

Improvement of postharvest quality of plum (*Prunus domestica* L.) using polysaccharide-based edible coatings

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Abstract

Polysaccharide-based edible coatings are served as an attractive preservation method for postharvest maintenance of most fruits. The current study examined the effect of carboxymethylcellulose (CMC)- and pectin (Pec)- based edible coatings on weight loss, firmness, total soluble solids (TSS), pH, titratable acidity (TA), vitamin C (vit C), total phenolics, anthocyanin and flavonoid contents, total antioxidant capacity (based on DPPH) and the activities of peroxidase (POD), polyphenol oxidase (PPO) and polygalacturonase (PG) enzymes during cold storage. The results showed that each coating and their combinations caused positive effects in all measured parameters except weight loss. The applied coatings preserved firmness and improved total phenols, anthocyanin and flavonoid contents, antioxidant capacity and POD activity. In addition, the coatings retarded TSS and pH enhancement and TA and vit C loss and decreased PPO and PG activities. It could be stated that CMC at 1 % and Pec at 1.5 % separately demonstrated the best results at most measured parameters; and among the combinations 0.5 % Pec + 1.5 % CMC acted better than the other treatments. Henceforth, application of CMC and/or Pec and/or

their combinations would be considered as favorable approaches to improve postharvest quality characteristics of plum fruit.

Keywords: Carboxymethylcellulose, Pectin, Plum, Qualitative attributes, Enzymatic activity, Postharvest.

1. Introduction

Numerous studies focus on detection of secondary metabolites from fruits for their health benefits and nutraceuticals. Fruits and vegetables have been invariably great source of antioxidants, anthocyanins, phenolic compounds, nutritional elements and some vitamins [1] associated with reduced rate of chronic health disorders like cardiac problems, aging, and cancers of respiratory tract, alimentary canal, lungs, bladder, and breast is well recognized [11, 12] [2,3]. Plums (*Prunus domestica* L.) is an important, among the functional foods and nutraceuticals [9]. Plum, as a good source of antioxidants, might help human body to fight various diseases even cancerous cells and heart diseases. However, their short postharvest life results in loss of valuable and nutritional elements [4] all due to their high respiration rate. Their quality rapidly declines after harvest and often don't reach consumers at their optimal status after extensive transport and marketing [5,6].

In recent times, applying 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. Safe strategies including edible coatings could improve fruit postharvest. So, a growing interest is there in the use of coatings with natural origin such as proteins and polysaccharides [7,8].

Polysaccharide-based edible coatings act as efficient oxygen blockers due to their well-ordered hydrogen bonded network structure but not that well as moisture barriers. The coatings are commonly colorless with oil-free appearance and minor caloric content that almost always prolong

postharvest period of fruit by diminishing dehydration and oxidative rancidity [7]. Moreover, polysaccharide-based edible coatings are highly stable, safe, nontoxic and biodegradable. Cellulose derivatives and pectin are two main groups of polysaccharide-based edible coatings [9].

Carboxymethylcellulose (CMC), a derivative of cellulose, is an anionic linear and long-chain compound with a high molecular weight [10,11]. CMC-based coatings are generally nontoxic, non-allergic, biodegradable, odorless and tasteless. They also are flexible, transparent, resistant to oil and fats, water-soluble and moderately permeable to moisture, oxygen and carbon dioxide [12].

Pectin (Pec), main compound of plant cell walls, is a complex polysaccharide with branching structure [13,14], amorphous and colloidal carbohydrate of high molecular weight [14]. Pec-based coatings are excellent barriers to oxygen and carbon dioxide, transparent, resistant to oil and fats, and water-soluble. They retard moisture loss and eventually maintain the sensory and quality of foods [14,15].

CMC-based edible coatings have been shown to be effective in maintaining postharvest quality of pear, papaya, mandarin and peach [16-19]. Pec-based ones preserved quality of peach, nectarine, fresh-cut apple and persimmon [20-23]. Some studies reported application of edible coatings on plum fruit, including the use of chitosan [24], carboxymethylcellulose in combination with irradiation [6] and carboxymethylcellulose [8].

Based on this literature review, no study (except those we did during shelf life (Panahirad *et al.* [8]) and the other one under decision for publication) was performed using these polysaccharide-based edible coatings (CMC and Pec) on plum fruit during cold storage. Also, no report of combination of the two coatings was observed on plum fruit. Accordingly, in this study, it is aimed to investigate the influence of CMC- and Pec-based edible coatings, alone and combined, on some

postharvest qualitative and enzymatic characteristic of plum to address postharvest losses of this fruit. Furthermore, the current survey might be a comprehensive evaluation of different qualitative characters especially antioxidant contents and enzymatic behavior of coated plum during cold warehousing.

2. Materials and methods

2.1. Plant materials

Fruit (*Prunus domestica* cv. “Golden Drop”) were collected free of any wound or scar and uniform 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 TSS/TA \approx 85 days after full bloom). The fruit were gently washed, cleansed with distilled water and placed on paper towels to dry at room temperature and subsequently coated with the following treatments; as described below.

2.2. Coating treatments of matured fruits

Experiments were performed using three concentrations of carboxymethylcellulose (CMC)- and pectin (Sigma, USA) and Pec (Sigma Aldrich Chemic, Steinhein-Germany) (0.5, 1 and 1.5 %), both alone and in combination (total 16 including control and 15 treatments) in three replications (Table 1). Each replication consisted of sixty fruits.

Table 1: Treatment combinations of CMC (%) and Pec (%) on fruits

CMC Pec	0	0.5	1.0	1.5
0	1	2	3	4
0.5	5	6	7	8
1.0	9	10	11	12
1.5	13	14	15	16

Sampling was done weekly intervals for six weeks, 10 fruits per each sampling. Coating treatment solutions (CMC and Pec) were prepared by dissolving them in distilled water, while stirring at 60 °C, and glycerol 0.3 % was added as a plasticizer and stirred. Then plum fruit were dipped into the homogenized solutions for 60 seconds, air-dried at room temperature for 1 h, placed on open plastic grids and stored at 4 °C and 75 % relative humidity for six weeks. The non-coated fruit, just treated with distilled water for 60 s, were used as the control.

Evaluation of fruit quality

2.3.1. Measurement of weight loss, firmness, total soluble solids (TSS), pH, titrable acidity (TA) and vitamin C (vit C)

Weight loss was calculated for each fruit-unit (four fruits/unit) 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. The samples (at least five fruits) were homogenized before measuring TSS, TA, and vit C. To determine the TSS of fruit samples, a refractometer (PR-1;

Atago Co., Ltd., Tokyo, Japan) was used at 20 °C (expressed as %). 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. The vitamin C content of the samples was determined using a titrimetric method based on the reduction of 2,6-dichlorophenolindophenol dye, as described by AOAC. The results of TA and vit C were expressed as g kg⁻¹ on a fresh weight basis. Three technical replicates were assessed for each measurement.

2.3.2. *Total phenolic compounds, total anthocyanin and flavonoid contents*

Total phenolic compounds were determined using Folin-Ciocalteu reagent was used as described by Singleton and Rossi [25]. Briefly, after digesting 1 g of the fleshy fruit with 2 mL 1% HCl-methanol and centrifuge (Hettich 320R, Germany) at 8000 g for 10 min at 4 °C, the supernatant was collected and used to quantify the total phenolic compounds. For this purpose, to the 50-mL extract, 450 mL distilled water and 2.5 mL 10 % Folin-Ciocalteu solution were added and incubated in dark, the absorbance was recorded after 1.5 h incubation in the dark at 760 nm using a spectrophotometer (Spekol 1500, Germany). The absorbance values were converted to total phenolics and expressed as g gallic acid kg⁻¹ on a fresh weight basis. Different concentrations of gallic acid in 95% methanol were used as standards. Peel and flesh (1 g) from five fruits were finely sliced and extracted with 2 mL of 1 % HCl-methanol to estimate the total anthocyanin content, as published in the literature [26]. After centrifuging, the extract absorbance was measured at 530 nm. Anthocyanin concentration was expressed as absorbance at 530 nm g⁻¹ on a fresh weight basis. Total flavonoid content was determined as per Woisky and Salatino method [27] with some modifications. To clarify, peel and flesh (1 g) from five fruits were extracted with 4 mL 96 % ethanol. After centrifuging and collecting supernatant, to 1300 µL of the extract, 700 µL 96 % ethanol, 100 µL 10 % aluminum chloride, 100 µL 1 M potassium acetate and at last 2.8 mL distilled

water were added and after 30 min incubation at room temperature, the absorbance of the solution was measured at 415 nm. The results were expressed as g quercetin kg⁻¹ on a fresh weight basis. Different concentrations of quercetin were used as standards.

2.3.3. *Total antioxidant activity*

1,1-Diphenyl-2-picryl-hydrazyl hydrate (DPPH) method was used to determine antioxidant activity. Peel and flesh (1 g) from five fruits were cut and extracted with 2 mL of 1 % HCl-methanol and then centrifuged, following the DPPH method to determine total antioxidant activity [28]. Absorbance was measured at 517 nm after 15 min and the activity was calculated and expressed as percentage (%) using the formula:

$$\% \text{ Total antioxidant activity} = (A_{\text{blank}} - A_{\text{sample}}) / (A_{\text{blank}}) \times 100$$

2.3.4. *Peroxidase (POD), Polyphenol oxidase (PPO) and Polygalacturonase (PG) activities*

Peel and flesh (1 g) from five fruits were homogenized in 3 mL of 0.1 M phosphate buffer in an ice bath. The homogenate was centrifuged and then the supernatant collected as the crude enzyme extract. POD activity was assayed through the procedure described by Arnnok et al. [29] with some modifications. The activity was determined in 2 mL reaction mixture containing 0.1 M phosphate buffer, guaiacol, extract and H₂O₂. Oxidation of guaiacol was monitored by the increase in absorbance at 470 nm. Results were expressed in terms of μmol tetraguaiacol min⁻¹ g⁻¹ on a fresh weight basis. PPO activity was estimated using Jiang et al method [26] with modifications. 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 for 90 s. Results were reported in terms of μmol oxidized catechol min⁻¹ g⁻¹ on a fresh weight basis.

