect antimicrobial properties. Nano-ZnO antimicrobial activity has been tested in the range of 0.002 to 0.5% (Brayner et al., 2006; Reddy et al., 2007). In the current study, three different concentrations (0, 0.1 and 0.2% w/v) of nano-ZnO (30–100 nm) were used according previous research (Sogvar et al., 2016), suspension were prepared by ultrasonically assisted dispersing of ZnO nanoparticle in distilled water. Arils were dipped in nano-ZnO solutions or distilled water at 20 °C for 4 min. After rinsing, excess water was removed. Then ZnO treated arils were dipped in 0.5% (w/v) CMC prepared gel for 4 min according preliminary test. 0, 0.5 and 1% CMC used in preliminary test and 0.5% yielded the lowest weight loss (data not shown). All arils were air-dried at room temperature for 30 min. Then, arils of each group were randomly distributed into 12 polystyrene boxes, each box contain about 150 g, and stored at 4 °C with 90% relative humidity. It should be noted that the box lids were not completely airtight. Sampling of either treated or control was carried out at 3, 6, 9 and 12 days. Three boxes were sampled at each sampling times as three replications.

2.3. Microbiological evaluations

Ten grams arils were obtained aseptically, homogenized and diluted with sterile peptone water (0.1% w/v) to obtain the microbial count. Serial dilutions were performed in triplicate. Total aerobic mesophilic bacteria and total yeasts + molds counts were enumerated using the pour plate method on the plate count agar (PCA, Scharlau Chemie, S.A., Barcelona, Spain) and potato dextrose agar (PDA, Scharlau Chemie, S.A., Barcelona, Spain), respectively. Incubation for bacteria and yeasts + molds was performed at 30 and 25 °C, respectively for 2 days. Each test was performed in duplicate and results were expressed as colony-forming units (CFU) per g (Sogvar et al., 2016).

2.4. Fruit quality measurements

About 30 g aril juice was extracted using a garlic press. Obtained juice was centrifuged for 10 min at 5000g and clear juice was used to solubilize solids content (SSC), titratable acidity (TA), pH, vitamin C and anthocyanin measurements. SSC was measured using an Atago Digital Refractometer (Brix 0–32%, Atago, Japan). TA was measured by titrating 30 mL of aliquoted juice (5 mL juice + 25 mL distilled water) with 0.1 N NaOH to an end point of pH 8.2 and expressed as a percentage of malic acid (Sayyari et al., 2010). The pH of fruit juice was measured using a pH meter (Metrohm 827, Switzerland).

All polystyrene boxes contain arils weight was recorded on day of treatments and at each sampling time. Cumulative weight losses were calculated as the percentage of initial fresh weight. Fruit juice content was measured by obtained juice from 50 g arils using garlic press and expressed as juice percent (JP).

2.5. Vitamin C assay

Vitamin C content was determined by adding 2 mL TCA (0.5%) to 2 mL aril juice and titration with 2,6- dichlorophenolindophenol (DCPIP) (AOAC, 2000), using different AA concentrations for the standard curve, and expressed as mg of vitamin C per 100 mL of fruit juice.

2.6. Total anthocyanin and total phenolic (TP) concentrations

Total anthocyanin concentrations (TAC) were determined using the pH differential method as described previously (Sogvar et al., 2016). The absorbance was measured spectrophotometrically (UV-2100, New Jersey) at 510 and 700 nm in buffers at pH 1.0 and 4.5 and the difference between the buffer systems was calculated using $A = [(A_{510} - A_{700}) \text{ pH1.0} - (A_{510} - A_{700}) \text{ pH4.5}]$. The results were expressed as mg of cyanidin-3 glucoside equivalents per 100 mL of fruit juice (mg 100 mL-1 fruit juice).

After weight loss evaluation, about 40 g aril per replicate were removed, immediately frozen in liquid nitrogen, ground (IKAG- Werke GmbH & Co. KG, A 11, Germany) and stored at 80 °C until used for extraction and analysis of the TP concentration and antioxidant activity. TP concentrations were measured by homogenizing 1 g of frozen arils powder from each replicate with 3 mL ice cold 1% HCl– methanol solution and then centrifuged at 4 °C for 15 min at 12,000 g. The supernatant was collected and used for phenol and antioxidant determination. TP concentration in the extracts was determined according to the Folin–Ciocalteu procedure (Singleton, Orthofer, & Lamuela-Raventos, 1999), using gallic
acid as the standard curve. Results were expressed as mg of gallic acid per 100g of fruit fresh weight.

2.7. Total antioxidant activity (TAA)

Antioxidant activity was measured by the 2,2-diphenyl-1-picryl-hidrazil (DPPH) radical-scavenging method (Sanchez-Moreno, Larrauri, & Saura-Calixto, 1999). For assay, 40 µL above described methanol extract was added to 960 µL of DPPH solution, and then the mixture was vortexed and allowed to stand at room temperature in darkness. The absorbance was measured at 517 nm, using a spectrophotometer (UV-2100) after 15 min. Total antioxidant activity was expressed as the percentage inhibition of the DPPH radical and was determined using the following equation: TAA = \[\frac{(Abs_{control} - Abs_{sample})}{Abs_{control}} \times 100.\]

2.8. Statistical analysis

Data were subjected to analysis of variance (ANOVA) with SAS 8.0 software (SAS Institute Inc., Cary, NC). Sources of variation were storage life (days) and treatments. Mean values were calculated and reported as the mean ± standard error (n = 3). The least significant difference (LSD) test at P = 0.05 was used to compare means among treatments.

