Shelf Life Quality of Plum Fruits (Prunus domestica L.) Improves with Carboxymethylcellulose-based Edible Coating

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Abstract. This study investigated the carboxymethylcellulose (CMC)-based edible coating effects on some quality parameters and enzyme activities of plum fruits (Prunus domestica L. cv. Golden drop) during their shelf life. Three concentrations of CMC (0.5%, 1%, and 1.5%), plasticized with glycerol (0.3% w/v), were applied to plum fruits plus a control treated with only distilled water. The results demonstrate that the CMC-based edible coating was significantly effective in maintaining firmness and titratable acidity (TA); vitamin C, anthocyanin, and flavonoid content; and the antioxidant capability of plum fruits. Enzymatic activity was affected significantly by the coating. Peroxidase (POD) activity increased, and polyphenol oxidase (PPO) and polygalacturonoase (PG) decreased. In general, the formulation consisting of 1% CMC showed the best results in most of the measured parameters. Taking into account the positive effects on qualitative and biochemical characteristics of CMC-based edible coatings on plums, their application can be a potentially promising method to enhance the shelf-life of this fruit.

Postharvest Biology and Technology

Plums (Prunus domestica L.) are a good source of antioxidants, anthocyanin, phenolic compounds, nutritional elements, and some vitamins (Cevallos-Casals et al., 2006) that may benefit human health (Gil et al., 2002; Wargovich, 2000), but plums have a short postharvest life. Plums are highly perishable and their quality deteriorates rapidly after harvest, with the fruit not often reaching consumers at its optimal quality stage after extensive transport and marketing (Abdi et al., 1997; Crisosto et al., 2004; Eum et al., 2009; Hussain et al., 2015).

Enzymes, essential biocatalysts in the physiology and metabolism of plants, remain active postharvest, which may be desirable or undesirable in some cases and may lead to changes in quality attributes such as texture and nutritional value (Terefe et al., 2014). One of these enzymes is PG, a cell wall-bound enzyme that causes the cleavage of bonds between two galacturonic acid residues in pectin, resulting in pectin depolymerization, which leads to degradation of textural quality and softening, and thus has great importance in postharvest senescence (Terefe et al., 2014). PPO, another postharvest active enzyme, causes loss of nutritional value by acting on phenols (phenol oxidation) (Terefe et al., 2014) as well as vitamin C, flavonoids (Zhou et al., 2008), and anthocyanin degradations (Jiang et al., 2005). On the other hand, PODs control the level of peroxides generated in oxygenation reactions to avoid excessive formation of radicals harmful to all living organisms (Terefe et al., 2014).

Of late, providing safer methods for fruit maintenance with no side effects on human and animal health, and no negative effects on the environment is a high priority (Panahirad et al., 2014). Innocuous strategies including edible films and coatings could improve fruit shelf-life (Bourtoom, 2008; Mu et al., 2012). There is growing interest in the use of coatings based on different natural-origin compounds such as lipids, proteins, and polysaccharides (Bourtoom, 2008; Dhall, 2013; Shit and Shah, 2014; Tanada-Palmu et al., 2004; Shit and Shah, 2014). Nowadays, edible coatings are applied successfully as multipurpose materials in postharvest-related areas for improving appearance, reducing water loss and respiration, delaying ripening, increasing firmness, and so on (Bifani et al., 2007; Zhou et al., 2008).