PG activity was assayed based on the release of reducing groups produced by PG and measured by spectrophotometer [30]. Peel and flesh (1 g) from five fruits were homogenized in 3 mL 50 mM

sodium acetate buffer and then centrifuged. The enzyme extract (50 mL) was mixed with 950 mL sodium acetate buffer and 1 mL 0.3 % polygalacturonic acid, then incubated at 30 °C for 45 min. To stop the reaction, 800 mL 0.1 M borate buffer (pH 9.0) at 0 °C and 200 mL of 1 % cyanoacetamide solution were added to the reaction mixture and boiled for 10 min. After cooling, the absorbance was measured at 276 nm. PG activity was expressed as $\mu\text{mol D-galacturonic acid min}^{-1} \text{ g}^{-1}$ on a fresh weight basis.

2.4. Statistical analysis

The study was performed as factorial experiment in completely randomized design (CRD). Following 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 16, SPSS Inc., Chicago, IL). Maximum likelihood-based estimated marginal means were reported and adjustment for multiple comparisons was performed based on least significant difference at $P \leq 0.05$ and finally 95% confidence intervals were shown as error bars in the presented graphs. The study emphasized on simple and interaction effects of Pec and CMC, omitting time course and the interaction of coating materials with time to just focus their influence on maintaining plum during cold storage. The results of those parts may be presented as a complementary paper soon.

3. Results

3.1. TA, firmness, vit C, TSS, pH and weight loss

Results showed that coating of Pec ($P \leq 0.05$), CMC and their combinations (Pec*CMC) ($P \leq 0.01$) maintained TA and prevented its loss. Amongst Pec concentrations, 1.5 % ($\approx 15.55 \text{ g kg}^{-1}$) was effective in maintaining TA (Fig. 1a), while all CMC concentrations significantly caused higher TA amounts, as the most of the effect was observed at 1 % CMC ($\approx 15.87 \text{ g kg}^{-1}$) (Fig. 1b), when treated the coating separately. Thus, CMC concentrations was significantly different from the

others compared to the control. All Pec and CMC combinations (except 1 % Pec + 1.5 % CMC \approx 14.57 g kg⁻¹) maintained TA contents of coated fruit with the high TA at 0.5 % Pec + 1 % CMC (\approx 16.44) and 1.5 % Pec + 1.5 % CMC (\approx 16.35 g kg⁻¹) (Fig. 1c).

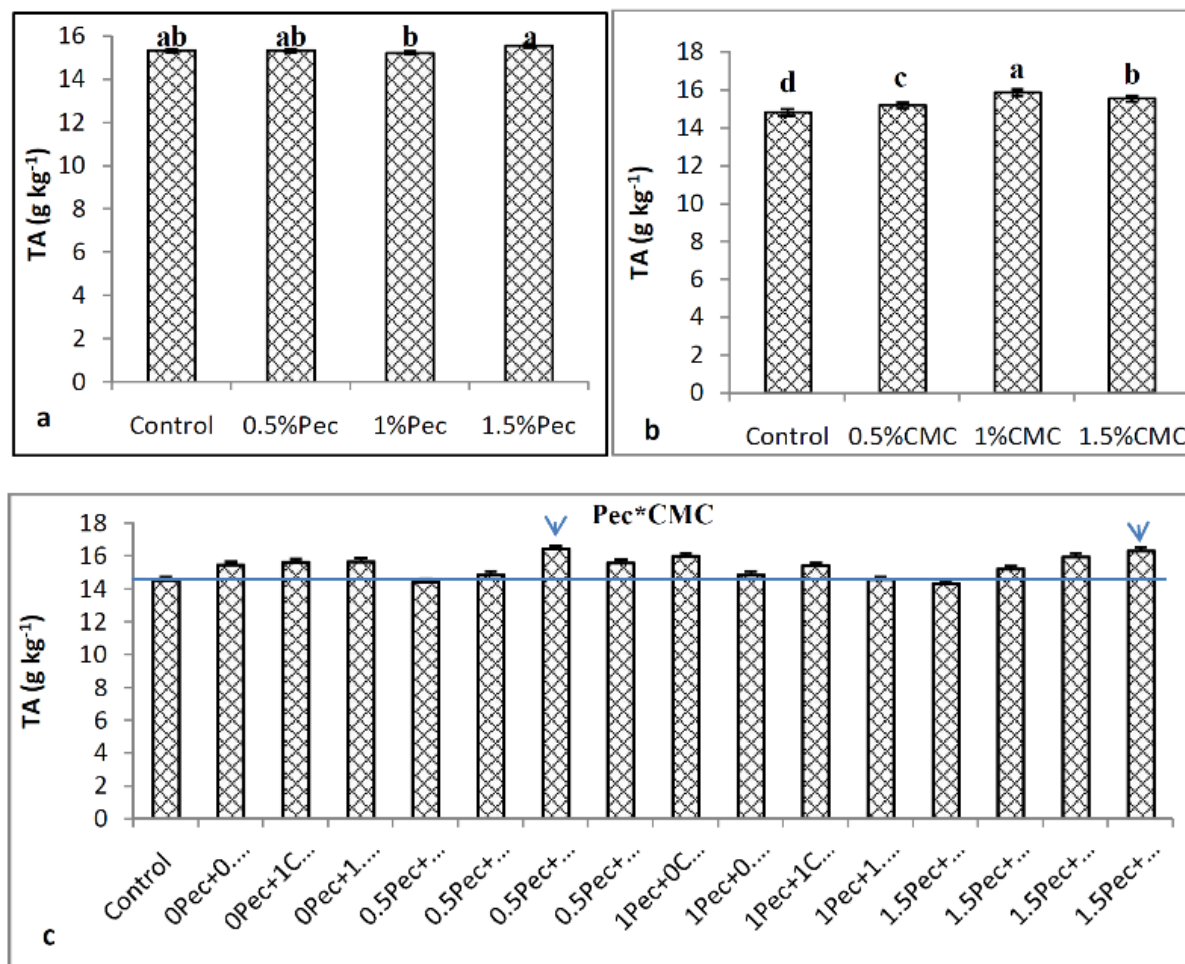


Fig.1: Effect of 0 (control), 0.5 (0.5%Pec), 1 (1% Pec) and 1.5 (1.5%Pec)% pectin-based edible coatings (a), 0 (control), 0.5 (0.5%CMC), 1 (1% CMC) and 1.5 (1.5%CMC)% carboxymethylcellulose-based edible coatings (b) and their combinations (c) on TA (titrable acidity) content during cold storage period. Data are the “estimated marginal means \pm 95% confidence intervals”. The results were expressed on a fresh weight basis.

The coating treatments (Pec, CMC and Pec*CMC) were good in firmness preservation ($P \leq 0.01$). Pec-based edible coatings at 1.5 (\approx 12.556) and 0.5 % (\approx 11.088 N) concentrations (Fig. 2a) and all CMC concentrations especially 1 (\approx 12.31) and 1.5 % (\approx 11.878 N) preserved plum firmness compared to the control (Fig. 2b). All Pec*CMC combinations caused better firmness compared

to the control (≈ 5.668 N). The best firmness preservation was observed at 1.5 % Pec + 1 % CMC (≈ 14.495 N) (Fig. 2c).