3. Results and discussion

3.1. Microbiological analysis

Total yeast + mold and total aerobic mesophilic bacteria counts were 1.81 and 1.98 Log CFU g⁻¹, respectively on day 0 and increased during pomegranate arils storage. Fig. 1 shows a decrease in yeasts + molds after 6 and 12 days and an increase in bacterial load after 12 days, in coated arils. Coating + 0.2% nano-ZnO was more effective to decrease yeast and mold than 0.1% nano-ZnO. There was significant differences in bacterial load of coated arils. Coating + 0.2% nano-ZnO decreased yeast + molds after 6 and 12 days and an increase in bacterial load after 12 days, in coated arils. Coating + 0.2% nano-ZnO was more effective to decrease yeast and mold than 0.1% nano-ZnO. There was significant differences in bacterial load of coated arils. Coating + 0.2% nano-ZnO was more effective to decrease yeast and mold than 0.1% nano-ZnO. There was significant differences in bacterial load of coated arils.

Microbial counts observed in this study was lower than the previous report (Hussein et al., 2015), that may be attributed to chemical constituent difference between pomegranate cultivars or sanitary condition during arils processing. Microbial population significantly increased on pomegranate arils during storage that was in agreement with previous report (Hussein et al., 2015). Total mesophilic bacteria population was higher than yeast and mold, that these findings were in contrast with previous reports (Caleb et al., 2013; Hussein et al., 2015).

In the current study coatings affected microbial counts similar to chitosan coating (Ghasemnezhad et al., 2012). In consistent with these findings, nano-ZnO treatment reduced microbial growth on strawberry (Sogvar et al., 2016). Nano-ZnO + coating were more effective on yeast + mold than that of total aerobic mesophilic bacteria. Also, it has been observed that zinc treatment completely inhibited or reduced mycelia growth of diverse species of fungi while decreased or increased bacteria survival depended to species. (Babich & Stotzky, 1978). In contrast, Sogvar et al. (2016) reported that nano-ZnO inhibitory impact on total aerobic mesophilic bacteria was greater than total yeast + molds during strawberry fruit storage.

The mechanism at which how nano-ZnO affected microbial load is not still fully understood although few mechanisms has been proposed according literature (Sogvar et al., 2016) and the core findings are reactive oxygen species generation (Opara, Atukuri, & Fawole, 2015; Sawai & Yoshikawa, 2004), and membrane disorganization follow by ZnO accumulation in bacteria membrane (Brayner et al., 2006; Opara et al., 2015; Xie et al., 2011). Nano-ZnO incorporation to bacteria affects cell membrane permeability and induced oxidative stress by generating hydrogen peroxide (H₂O₂), OH⁻ or O₂⁻ (Jones et al., 2008; Xie et al., 2011) which might be resulting in the inhibition of microbial cell growth and eventually in cell death (Xie et al., 2011). Whereas the nano-ZnO toxicity for microbial cells has been observed, it’s adverse effects on human health have not been reported yet.

3.2. Fruit quality (SSC, TA, pH, WL and JP)

SSC decreased during aril storage regardless of treatments. It was in agreement with those reported in arils stored in modified atmosphere packaging (MAP) (Ayhan & Eştürk, 2009). TA decreased by first 3 days of storage while the greatest decrease was in control. TA increased thereafter and there was no difference between either coated or uncoated arils at last sampling time. TA changes in pomegranate arils varied in different cultivars and storage temperature (Caleb et al., 2013; O’Grady et al., 2014a). In the current study coatings decreased TA changes. Fruit pH changes were not significant during storage (Table 1).

Weight loss (WL) substantially increased during first three storage days and as Fig. 2A shows the weight loss was greater in control compared to the treated fruit. The least WL was recorded in CMC + 0.2% ZnO at 9 days of storage (Fig. 2A). Fruit juice percent changes were not significant during storage although the juice percent of coated aril were greater than uncoated at last sampling time (Fig. 2B). The amount of water lost by the arils was most likely
not sufficient to cause a difference in juice content between treatments.

WL is important because could affect both visual and nutritional quality of fruit. The first initial increase in WL could be attributed to moisture evaporation from pomegranate aril surface similar to those previously reported (Caleb et al., 2013; Gil et al., 1996). Edible coatings have been used extensively to conquer WL problem during fruit storage and in agreement with others findings, in the current study coating treatment could reduce WL in pomegranate arils. Nano-ZnO as an additive to CMC enhanced the coating effectiveness. These ZnO effects might describe in tow way: 1) ZnO by itself, enhanced the barrier attribute of coating and reduced moisture diffusion of fruit to surrounding atmosphere as reported in strawberry (Sogvar et al., 2016) and kiwifruit (Meng, Zhang, & Adhikari, 2014), and 2) ZnO decreased microbial load and fruit physiological activity that are related to fruit deterioration and weight loss.