CMC, a derivative of cellulose, is an anionic linear and long-chain compound with a high molecular weight (Bifani et al., 2007; Tongdeesoontorn et al., 2011). Natural coatings based on CMC are generally nontoxic, nonallergenic, biodegradable, odorless, and tasteless. These flexible and transparent bio-coatings are resistant to oils and fats. However, they are water soluble and are moderately permeable to moisture, oxygen, and carbon dioxide (Bourtoom, 2008; Dhall, 2013; Jafarizadeh-Malmiri et al., 2011; Nie et al., 2004). Interestingly, CMC coatings act as antisenescent and antifungal ingredients and delay ripening (Hussain et al., 2016), which preserves original fruit firmness (Vyas et al., 2014). The coatings are also applied to harvested fruit as a barrier against gas exchange between fruit and the environment, and thus are used successfully for adjusting oxygen and water transfer (Shit and Shah, 2014). In short, CMC is a good choice for maintaining fruit quality and extending shelf-life (Hussain et al., 2010, 2016) because it is commercially available and inexpensive (Hussain et al., 2010; Lim et al., 2011). CMC-based edible coatings have been used on fruit such as apples, mandarins, fresh-cut mangos, pears, sweet cherries, and papayas (Arnon et al., 2015; Hussain et al., 2010; Lim et al., 2011; Moldão-Martins et al., 2003; Plotto et al., 2004; Togrul and Arslan, 2004; Vyas et al., 2014); and in combination with irradiation in plums (Hussain et al., 2015), peaches (Hussain et al., 2016), and fresh-cut mangos (Salinas-Roca et al., 2018).

This research reports, for the first time, how the application of a CMC-based edible coating could improve qualitative and biochemical (especially enzymatic) properties of plum shelf-life.

Materials and Methods

Plant materials

Plums (Prunus domestica cv. Golden drop) were collected ripe, colored, intact (i.e., free of any wound and scar), and homogeneous in size and maturity from a commercial orchard in Shabestar city, northwest of Iran, at their commercial harvest stage [ripe, firm, and acceptable amount of total soluble solids (TSS)/TA]. The fruit were washed gently with distilled water, placed on paper towels to dry at room temperature, and subsequently coated.

Coating treatments

Experiments were performed using three concentrations of CMC (Sigma, USA) at 0.5%, 1%, and 1.5% in three replications (each replication consisted of 60 fruit, 12 fruit for each sampling period). Sampling was done every other day for 8 d. Solutions
were prepared by dissolving CMC in distilled water and stirring at 60 °C. After dissolving completely, 0.3% glycercol (w/v), as a plasticizer, was added and stirred. Plums were then dipped into the solutions for 60 s, air-dried at room temperature for 1 h, placed on open plastic grids, and then stored at 19 ± 2 °C and 65% relative humidity for 8 d. The noncoated fruit, treated using distilled water for 60 s, were used as the control. The concentration of CMC and type of plasticizer (glycercol) were chosen by preliminary experiments (data not shown) and based on the literature.

**Evaluation of fruit quality**

**Weight loss, firmness, TSS, pH, TA, and vitamin C.** Weight loss was calculated for each fruit unit (four fruit) as percentage loss of initial weight. Firmness was measured on both sides of each fruit, after peeling, using a manual penetrometer (Effegi, Italy) with an 8-mm plunger. To determine the TSS of fruit samples, a refractometer (PR-1; Atago Co., Ltd., Tokyo, Japan) with a scale of 0 to 32°Brix was used at 20 °C. pH was recorded with a pH meter (Hanna Instrument, Italy) and TA was measured by titration with 0.1 N NaOH up to pH 8.1 and expressed as grams of malic acid equivalent per 100 g fresh weight. The vitamin C content (measured in milligrams per 100 g) of the samples was determined using a titrimetric method based on the reduction of 2,6-dichlorophenolindophenol dye, as described by the Association of Official Analytical Chemists (2000). Three technical replicates were assessed for each measurement. The samples were homogenized before measuring TSS, TA, and vitamin C.

**Total phenolic compounds.** Folin-Ciocalteu reagent was used for determining total phenolics as described by Singleton and Rossi (1965). Briefly, after digesting 1 g of the fleshy part of the fruit with 2 mL 1% HCl-methanol (1 mL HCl in 99 mL methanol) and centrifuging (Hettich 320R, Germany) at 12,000 rpm for 10 min at 4 °C, the supernatant was collected and used to quantify the total phenolic compounds. For this purpose, to the 50-μL extract, 450 μL distilled water and 2.5 mL 10% Folin-Ciocalteu solution were added. After 10 min, 2 mL 7.5% sodium bicarbonate was again added, and after 1.5 h in the dark, the absorbance was recorded at 760 nm using a spectrophotometer (Spekol 1500, Germany). The absorbance values were converted to total phenolics and were expressed as milligrams of gallic acid per 100 g fresh weight. Different concentrations of gallic acid in 95% methanol were used as standards.