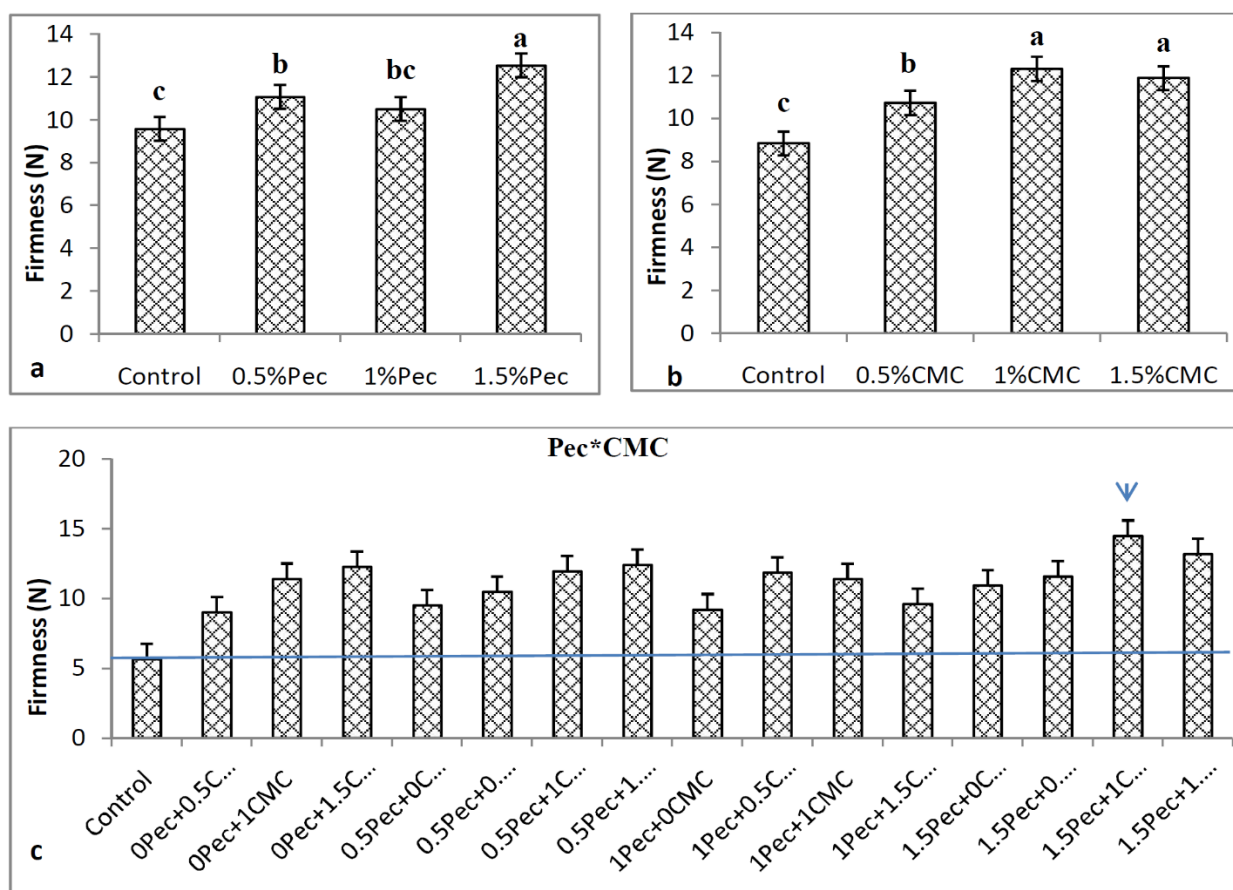


Fig. 2: Effect of 0 (control), 0.5 (0.5%Pec), 1 (1% Pec) and 1.5 (1.5%Pec)% pectin-based edible coatings (a), 0 (control), 0.5 (0.5%CMC), 1 (1% CMC) and 1.5 (1.5%CMC)% carboxymethylcellulose-based edible coatings (b) and their combinations (c) on firmness during cold storage period. Data are the “estimated marginal means \pm 95% confidence intervals”.

The vit C values significantly ($P \leq 0.01$) affected by Pec (1.5 ≈ 0.126 and 1 % ≈ 0.123 g kg⁻¹) (Fig. 3a), all concentrations of CMC (Fig. 3b), either treated alone or in combinations. Among Pec*CMC-based edible coatings, 1.5 % Pec + 1.5 % CMC (≈ 0.133), 1 % Pec + 0.5 % CMC (≈ 0.129) and 1 % Pec + 1 % CMC (≈ 0.129 g kg⁻¹) showed higher levels of vit C contents (Fig. 3c). The results suggest that coatings either alone or in combination resulted in vit C preservation when compared to the control.

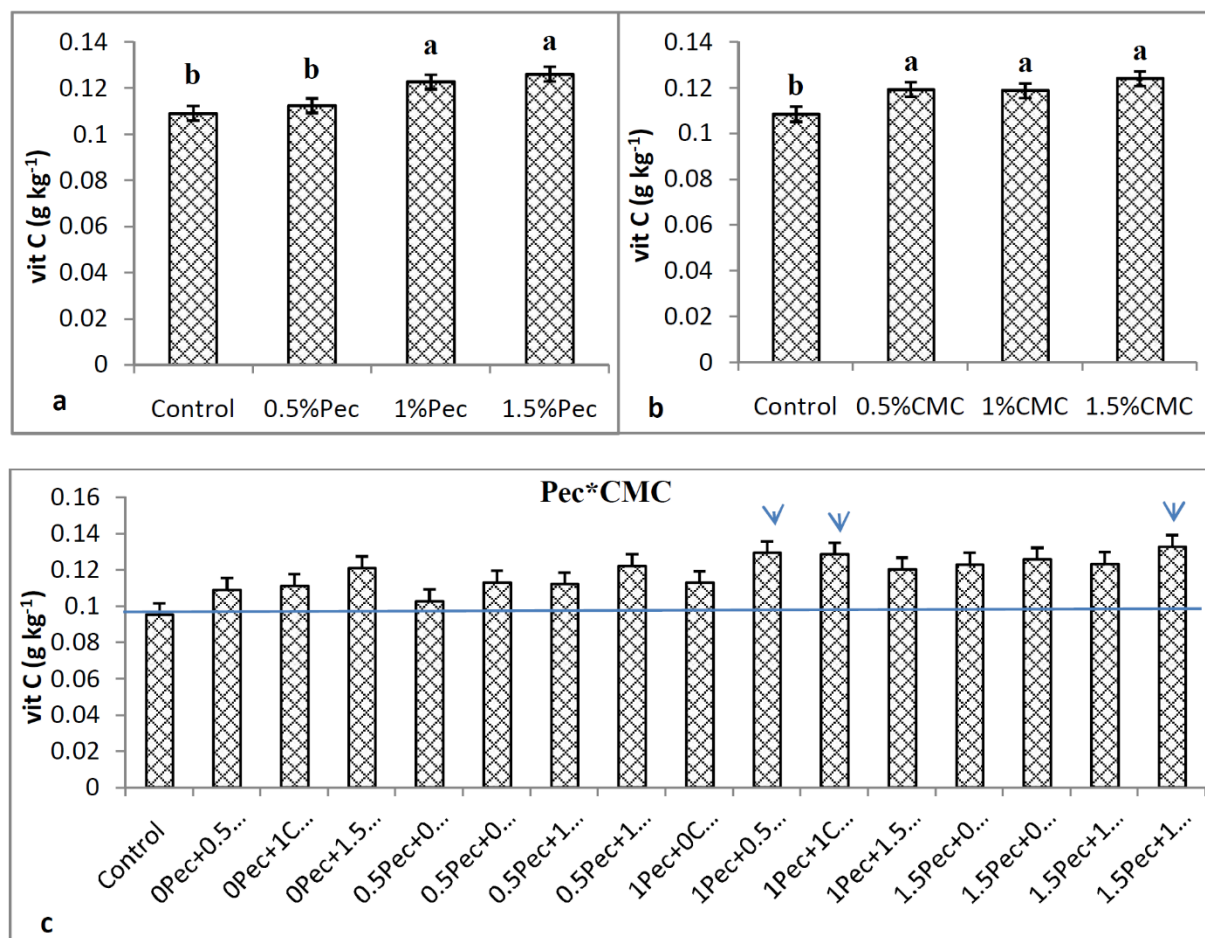


Fig. 3: Effect of 0 (control), 0.5 (0.5%Pec), 1 (1% Pec) and 1.5 (1.5%Pec)% pectin-based edible coatings (a), 0 (control), 0.5 (0.5%CMC), 1 (1% CMC) and 1.5 (1.5%CMC)% carboxymethylcellulose-based edible coatings (b) and their combinations (c) on vitamin C (vit C) contents during cold storage period. Data are the “estimated marginal means \pm 95% confidence intervals”. The results were expressed on a fresh weight basis.

The results for TSS amounts revealed that all Pec concentrations (with the best result at 1.5 % \approx 9.731 %) (Fig. 4a), CMC concentrations (Fig. 4b) and their combination of 0.5 % Pec + 1.5 % CMC (\approx 9.443 %) (Fig. 4c) were significantly ($P \leq 0.01$) effective on preventing TSS enhancements.

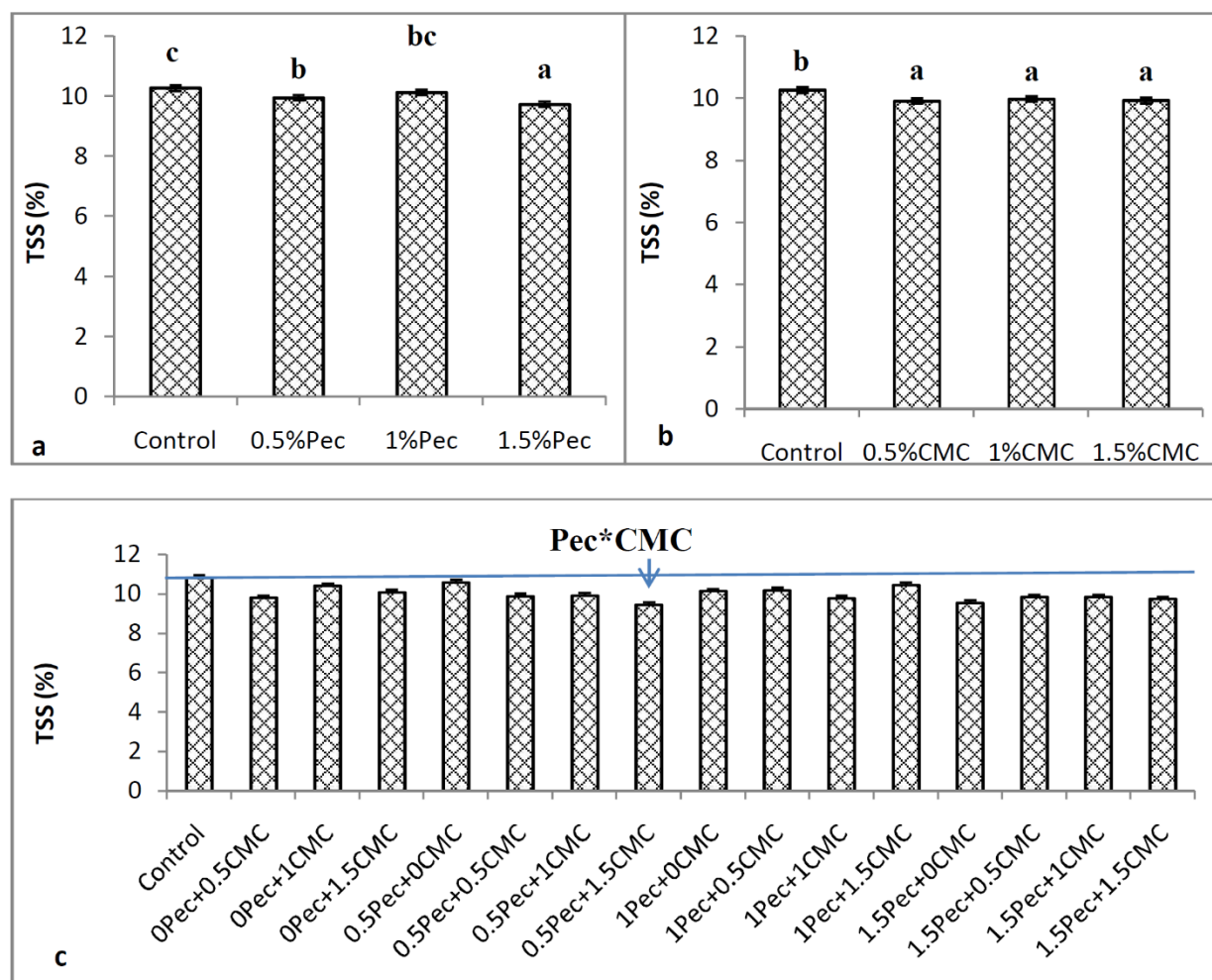


Fig. 4: Effect of 0 (control), 0.5 (0.5%Pec), 1 (1% Pec) and 1.5 (1.5%Pec)% pectin-based edible coatings (a), 0 (control), 0.5 (0.5%CMC), 1 (1% CMC) and 1.5 (1.5%CMC)% carboxymethylcellulose-based edible coatings (b) and their combinations (c) on total soluble solids (TSS) contents during cold storage period. Data are the “estimated marginal means \pm 95% confidence intervals”. The results were expressed on a fresh weight basis.

