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Effect of Edible Coating on the Quality and Antioxidant Enzymatic Activity of Postharvest Sweet Cherry (*Prunus avium* L.) during Storage

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Abstract: The effects of edible chitosan coating (0.1%, 0.3%, 0.5% and 0.75% *w*/*v*) on the changes in the quality, respiration rate, total phenolic content and anthocyanin of postharvest sweet cherry (*Prunus avium* L.) at 10 °C were investigated. The activities of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) were also determined. The result showed that the treatments of chitosan edible coating were effective at delaying the evolution of the parameters related to postharvest ripening, such as color and firmness, and respiration rate. The edible coatings also showed that the lower total phenolics and total antioxidant activity were maintained compared to that in the control associated with the overripening. It was suggested that the optimal quality and enhanced antioxidant enzymatic activities of postharvest cherry fruits were obtained by an edible coating of chitosan 0.5% up to 24 days at 10 °C. The chitosan edible coating could be favorable for extending shelf-life, maintaining the quality of sweet cherries.

Keywords: sweet cherry; edible coating; quality; antioxidant enzymatic activities

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1. Introduction

Sweet cherries (*Prunus avium* L.) are more and more popular fruits due to their color, nutritional value and taste. Sweet cherries have been such an attractive fruit crop for consumption due to their precocity and excellent quality. Recent attention on the health benefits of cherries has further helped boost their consumption. Sweet cherries, in particular, have been found to offer a good source of phenolic compounds that boost the fruit antioxidant activity including fiber, carotenoids, vitamin C and anthocyanins [1].

Fruit size, bright red color, firmness and flavor are major postharvest sweet cherry quality attributes [2]. The quality of cherries deteriorates rapidly after harvest. The main cause is their relatively high metabolic activity, which leads to loss of weight, changes of chroma, surface pitting, stem browning, softening and loss of acidity [3,4]. The food industry is constantly searching for effective and safe means to control these problems. Of the various preservation techniques, sweet cherry stored at low temperatures is the most preferred by the consumers. The preservation method reduces water loss rate and inhibits pathogen growth and maintains the freshness of sweet cherry. However, biochemical reactions of the quality deterioration of sweet cherry is not inhibited completely at low temperatures. Moreover, temperature-sensitive fruits such as sweet cherries need to be stored at an appropriate temperature to prevent cold damage [4]. The application of fruit edible coatings is another new trend in sweet cherry preservation. Edible coatings including various polysaccharides, proteins, lipids composite coatings have certain advantages in maintaining quality attributes. The edible coating has been widely used in recent years to maintain quality of fruit [5]. It leads to reductions in respiration rate and transpiration

and to slowing the ripening process by decreasing permeability to O_2, CO_2, and water vapor [6–8].

Chitosan has received a great deal of attention from the food industries. This derivative of chitin is a copolymer of N-acetylglucosamine and glucosamine residues linked by β-1,4-glycosidic bonds and is insoluble in dilute acids. Chitosan has been proven one of the best edible and biologically safe preservative coatings for different types of fruits and vegetables because of its film forming properties, antimicrobial actions, nontoxic, biodegradability and biocompatible properties [9]. Chitosan edible coatings can modify the internal atmosphere, decrease transpiration loss, and delay the ripening of fruits and vegetables due to its ability to form a semipermeable film that is durable and flexible. All these properties provide advantages to chitosan compared to other edible coatings [10]. Several studies have indicated that chitosan has the beneficial effects for food preservation in recent years [8–11]. A study showed chitosan treatment significantly inhibited the bacterial growth on the surface of Indian oil sardines and reduced the formation of volatile bases and oxidation products [12]. Another study demonstrated that the combination of lemon essential oil and chitosan induces a beneficial preservation of strawberries with fungal decay [13].

It is worth noting that when chitosan comes into contact with plant tissue, it is associated with the antioxidant capability of the fruit and the control of oxidative stress in fresh fruit [14]. In general, enzymatic and non-enzymatic systems are two types of antioxidant defense systems. Superoxide dismutases (SOD), ascorbate per-oxidases (APX) and catalases (CAT) are major enzymatic antioxidants. These enzymes are effective in scavenging reactive oxygen species (ROS). Although some reports showed the impact of the application of several edible coatings on the quality of food, no information was shown on the effect of chitosan coating on sweet cherry in terms of antioxidant capacity. Thus, the aim of this study was to analyze the effect of chitosan, applied as an edible coating at four concentrations (0.1%, 0.3%, 0.5% and 0.75% w/v), on the quality, antioxidant activity and bioactive compounds of sweet cherry cultivar during storage time.

2. Materials and Methods

2.1. Fruit Materials and Treatments

The sweet cherry (*Prunus avium* L. cv. Summit) used in this study was obtained from Yingyuan farm near Jinzhou, Dalian, Liaoning, China. Sweet cherries with the same size and color were selected and randomized for the experiments. The quality of sweet cherry involving chroma, firmness, total phenols and total anthocyanins were analyzed. Chitosan film-forming solution was prepared according to the procedure of Petriccione et al., (2015) [15]. Chitosan (Solarbio, Beijing, China) was dissolved in an aqueous solution including acetic acid (0.5% v/v). The solution was heated and stirred at 45 °C to dissolve the chitosan. The pH was adjusted to 5.6 with 1.0 mol/L NaOH, and Tween-80 (0.05 g/100 mL) for all solutions. Sweet cherries were soaked and coated with different concentrations of 0.1%, 0.3%, 0.5% and 0.75% w/v chitosan in this study. The treatments consisted of immersing the sweet cherry sample for 5 min at 20 °C in: (a) 0.1% w/v chitosan; (b) 0.3% w/v chitosan; (c) 0.5% w/v chitosan; and (d) 0.75% w/v chitosan. The uncoated sweet cherry was the control. The treated and control samples were air-dried for 2 h at 20 °C, then packaged into plastic polyethylene bags at 10 °C.