**Total anthocyanin content.** Peel and flesh (1 g) from five fruit were finely sliced and extracted with 2 mL 1% HCl-methanol. The extract was centrifuged and the absorbance was measured at 530 nm. Anthocyanin concentration was expressed as absorbance at 530 nm g⁻¹ fresh weight (Jiang et al., 2005). Total flavonoid content. Peel and flesh (1 g) from five fruit were extracted with 4 mL 96% ethanol. After centrifuging and collecting the supernatant, 700 μL 96% ethanol, 100 μL 10% aluminum chloride, 100 μL 1 M potassium acetate, and 2.8 mL distilled water were added to 1300 μL of the extract (supernatant). After 30 min at room temperature, the absorbance of the solution was measured at 415 nm vs. a blank. The results are expressed as milligrams of querceitin per 100 g fresh weight as recommended by Woisky and Salatino (1998), with some modifications.

**Total antioxidant activity.** The 1,1-Diphenyl-2-picryl-hydrazyl hydrate (DPPH) method was used to determine antioxidant activity (Shiri et al., 2013). Peel and flesh (1 g) from five fruit were cut and extracted with 2 mL 1% HCl- methanol and then centrifuged. An aliquot of the extract (100 μL) was added to 1.8 mL DPPH (0.1 N in methanol). Absorbance was measured at 517 nm after 15 min (vs. a blank, which contained 1 mL DPPH and 1 mL 1% HCl-methanol). The antioxidant activity was then calculated and the results are expressed as a percentage according to the following equation:

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\text{Total antioxidant activity (％)} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100.
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**POD activity.** Peel and flesh tissue (1 g) from five fruit were homogenized in 3 mL 0.1 M phosphate buffer (pH 7.0) and the supernatant after centrifugation (12,000 rpm, 15 min, 4 °C) was collected as a crude enzyme. POD activity was determined in a 2-mL reaction mixture containing 0.1 M phosphate buffer, guaiacol, extract, and H₂O₂. Oxidation of guaiacol was monitored by the increase of absorbance at 470 nm, as recommended by Armok et al. (2010), with modifications. Results are expressed as micromoles tetraguaiacol per minute per gram fresh weight.

**PPO activity.** Peel and flesh (1 g) from five fruit were homogenized in 3 mL 0.1 M phosphate buffer (pH 7.0) in an ice bath. The homogenate was centrifuged and then the supernatant collected as the crude enzyme extract. The assay of PPO activity was performed using 0.1 M phosphate buffer, 1 M 4-methylcatechol, and enzyme solution. The increase in absorbance at 420 nm was recorded automatically for 90 s, as recommended by Jiang et al. (2005) with modifications. Results are reported as micromoles oxidized catechol per minute per gram fresh weight.

**Statistical analysis**

After data collection, a generalized linear mixed model was used for analysis of variance, assuming subjects as random and time as repeated measures, using IBM SPSS software (version 21, SPSS Inc., Chicago, IL). Maximum likelihood-based estimated marginal means are reported, and adjustment for multiple comparisons was performed based on least significant difference at $P \leq 0.05$. Last, 95% confidence intervals are shown as error bars in the figures.