The pH value demonstrated that Pec and CMC treated increased pH values (Fig. 5a). CMC and Pec*CMC combinations significantly ($P \leq 0.01$) affected pH (Fig. 5 b and c). Pec alone at 1.5 % concentration CMC treatments caused retarding pH enhancement with the best effect at 1 % (≈ 3.503) (Fig. 5b). Combinations of Pec and CMC caused maintaining pH value except 1 % Pec + 1.5 % CMC (≈ 3.575), 1.5 % Pec + 0.5 % CMC (≈ 3.559) and 1.5 % Pec + 1.5 % CMC (≈ 3.57) as compared to the control (≈ 3.558) (Fig. 5c).

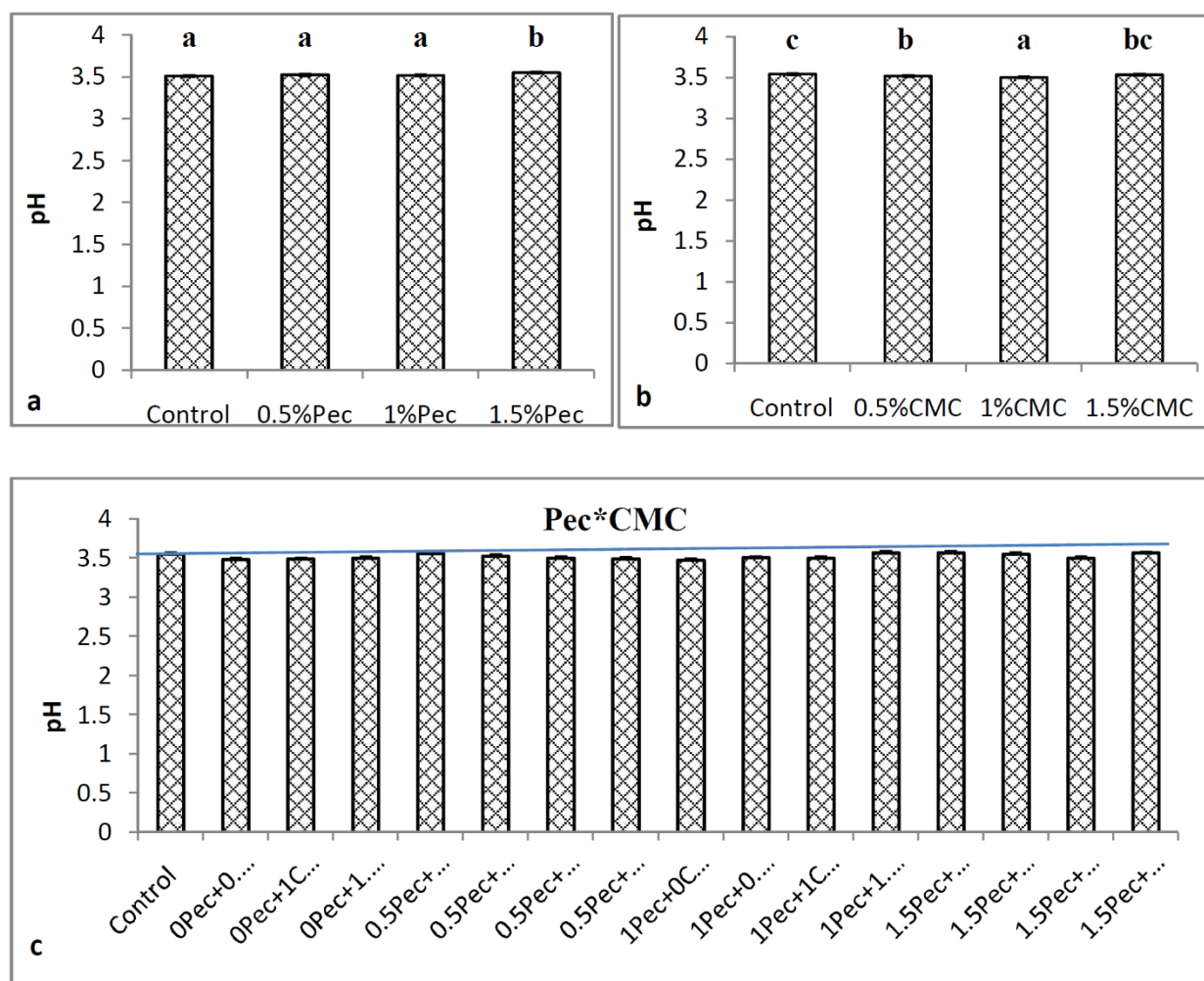


Fig. 5: Effect of 0 (control), 0.5 (0.5%Pec), 1 (1% Pec) and 1.5 (1.5%Pec)% pectin-based edible coatings (a), 0 (control), 0.5 (0.5%CMC), 1 (1% CMC) and 1.5 (1.5%CMC)% carboxymethylcellulose-based edible coatings (b) and their combinations (c) on pH during cold storage period. Data are the “estimated marginal means \pm 95% confidence intervals”.

No considerable difference was detected between the control and coated fruit (CMC, Pec and Pec*CMC) in weight loss parameter (Data not shown).

Total phenolic compounds, total anthocyanin and flavonoid contents

All Pec-based edible coatings especially at 0.5 % (≈ 0.989 g kg⁻¹ gallic acid) (Fig. 6a) and CMC-based edible coating just at 0.5 % (≈ 0.954 g kg⁻¹ gallic acid) (Fig. 6b) caused a higher amount of phenolic compounds compared to the control ($P \leq 0.01$). All Pec*CMC combinations except 1.5 %

Pec + 1 % CMC ($\approx 0.752 \text{ g kg}^{-1}$ gallic acid) showed higher phenolic content than the control ($\approx 0.749 \text{ g kg}^{-1}$ gallic acid) (Fig. 6c).

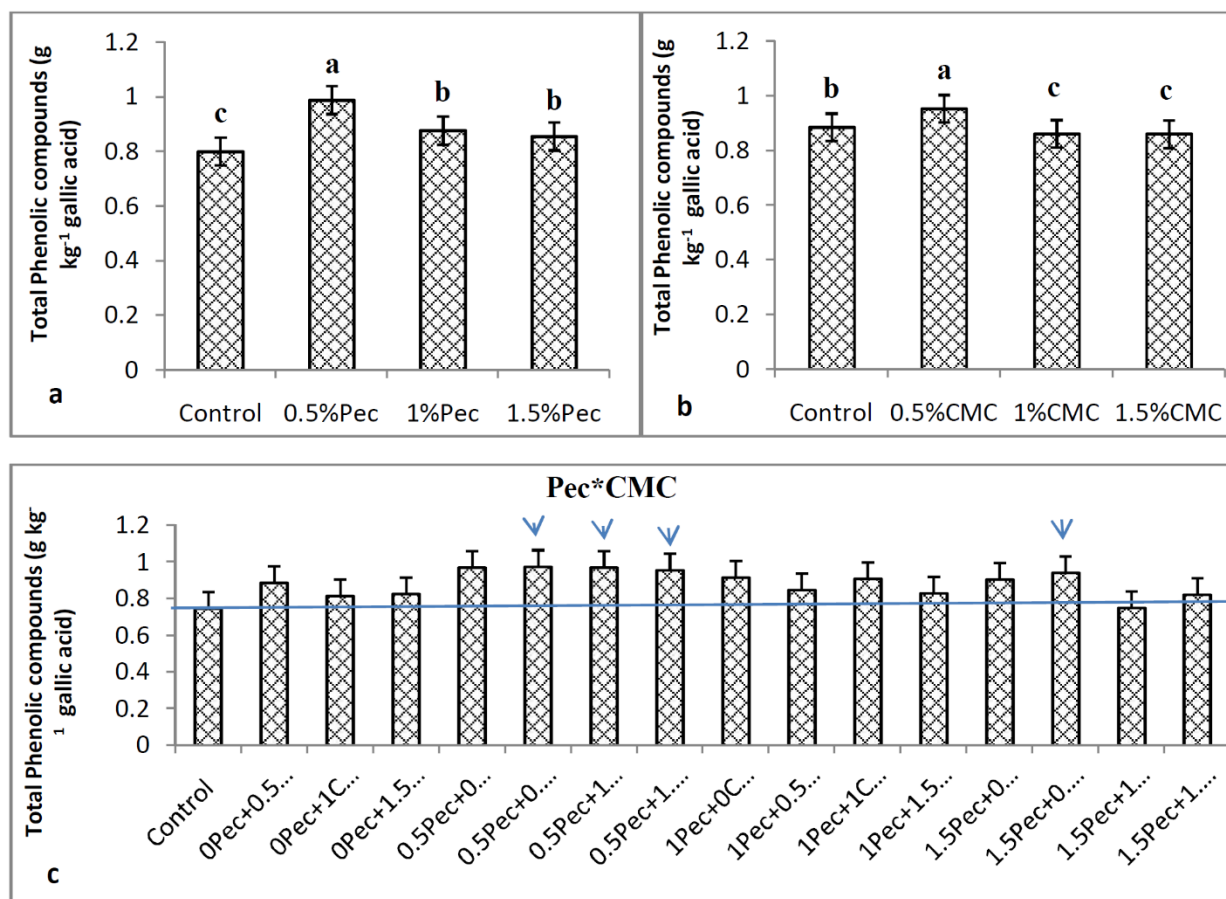


Fig. 6: Effect of 0 (control), 0.5 (0.5%Pec), 1 (1% Pec) and 1.5 (1.5%Pec)% pectin-based edible coatings (a), 0 (control), 0.5 (0.5%CMC), 1 (1% CMC) and 1.5 (1.5%CMC)% carboxymethylcellulose-based edible coatings (b) and their combinations (c) on total phenolic compounds during cold storage period. Data are the “estimated marginal means \pm 95% confidence intervals”. The results were expressed on a fresh weight basis.