3.3. Vitamin C

Vitamin C content was increased at first 3 storage days but gradually decreased thereafter. There was significant difference among treatments and control at last sampling time, with the greatest and least vitamin C was in 0.5% CMC + 0.1% ZnO and control arils, respectively (Fig. 3A).

In consistent with our findings, an increase in vitamin C was observed at day 7 of storage while it declined across aril storage (O’Grady, Sigge, Caleb, & Opara, 2014b). Ascorbic acid is a very complex molecule and the activity of ascorbate oxidase (Agar, Streif, & Bangerth, 1997), phenolaze, light, temperature, and oxygen affect its stability. Those enzymes induced the oxidation of ascorbic acid to dehydro ascorbic acid and are responsible for vitamin C losses. Fruit coating could reduce endogenous oxygen and thus delay oxidative reaction. Furthermore, it has been reported a relation between fruit weight loss and ascorbic acid oxidation (Maftoonazad & Ramaswamy, 2005; Nunes, Brecht, Morais, & Sargent, 2006). In the current study, coating reduced aril weight loss and it may attribute to the ascorbic acid decline.

3.4. TAC and TP

Cyanidin, plargonidin and delphinidin are the major anthocyanins in pomegranate juice and formed its color and provide beneficial effects for human health (Varasteh, Arzani, Barzegar, & Zamani, 2012). TAC increased in all samples during first 3 days of storage and then gradually decreased. TAC was greater in coated arils than control. TAC of 0.5% CMC + 0.2% ZnO was higher than 0.5% CMC + 0.1% ZnO at last sampling time (Fig. 3B).

A similar trend has been reported for pomegranate arils during storage (Ghasemnezhad et al., 2012). The TAC decline might be related to its oxidation (O’Grady et al., 2014a) because, a 65% anthocyanins reduction has been reported in high temperature and in the presence of oxygen while it was relatively heat stable in the absence of oxygen. Coatings provide an oxygen barrier (Bourtoom, 2008) thus might suppress TAC reduction via endogenous gas modification.

The highest TP level was at harvest and decreased during storage but the rate of decrease was greater in uncoated than that of coated arils. TP changes in 0.5% CMC + 0.2% ZnO was less than 0.5% CMC + 0.1% ZnO at last sampling time (Fig. 3B).

A trend has been reported for pomegranate arils during storage (Ghasemnezhad et al., 2012). The TAC decline might be related to its oxidation (O’Grady et al., 2014a) because, a 65% anthocyanins reduction has been reported in high temperature and in the presence of oxygen while it was relatively heat stable in the absence of oxygen. Coatings provide an oxygen barrier (Bourtoom, 2008) thus might suppress TAC reduction via endogenous gas modification.

The highest TP level was at harvest and decreased during storage but the rate of decrease was greater in uncoated than that of coated arils. TP changes in 0.5% CMC + 0.2% ZnO was less than the others (Fig. 3C).

Generally, TP of fruits and vegetables may either increase or decrease depending on the crop or storage conditions. The TP decline (Fig. 3C) is similar to those previously reported in pomegranate arils (Ghasemnezhad et al., 2012). TP reduction in pomegranate arils might be related to oxidation or polymerization of phenolic compounds similar to those reported in apples or quince.
Coating in combination with nano-ZnO provides a protective barrier on the arils surface thus might reduce the oxygen supply for enzymatic oxidation of phenolic compounds and also decrease microbial population that could accelerate arils senescence and decay.

3.5. TAA

TAA increased in coated arils at first 3 storage days and was almost stable thereafter. TAA of uncoated arils gradually increased at 9 days of storage and was almost stable thereafter. TAA in 0.5% CMC + either 0.1 or 0.2% ZnO was greater than control across the storage (Fig. 3D).
Antioxidant capacity of ‘Tarom’ pomegranate decreased (Ghasemnezhad et al., 2012), while that of ‘Mollar of Elche’ pomegranate was stable (López-Rubira et al., 2005) during storage. In the current study the antioxidant capacity increased at first 3 days and it might be related to TAC or in partially to vitamin C increments at same time. Minimally processed fruit coating were effective to maintain or reduce antioxidant changes during storage (Koushesh Saba & Sogvar, 2016; Oms-Oliu, Soliva-Fortuny, & Martin-Belloso, 2008). Our findings similarly showed antioxidant capacity were higher in coatings, also the greatest value was in higher nano-ZnO treatment similar to those has been observed in strawberry (Sogvar et al., 2016).

4. Conclusion

CMC as a coating is a white to cream colored, odorless and tasteless. Nano-ZnO reduced microbial load on ready-to-use pomegranate arils. The combination of nano-ZnO with CMC reduced weight loss and bioactive constituent decline during aril storage. The present study findings suggest that nano-ZnO combination with CMC may be a useful method for maintaining reedy-to-use pomegranate quality, extending its shelf life and promoting its greater consumption.

References