**Results and Discussion**

**TA, firmness, vitamin C, TSS, pH, and weight loss.** The analysis of the data obtained revealed a slight decrease in TA by the end of the storage time, with the maximum decrease at 1.5% CMC (Fig. 1A). The 1% CMC-based edible coating was the most effective ($\approx 1.544$ g/100 g) in TA maintenance of plums during their shelf life, and the 1.5% concentration was, surprisingly, the least effective ($\approx 1.361$ g/100 g) (Fig 1B). TA maintenance by coating could be the result of a delay in ripening and ripening.
maturity that reduces the metabolism involved in TA loss (Chiabrando and Giacalone, 2015; Eum et al., 2009). The carboxylic acid production resulting from fixation of CO₂ could be the main reason for the TA increase (Togrul and Arslan, 2004). So, TA enhancement at the 1% CMC-based edible coating in the current study could be explained by these reasons. Usually, TA decreases during the postharvest period because of degradation of organic acids during the respiration process throughout ripening and storage (Hussain et al., 2010; Togrul and Arslan, 2004). In addition, use of organic acids as the carbon skeleton for synthesis of new compounds during ripening (Hussain et al., 2010) is another reason that may explain the reduction of TA on the second day of storage, and the ineffectiveness of 0.5% CMC. Also, at greater concentrations of CMC, anaerobic respiration, which results in alcohol production resulting from diminishing in O₂ transfer into the fruit, could be the main reason for the TA decrease (Togrul and Arslan, 2004) that might describe the negative influence of the 1.5% concentration compared with control fruit.

As expected, the firmness parameter showed a reducing trend and greater differences were observed among the sampling times, with the maximum and minimum amounts at the end of the storage time in 1% CMC-based coated fruit and the control, respectively (Fig. 2A). The coating treatments were good in firmness preservation. The 1.5% CMC (≈9.13 N) and 1% (≈9 N) CMC-based edible coatings had a significant influence on fruit firmness preservation, and noticeable differences were also observed between 0.5% CMC (≈7 N) and the control (≈5.53 N) (Fig. 2B). The effectiveness of CMC coatings on maintaining fruit firmness could be the result of the existence of carboxylic groups in the chemical structure of CMC that causes hydrogen bonding inside the coating matrix and between the coating with the fruit peel, which in turn causes positive effects on firmness preservation (Arnon et al., 2015). Also, this positive effect is attributed to a reduction in the activity of some enzymes such as pectinmethylesterase, which itself is a result of a delay in ripening (Hussain et al., 2015) that in turn reduces soluble pectic fractions and retards conversion of insoluble pectins to soluble ones (Hussain et al., 2010, 2015). Polygalacturonase, pectin-methylesterase, 1,4-β-D-glucanase/glucosidase, and β-galactosidase are the main enzymes that cause softening in plums (Manganaris et al., 2008). Reduction of PG activity in our study by a CMC-based edible coating also indicates that this enzyme plays an important role in fruit softening. Activity of PG depends on respiration and ethylene production. Lack of O₂ delays the biosynthesis of ethylene and subsequent changes in fruit texture. Thus, edible coatings can improve fruit quality by restricting O₂ availability and modifying internal gas composition, decreasing oxidative metabolism and delaying textural changes in coated fruit (Chiabrando and Giacalone, 2015; Kowalczyk et al., 2017).

The vitamin C values reached a maximum at day 4 and then slowly declined. The 1% CMC and control had the greatest and least values at the end of maintenance, respectively (Fig. 3A). The vitamin C content was affected by 1% (≈8.8 mg/100 g), 1.5% (≈8.4 mg/100 g), and 0.5% (≈8.27 mg/100 g) CMC-based edible coatings compared with the control (≈6.87 mg/100 g) (Fig. 3B). Vitamin C amounts decrease postharvest, and this loss is the result of the antioxidant activity during postharvest storage (Hussain et al., 2015, 2016). Vitamin C is modified primarily by ascorbic acid oxidase and polyphenol oxidase, the activities of which directly depend on the O₂ content of the environment. Thus, a lessening in respiration rate of coated fruit could be a reason for vitamin C preservation (Zhou et al., 2008). The greater amount of the vitamin C in CMC-coated fruit could be explained by the O₂ and CO₂ transmission rates through the coating layer (Togrul and Arslan, 2004). Also, reduction in PPO activity in our study could describe the vitamin C preservation in coated plums.