Changes in anthocyanins demonstrated that all treatments including Pec, CMC and Pec*CMC showed significantly ($P \leq 0.01$) higher anthocyanin contents than the control measured as absorbance at 530 nm. Pec at 0.5 % (≈ 0.5 [absorbance]) (Fig. 7a) and CMC at 1 % (≈ 0.484 [absorbance]) (Fig. 7b) caused the highest anthocyanin values. Also, all Pec*CMC concentrations caused higher anthocyanin amounts compared to the control (≈ 0.32 [absorbance]) with the best result at 0.5 % Pec + 1 % CMC (≈ 0.543 [absorbance]) (Fig. 7c).

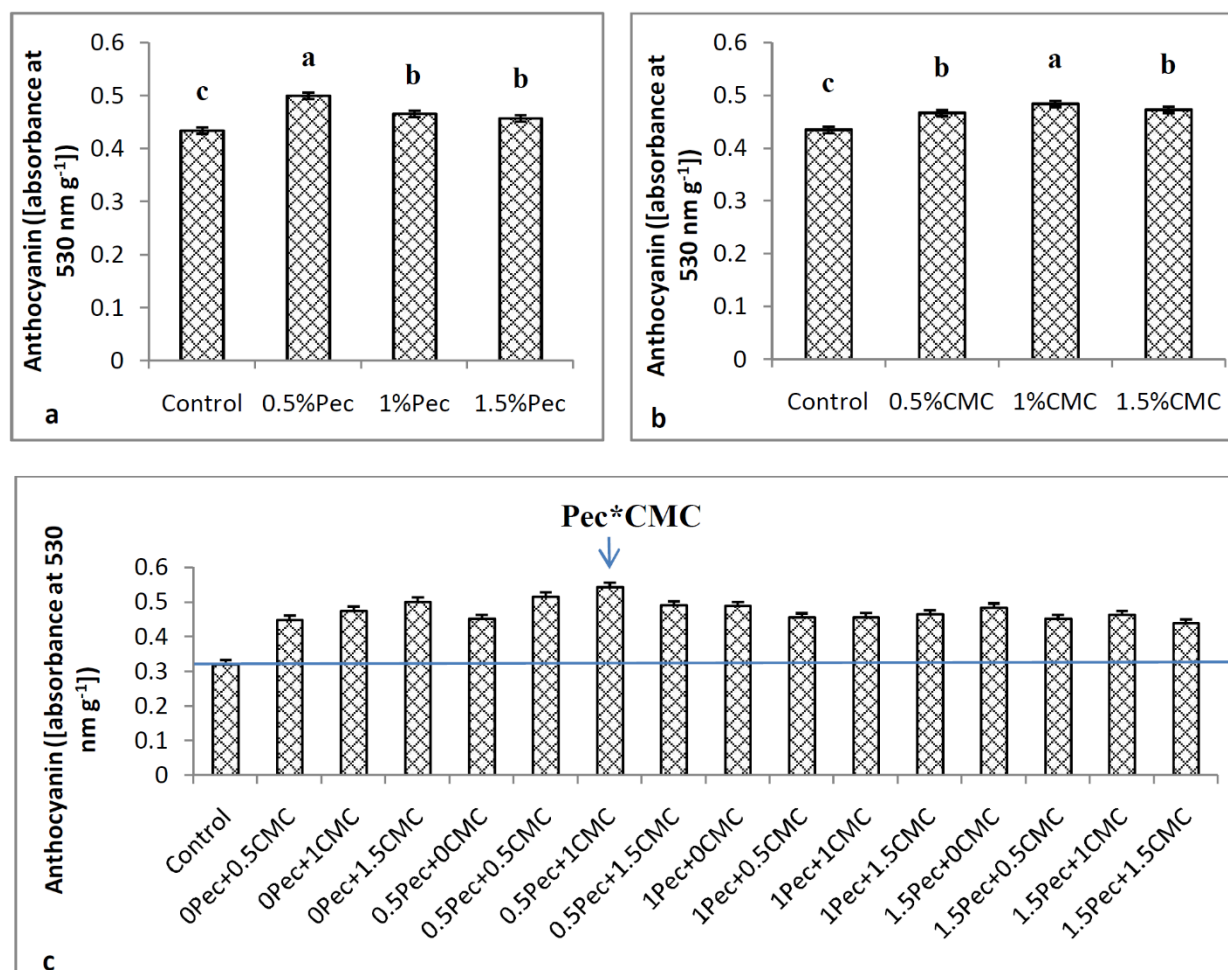


Fig. 7: Effect of 0 (control), 0.5 (0.5%Pec), 1 (1% Pec) and 1.5 (1.5%Pec)% pectin-based edible coatings (a), 0 (control), 0.5 (0.5%CMC), 1 (1% CMC) and 1.5 (1.5%CMC)% carboxymethylcellulose-based edible coatings (b) and their combinations (c) on anthocyanin during cold storage period. Data are the “estimated marginal means \pm 95% confidence intervals”. The results were expressed on a fresh weight basis.

Significant differences ($P \leq 0.01$) in flavonoids content were observed between 0.5 % (≈ 2.02) and 1.5 % (≈ 2.00) Pec (Fig. 8a) and 0.5 % (≈ 2.04 g kg⁻¹ quercetin) CMC-coated fruit (Fig. 8b) with the control and the others. In combination of Pec and CMC, significant enhancement ($P \leq 0.01$) in flavonoid content was observed at 0.5 % each (≈ 2.07), 0.5 and 1% (≈ 2.05) and 0.5 and 1.5 % (≈ 2.07), when compared to control the control (≈ 1.83 g kg⁻¹ quercetin) (Fig. 8c).

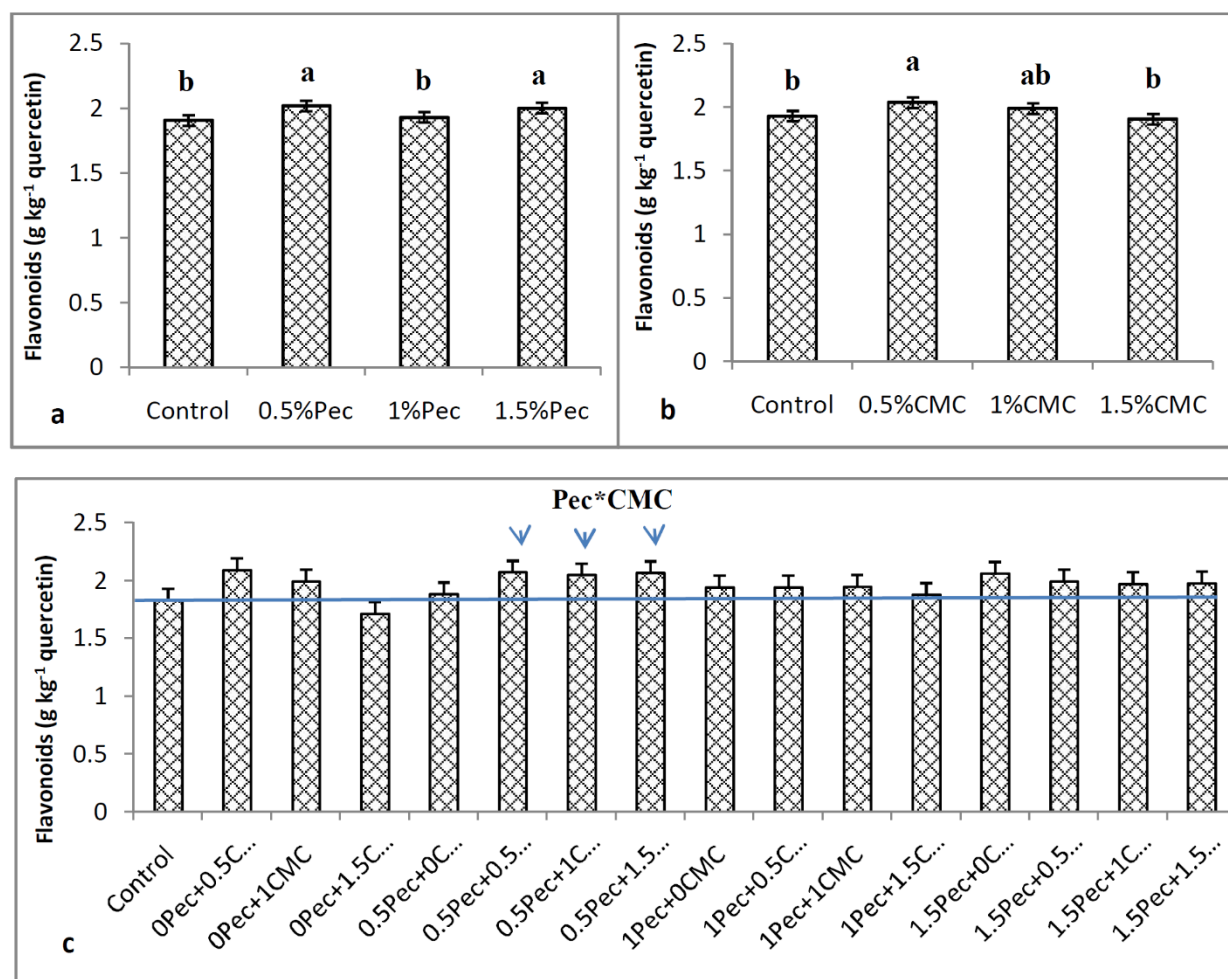


Fig. 8: Effect of 0 (control), 0.5 (0.5%Pec), 1 (1% Pec) and 1.5 (1.5%Pec)% pectin-based edible coatings (a), 0 (control), 0.5 (0.5%CMC), 1 (1% CMC) and 1.5 (1.5%CMC)% carboxymethylcellulose-based edible coatings (b) and their combinations (c) on flavonoids contents during cold storage period. Data are the “estimated marginal means \pm 95% confidence intervals”. The results were expressed on a fresh weight basis.