For the other parameters—weight loss (control, ≈16.106%; 0.5% CMC, ≈13.154%; 1% CMC, ≈10.298%; and 1.5% CMC, ≈12.589%), pH (control, ≈3.694; 0.5% CMC, ≈3.639; 1% CMC, ≈3.633; and 1.5% CMC, ≈3.66), and TSS (control, ≈11.147, 0.5% CMC, ≈10.773, 1% CMC, ≈10.727 and 1.5% CMC, ≈10.44 Brix)—no considerable difference was detected between coated and control fruit (data not shown).

**Total phenolic compounds, total anthocyanin, and flavonoid content.** No significant difference in total phenolic compounds between the coated and control fruit were witnessed (control, ≈147.91, 0.5% CMC, ≈166.94, 1% CMC, ≈147.26 and 1.5% CMC, ≈162.1 mg gallic acid/100 g fresh weight) (data not shown). Investigation of anthocyanins and flavonoids demonstrated surprising variation. With regard to the anthocyanin content, fruit coated with 1% (≈0.4712), 1.5% (≈0.4588), and 0.5% (≈0.4368 absorbance at 530 nm g⁻¹ fresh weight) CMC-based edible coatings had a greater amount of anthocyanins than the control (≈0.3188 absorbance at 530 nm g⁻¹ fresh weight), but no noticeable differences were observed among coating treatments (Fig. 4B). In this case, no recognizable differences were noticed during shelf life, and significant changes were detected between harvest day and the other days. However, over time, anthocyanin enhancement occurred in the coated fruit, and the fruit had the maximum content of anthocyanin at the end of the storage time (Fig. 4A). Regarding flavonoid content, fruit treated

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**Fig. 2.** (A) Firmness values at harvest (day 0) and on days 2, 4, 6, and 8 of control and plums coated with carboxymethylcellulose (CMC) at 0.5%, 1%, and 1.5%. Analysis results = 5.53 (c), 7.0 (b), 9.0 (a), and 9.13 (a) for control, 0.5%, 1%, and 1.5% CMC, respectively. (B) Data are the estimated marginal means ± 95% confidence intervals.

**Fig. 3.** (A) Vitamin C contents at harvest (day 0) and on days 2, 4, 6, and 8 at 19 °C of control and plums coated with carboxymethylcellulose (CMC) at 0.5%, 1%, and 1.5%. Analysis results = 5.53 (c), 7.0 (b), 9.0 (a), and 9.13 (a) for control, 0.5%, 1%, and 1.5% CMC, respectively. (B) Data are the estimated marginal means ± 95% confidence intervals.
with 0.5% (≈0.202 mg quercetin/100 g fresh weight) CMC-based edible coating showed a substantial enhancement in flavonoid content whereas the 1% (≈173) and 1.5% (≈172) CMC concentrations resulted in less content than the control (≈185 mg quercetin/100 g fresh weight). So, significant differences were noticed between 0.5% CMC and the other CMC concentrations (Fig. 4B). Flavonoid content increased over time, and the greatest flavonoid content was observed at day 4, but it decreased thereafter (Fig. 4A). The increase in anthocyanin amount was associated with less PPO activity in coated fruit. PPO causes phenol oxidation (Jiang et al., 2005; Terefe et al., 2014) and anthocyanin degradation (Jiang et al., 2005). Flavonoids are also oxidized by PPO (Zhou et al., 2008). Thus, in the case of high PPO activity, the amount of phenolic compounds and derivatives, including flavonoids and anthocyanins, reduces. In the current study, again, the decrease in PPO activity in coated plums could be the reason for anthocyanin and flavonoid enhancement.

Total antioxidant capacity and POD activity. Data obtained for antioxidant capacity, based on the DPPH radical scavenging activity method, revealed that all concentrations of CMC-based edible coating increased total antioxidant activity effectively. The 1.5% CMC-based edible coating had the greatest activity (≈202.66%), followed by the 1% (≈15.48%) and 0.5% (≈13.92%) concentrations. Control fruit showed minimum (≈6.85%) antioxidant capacity (Fig. 5B). Regarding this property, the least activity was noticed on day 0, then improved meaningfully (significantly at 1.5% CMC), but at the end of the period it reduced (significantly in the control) (Fig. 5A).