3.2. Total antioxidant capacity

Total antioxidant studies revealed that, both Pec- and CMC -based edible coatings increased their contents significantly at their higher concentrations. (Fig. 9 a and b). Further in combination of both coatings at 1.5 % Pec + 1 % CMC demonstrated noticeably ($P \leq 0.01$) a further increase in the antioxidant capacity levels compared over that control higher antioxidant capacity than the control (Fig. 9c).

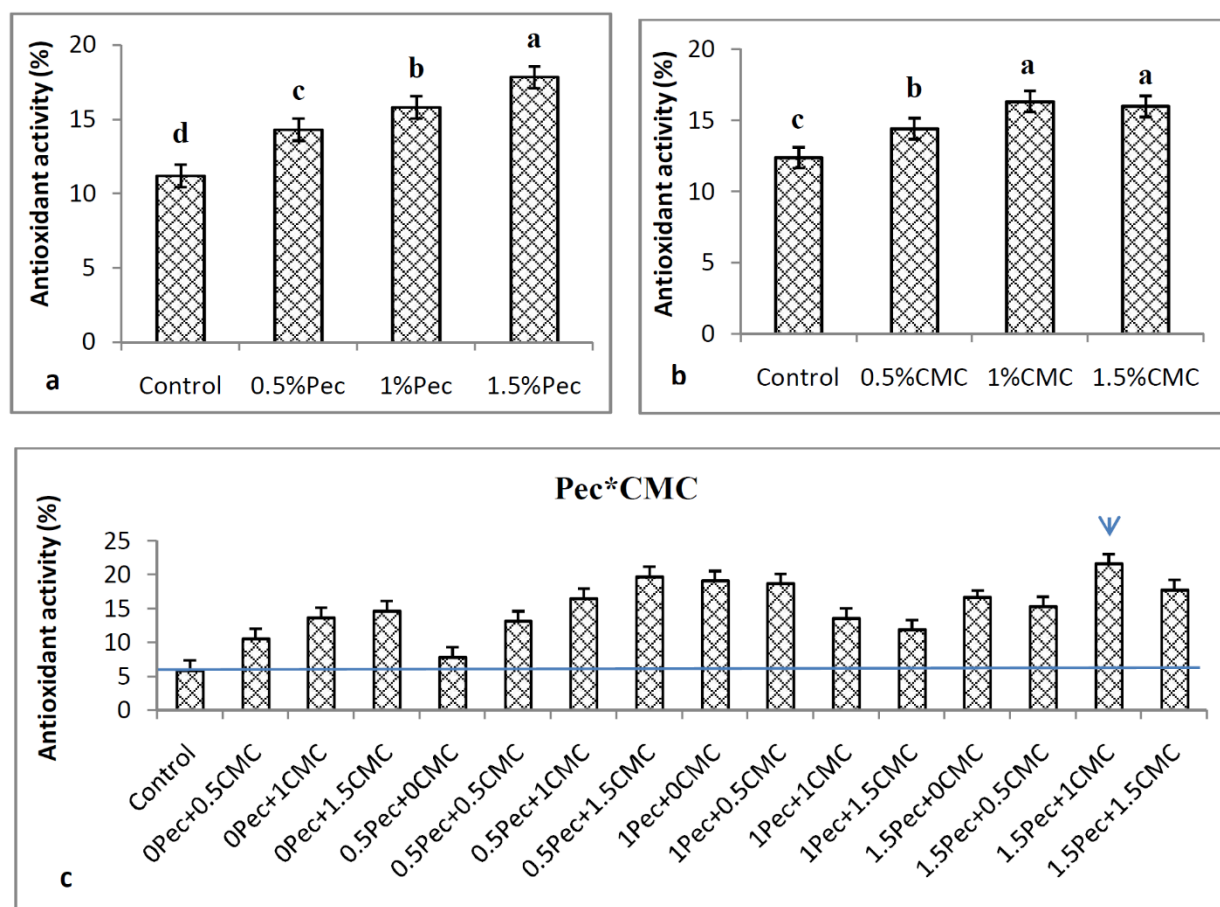


Fig. 9: Effect of 0 (control), 0.5 (0.5%Pec), 1 (1% Pec) and 1.5 (1.5%Pec)% pectin-based edible coatings (a), 0 (control), 0.5 (0.5%CMC), 1 (1% CMC) and 1.5 (1.5%CMC)% carboxymethylcellulose-based edible coatings (b) and their combinations (c) on antioxidant capacity based on DPPH method during cold storage period. Data are the “estimated marginal means \pm 95% confidence intervals”.

3.3. POD and PPO activities

POD one of the major antioxidant enzymes, increase ($P \leq 0.01$) in response to CMC, Pec treated either alone or in combinations. All concentrations of Pec-based edible coating caused higher POD activities with no significant differences among concentrations (Fig. 10a). Also, all CMC-based edible coatings demonstrated higher POD activities compared to the control with the maximum activity at 1 % concentration ($\approx 0.287 \mu\text{mol tetraguaiacol min}^{-1} \text{g}^{-1}$) (Fig. 10b). All combinations caused higher POD activities than the control (≈ 0.137) with the highest activity at 1.5 % Pec + 1 % CMC ($\approx 0.383 \mu\text{mol tetraguaiacol min}^{-1} \text{g}^{-1}$) (Fig. 10c).

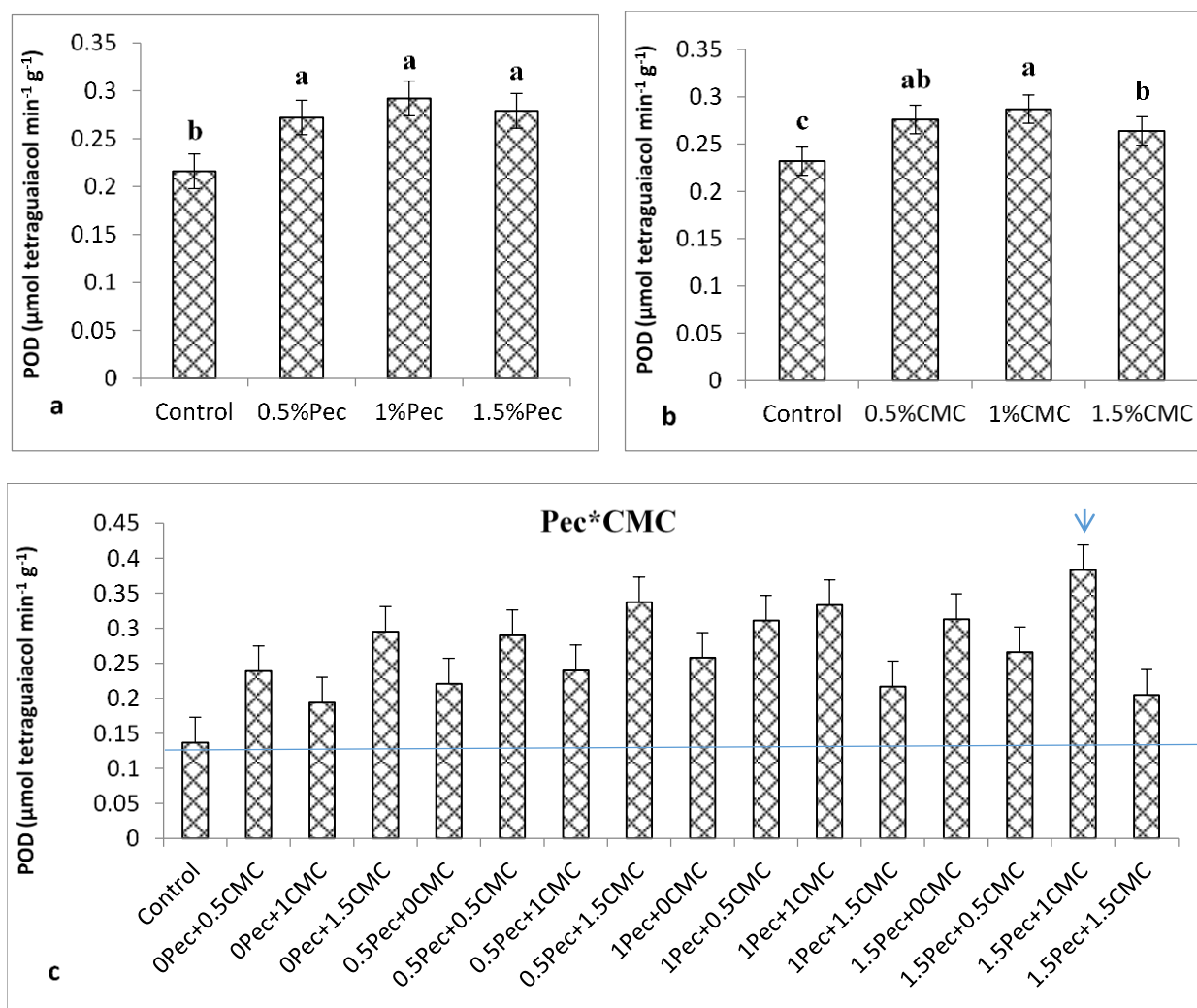


Fig. 10: Effect of 0 (control), 0.5 (0.5%Pec), 1 (1% Pec) and 1.5 (1.5%Pec)% pectin-based edible coatings (a), 0 (control), 0.5 (0.5%CMC), 1 (1% CMC) and 1.5 (1.5%CMC)% carboxymethylcellulose-based edible coatings (b) and their combinations (c) on peroxidase (POD) enzyme activity during cold storage period. Data are the “estimated marginal means \pm 95% confidence intervals”. The results were expressed on a fresh weight basis.