POD activity, as an antioxidant enzyme, illustrated almost the same behavior in response to CMC concentrations. The 1.5% (≈0.274 μmol tetrugaiacol/min/g fresh weight) CMC-based edible coated fruit had the maximum enzyme activity, followed by the 0.5% (≈0.223) and 1% (≈0.182 μmol tetrugaiacol/min/g fresh weight) concentrations, which were different from each other and the control (≈0.13 μmol tetrugaiacol/min/g fresh weight) (Fig. 6B). Moreover, POD activity enhanced noticeably over time, and the maximum activity was detected at the end of the shelf life period except at the 1.5% concentration and in the control (Fig. 6A).

Phenolic compounds, including anthocyanins and flavonoids, have strong antioxidant activities, and a close relationship was realized between them and antioxidant capacity. Likewise, vitamin C can cause this effect (Manganaris et al., 2008). Thus, enhancement in the amounts of phenolic compounds, anthocyanins, flavonoids, and vitamin C improves correspondingly the antioxidant capacity of antioxidant enzymes such as POD (Jiang et al., 2005; Prior and Cao, 2000; Zhou et al., 2008). In our study, the greater amounts of vitamin C, flavonoids, and anthocyanins—in addition to the greater POD (which controls the level of peroxides and avoids formation of radicals) and lesser PPO (which oxidizes phenols and degrades anthocyanin and flavonoids) activity—could describe the greater total antioxidant capacity. This increasing trend in antioxidant activity using the DPPH method in plums during storage has been reported previously (Singh et al., 2012). Enhancement in POD activity by CMC coatings, as a desired characteristic, lessens the level of harmful radicals that could improve the shelf life as well as antioxidant capacity.

PPO and PG activity. PPO activity demonstrated a general increase over time; the least and greatest activity were reported on the first and last days of storage (Fig. 7A). Results obtained from PPO activity demonstrated that 1% (≈0.0135), 1.5% (≈0.0137) and 0.5% (≈0.0156 μmol oxidized catechol/min/g fresh weight) CMC-based edible coatings had the least activity and the control had the greatest (≈0.0181 μmol oxidized catechol/min/g fresh weight). In other words, significant differences in PPO activity were observed between coated fruit and the control (Fig. 7B). During storage, senescence, solubilization of cell wall pectic ingredients, and microbial infestation disrupt subcellular compartmentation, membrane integrity, and oxygen penetration, leading to greater PPO activity and oxidation of phenols. Furthermore, senescence or injury can destroy the biologic barrier between PPO and...
polyphenols, and can activate the enzyme. The quinones produced from PPO oxidation reactions can generate free radicals, which in turn use phenols to be scavenged (Hussain et al., 2016). Thus, in the current study, retarding senescence and maintaining membrane integrity by CMC coatings may describe the decline in PPO activity compared with the control.

PG activity was significantly high at the beginning and end of shelf life, with the greatest activity seen in the control. The best results for PG activity were achieved during the first week of storage, in which coated fruit demonstrated the least activity (Fig. 8A). Considering the obtained results for PG activity in plums, all concentrations of CMC-based edible coating reduced the enzyme activity, as a favorite quality. The difference in reduction was considerable among the three concentrations and between the concentrations and the control. The fruit with the 1.5% CMC-based edible coating and the control fruit showed the least (>=0.1906) and greatest (>=0.2398 μmol D-galacturonic acid/min/g fresh weight) amounts of the enzyme activity, respectively (Fig. 8B) as discussed previously.

Conclusion

CMC-based edible coatings may be applied to plums after harvesting as an innocuous and natural treatment to maintain quality and delay ripening during storage. In general, in our study the coatings were effective in preserving all qualitative properties, especially TA, vitamin C, firmness, total anthocyanin and flavonoid content, total antioxidant capacity, and POD activity. Moreover, the coatings decreased PPO (compared with the control) and PG activity, which altogether promoted better qualitative properties in the plums. It can be concluded that the application of CMC-based edible coatings is a promising method for increasing plum fruit quality and shelf life.

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