Pec at 1 (≈ 0.0141) and 1.5 % ($\approx 0.014 \mu\text{mol oxidized catechol min}^{-1} \text{ g}^{-1}$) (Fig. 11a) and CMC at all concentrations had a lower ($P \leq 0.01$) PPO activity than the control with the lowest activity at the concentration of 1 % ($\approx 0.0137 \mu\text{mol oxidized catechol min}^{-1} \text{ g}^{-1}$) (Fig. 11b). Coatings with combinations demonstrated lower PPO activities than the control (≈ 0.019) with the lowest activity at 1.5 % Pec + 1.5 % CMC ($\approx 0.013 \mu\text{mol oxidized catechol min}^{-1} \text{ g}^{-1}$).

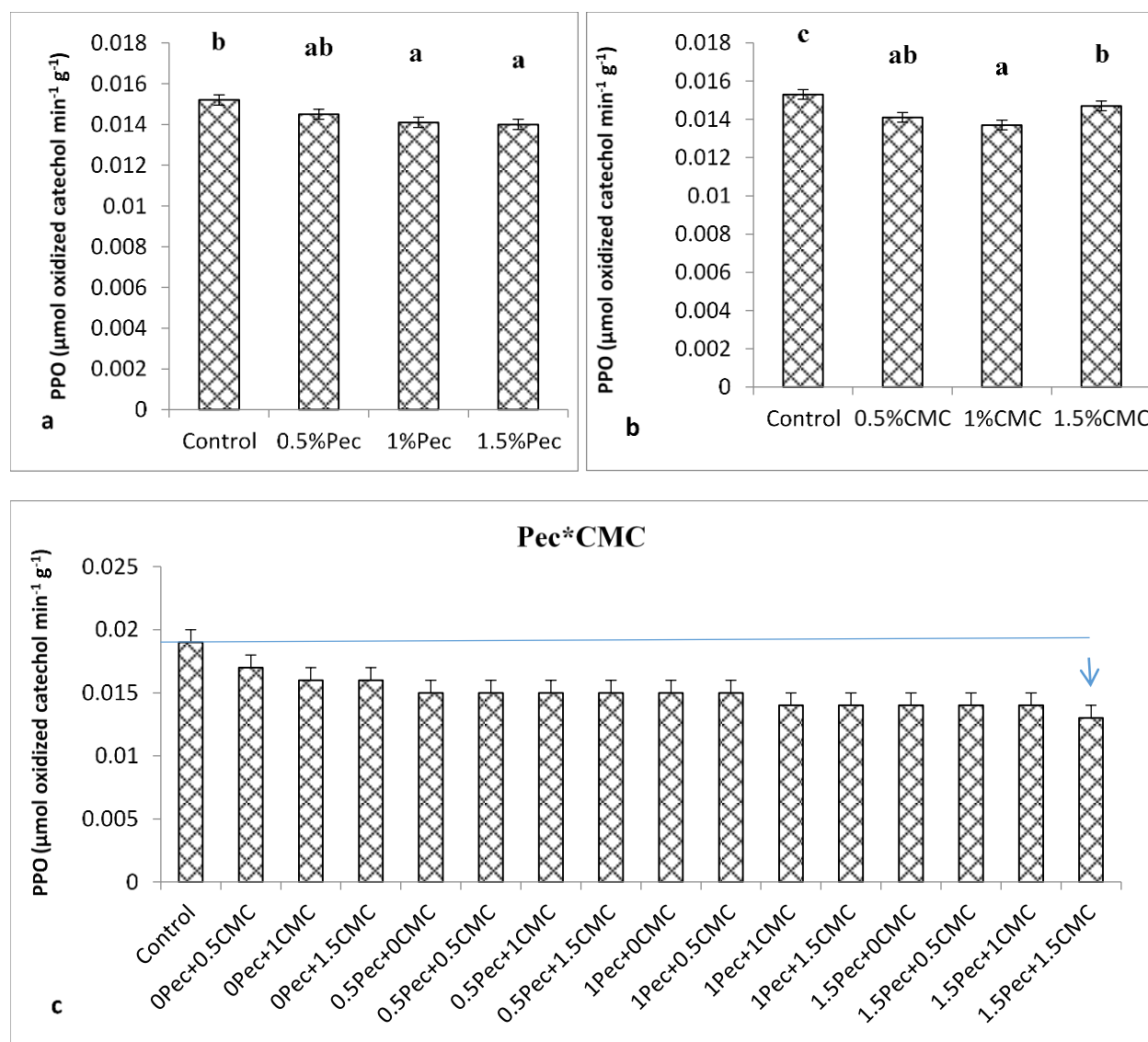


Fig. 11: Effect of 0 (control), 0.5 (0.5%Pec), 1 (1% Pec) and 1.5 (1.5%Pec)% pectin-based edible coatings (a), 0 (control), 0.5 (0.5%CMC), 1 (1% CMC) and 1.5 (1.5%CMC)% carboxymethylcellulose-based edible coatings (b) and their combinations (c) on polyphenoloxidase (PPO) enzyme activity during cold storage period. Data are the “estimated marginal means \pm 95% confidence intervals”. The results were expressed on a fresh weight basis.

3.4. PG activity

CMC, Pec and their combinations considerably reduced the PG enzyme activity ($P \leq 0.01$), affecting the fruit quality. Pec at 0.5 (≈ 0.719) and 1.5 % (≈ 0.718 $\mu\text{mol D-galacturonic acid min}^{-1} \text{g}^{-1}$) reduced PG activity whereas 1 % Pec showed no difference as compared to the control (Fig. 12a). CMC concentrations showed similar effect showing slight reduction in PG content in all

CMC treatments (Fig. 12b). The fruit coated with Pec*CMC-based edible coatings demonstrated lower PG activities than the control (≈ 0.847) with the lowest activity at 0.5 % Pec + 1.5 % CMC ($\approx 0.696 \mu\text{mol D-galacturonic acid min}^{-1} \text{g}^{-1}$) (Fig. 12c) as previously discussed.

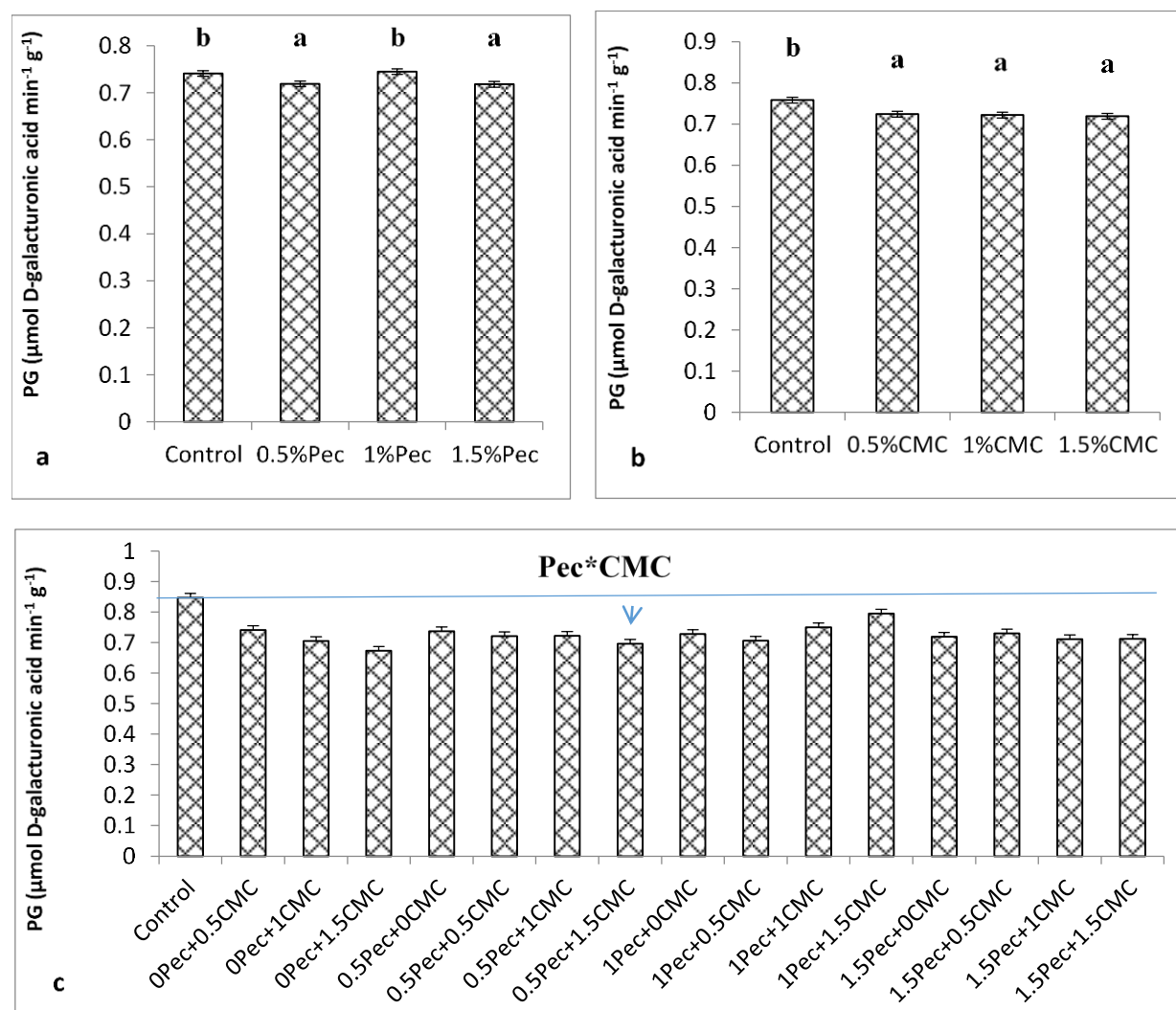


Fig. 12: Effect of 0 (control), 0.5 (0.5%Pec), 1 (1% Pec) and 1.5 (1.5%Pec) % pectin-based edible coatings (a), 0 (control), 0.5 (0.5%CMC), 1 (1% CMC) and 1.5 (1.5%CMC)% carboxymethylcellulose-based edible coatings (b) and their combinations (c) on polygalacturonase (PG) enzyme activity during cold storage period. Data are the “estimated marginal means \pm 95% confidence intervals”. The results were expressed on a fresh weight basis.

4. Discussion

In general, degradation of organic acids into sugars through respiration process decreases TA after harvesting [16,31]. Moreover, organic acids utilization as carbon skeleton for synthesis of new

compounds could be another reason for TA reduction [16]. Delay in fruit ripening [5] and maturation [32] caused by coating might reduce respiratory metabolisms involved in TA loss that in turn could enhance TA maintenance. Positive effects of coatings on TA maintenance have been previously reported [24,33]. Although, Panahirad *et al.* [8] reported positive effect of 1 % CMC on retarding TA loss of plum during shelf life; however, 0.5 and 1.5 % were not effective in this regard. This difference between results of two studies could be referred to different storage conditions.

Decrease in cell wall enzymes activities might be a probable reason for firmness preservation as stated by Sanchis *et al.* [23] and Kumar *et al.* [24] due to ripening delay by coating application [6]. Polygalacturonase (PG) enzyme is one of the main softening enzymes in plum [34]. Reduction in PG activity after coating (Fig. 12) also reflects importance of this enzyme in plum softening. PG activity depends on respiration and ethylene production. Therefore, lack of O₂ delays ethylene biosynthesis and subsequent changes in the fruit texture. Consequently, controlling O₂ availability and henceforth modifying internal gas composition by edible coatings decrease oxidative metabolism and delay textural changes in coated fruit [32,35]. This can be another possible reason for firmness preservation here. The existence of carboxylic groups in chemical structure of CMC may cause a positive effect on firmness preservation [18]. Also, retarding conversion of insoluble pectins to soluble ones that reduce soluble pectic fractions might be another reason for this positive effect [6,16]. Positive effect of CMC- and Pec- based edible coatings on firmness preservation has been previously reported [8,33]. Martinez-Romero *et al.* [36] also reported positive effect of coatings on plum firmness.

Antioxidant activity of vit C causes its own decrease during postharvest storage [6,19]. Ascorbic-acid oxidase and polyphenol oxidase modify vit C content whose activities directly depend on O₂

content of environment. Consequently, decrease in the respiration rate of coated fruit could be a reason for vit C preservation [37]. The higher amount of vit C in coated fruit could be explained by O₂ and CO₂ transmission rate through coating layer [31] as an apparent character of edible coatings by forming a semi-permeable barrier against gases and water vapor as well as solute movements. Polysaccharide-based edible coatings provoke low O₂ permeability properties and decrease the respiration rate in case of forming a good coating [22]. Oms-Oliu *et al.* [38] reported that Pec-based edible coating caused a lower O₂ and an upper CO₂ in the coated pears. Also, retention of vit C in Pec-coated pear (containing additives) was related to the loss or reduction of O₂ diffusion. It seems that our vit C results, in most aspects, could be interpreted by mentioned explanations. Also, the reduction in PPO activity (Fig. 11) by the applied coatings could describe vit C preservation in the coated plums. Menezes and Athmaselvi [33] reported similar result in retention of vit C content by Pec coating. Kowalczyk *et al.* [35] and Kumar *et al.* [24] stated retention in vit C after coating application. The vit C results of the current study are in agreement with our previous study [8].

TSS is a crucial quality factor and its amount at harvest time and its changes during warehousing are important for customer approval [34]. Forming a thin layer on fruit surface by coating reduces evaporation, delays degradation and diminishes respiration rate, which altogether might account for its positive effect on prevention of TSS changes during storage. Positive effect of different edible coatings on TSS has been previously reported [24,31,33].

Eum *et al.* [5] stated that versasheen[®] (a carbohydrate-based coating) edible coating significantly delayed pH enhancement in plum fruit and hence the coated fruit had a lower pH. Vyas *et al.* [17] reported that the control fruit had a higher pH than CMC-coated ones and the least pH amounts was recorded at 1 % CMC-coated papayas. The pH changes in mandarins coated with CMC

depended on the concentration of coating; the higher CMC concentration, the higher pH and the lower concentration along with beeswax, the lower pH. So, the control fruit had higher pH than the coated ones [31]. Menezes and Athmaselvi [33] reported a lower pH in Pec-coated fruit (in combination with additives). Aitboulahsen *et al.* [39] also stated a positive effect of coating on pH. Therefore, our pH outcomes were in agreement with the mentioned studies but in disagreement with our previous result.

Phenolic content contributes directly to antioxidative action [3,40]. The increase in phenolic, anthocyanin and flavonoid contents was associated with less PPO activity in the coated fruit as PPO causes phenol oxidation and anthocyanin degradation [26] and flavonoids oxidization as well [37]. In current study, the reduction in PPO activity in the coated plums could be the reason for the enhancement of phenolics, anthocyanins and flavonoids. Previously, it has been reported that Pec-based edible coating containing additives (such as anti-browning, apple fiber and antioxidants) caused higher phenolic compounds [20,38]. CMC-based coating has also resulted in higher phenolic compounds [17]. Positive effect of different coatings (alone or in combination with additives) on total phenolics has been reported [22,24,35,39]. Panahirad *et al.* [8] reported no positive effect of CMC on total phenolics; nevertheless, CMC-coating caused a positive effect on anthocyanins and flavonoids contents. Chitosan coating caused higher quantities of phenolic compounds, anthocyanins and flavonoids [41] and a decline in the activity of PPO [26,42]. Ayala-Zavala *et al.* [20] reported higher flavonoids content in Pec-coated peaches. Guerriero *et al.* [22] also reported flavonoids enhancement in coated fruit.

The vit C has also antioxidative properties, however phenolic compounds along with anthocyanins and flavonoids are the main radical scavenging molecules [3,34,40]. Correspondingly, once the amount of all mentioned compounds increases, the overall antioxidative capacity and the activity

of antioxidant enzymes like POD improve [26,37,43]. Delay in vit C diminution might be another reason for elevated antioxidative capacity [41]. The coatings, which diminish respiration, cause lower PPO and ascorbic acid oxidase activities [37] that may preserve or enhance the antioxidant capacity. Thus, preservation and enhancement of vit C, phenolic compounds, anthocyanins and flavonoids by the coatings could be the main reason for total antioxidant capacity enhancement in the current study. In addition, upper POD (Fig. 10) and lower PPO activities caused by the coating could be other reasons for this property. The increasing trend using DPPH method in plum fruit during storage was previously reported [8,44]. The antioxidant capacity enhancement was also observed in apple pieces coated with pectin in combination with pulse light treatment [45]. Oms-Oliu *et al.* [38] and Ayala-Zavala *et al.* [20] reported the same positive results using DPPH method on Pec-coated fruits. Ali *et al.* [46], Guerriero *et al.* [22] and Kumar *et al.* [24] all reported maintaining or enhancement in antioxidant capacity of coated fruits.

POD activity enhancement after application of Pec and CMC coatings has been reported by Ramirez *et al.* [21] and Panahirad *et al.* [8] in nectarine and plum, respectively. Enhancement in POD activity could reduce the level of harmful radicals and consequently improve antioxidant properties and postharvest quality of fruit life by preventing loss of nutritional values. The positive effect of the applied coatings might be referred to formation of a semi-permeable barrier on fruit surface that restricts gas exchange and reduces water loss. This property delayed physiological and biochemical changes that could result in quality preservation and strengthening antioxidant defense system. In fact, slowing down the procedures involved in senescence, ripening and decay might be considered as the main reason for POD enzyme enhancement by the applied coatings.

In storage, higher PPO activity and phenolics oxidation happen due to senescence-related processes especially the destruction of biological barriers between PPO and polyphenols that

activates the enzyme [19]. The coatings that cause a higher CO₂ and a lower O₂ decrease PPO activity [23,37]. In addition, reduction in pH can decrease the enzyme activity [23]. Decline in PPO activity by coating has been previously reported [8,23,26,42]. Storage improvement by decreasing O₂ availability, preservation of cellular compartmentation, protecting membrane structure from peroxidation and pH reduction due to slowing down senescence and delaying softening can be considered as possible reasons for the lower PPO activity.

5. Conclusion

The current survey reported positive effects of CMC- and Pec-based edible coatings, either alone or in combination with each other, on plum fruit through cold storage in terms of the measured parameters, except weight loss. The coatings especially improved vit C, total phenolics, anthocyanins and flavonoids contents and POD enzyme activity and decreased PPO and PG enzymes activities. Thus, application of CMC and/or Pec and/or their combinations might be considered as a favorable and safe approach for extending and improving postharvest qualitative characteristics of plum fruit.

Author Contributions

N.M. and R.N.H. and S.P. designed the experiment. S.P. performed the work and wrote the first draft of the manuscript. N.M. and S.P. analyzed data. N.M. overall supervised the work and reviewed and edited the manuscript. R.N.H. and R.K. read the manuscript, gave valuable comments and improved its quality. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

Submitted work was not carried out in the presence of any personal, professional, or financial relationships that could be potentially construed as a conflict of interest.

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