2.2. Extraction

Sweet cherry (100 g) was soaked in liquid nitrogen and ground using a blender. Samples were mixed with an extraction solution of 10mL including acetic acid 2%, water 28%, and acetone 70%. Cherry tissues were centrifuged for 15 min at 10,000× g in a Multifuge 3S-R (Kendro, Hanau, Germany). The extract of cherry was made up to 25 mL with acidified water (0.01% HCl [v/v] in deionized, distilled water) and stored at −80 °C until subsequent analyses.

2.3. Determination of Fruit Colour

Surface color (L^*, a^* and b^* values) was detected using a colorimeter (Minolta, Model CR-400, Osaka, Japan). L^* represents color lightness, 0 is black and 100 is white. The color of each sample was detected at 0, 6, 12, 18, 24 days. Chroma was calculated as $C^* = (a^{*2} + b^{*2})^{1/2}$ for color change measurements in the fruit [16]. The measurements were performed at the same marked sample zone of each sweet cherry. A high chroma value represents a highly saturated and intense color while a low value stands for dull colors.

2.4. Determination of Fruit Firmness

The firmness of the sweet cherries was determined using the texture analyzer (model TA.XT2; Stable Micro Systems Texture Technologies, Scarsdale, NY, USA). Firmness was measured using a 3 mm-diameter flat-plate probe. The maximum force (N) generated during probe travel was used for data analysis. Results were expressed as the slope of the curve in N cm^{-2}.

2.5. Determination of Respiration Rate

The respiration rate was measured with a CO_2 Gas Detector (Alnor Compu-flow, Model 8650, Alnor USA, Los Angeles, CA, USA). For 20 min, 200 g of sweet cherries were enclosed in tightly-sealed hermetic flasks. The sensor was used to measure CO_2 and calculate the respiration rate. Results were expressed as milligrams CO_2 per kilogram per hour.

2.6. Determination of Antioxidant Contents

2.6.1. Total Phenol (TP) Content

Chitosan coating samples (100 g) were mixed with 400 mL of 80% ethanol and homogenized until reaching a uniform consistency using a digital homogenizer (T25, Guangzhou Guangpeng, Guangzhou, China) to obtain 0.25 g mL^{-1} ethanol extract. The obtained mixtures were preserved in covered centrifuge tubes for ultrasound extraction for 40 min in darkness at 40 °C and were subsequently centrifuged at 12,000× g for 20 min and the TP Content of the supernatant was determined using a modification of the Folin–Ciocalteu method [17]. One milliliter of 0.25 g mL^{-1} supernatant was added to 1 mL of Folin–Ciocalteu reagent. After that, 10 mL of 7.5% (w/v) Na_2CO_3 solution and 13 mL of distilled water was added, and the mixtures were incubated at 25 °C for 90 min before measuring at 765 nm. TP content was expressed as mg kg^{-1} of gallic acid equivalent on a fresh weight tissue basis, based on a standard curve ($Y = 0.0051\ X + 0.0178$, $R^2 = 0.99$) prepared with a standard gallic acid solution.

2.6.2. Total Anthocyanin Content

Anthocyanin quantification of the chitosan film samples (100 g) was performed on a UV-visible spectrophotometer (Shimadzu UV-1601, Norcross, GA, USA) by the pH-differential method [18]. Calculation of the anthocyanins concentration was based on a cyanidin-3-glucoside molar extinction coefficient of 26,900 and a molecular mass of 449.2 g/mol. Measurements were replicated three times with means being reported. Results were expressed as milligrams of cyanidin-3-glucoside equivalent (CGE) per 100 g of fresh weight.

2.7. Determination of Antioxidant Enzyme Activity

Freeze-dried samples (5 g) were homogenized with a potassium phosphate buffer (pH 7.0) of 50 mM containing EDTA of 3 mM and polyvinyl pyrrolidone (PVP) of 0.1 g. The mixture was centrifuged for 20 min at 15,000× g at 4 °C. The activity of catalase (CAT), superoxide dismutase (SOD) and ascorbate peroxidases (APX) was determined immediately.

SOD activity was assayed by the method of Duan et al., (2011) [19]. One unit (U) of SOD activity was defined as the amount of enzyme required to cause a 50% inhibition of the reduction of nitro blue tetrazolium (NBT), as monitored at 560 nm. APX activity was

determined spectrophotometrically at 290 nm according to the method of Karagiannis et al., (2018) [20]. CAT activity was measured by monitoring the disappearance of H_2O_2 by recording the decrease in absorbance at 240 nm of a reaction mixture containing 50 mmol/L sodium phosphate buffer (pH 7.0), 12.5 mmol/L H_2O_2, and 20 µL of enzyme extract. One unit of CAT activity is defined as the amount of enzyme that decomposes 1 µmol of H_2O_2 per minute per milligram of protein under the conditions of the assay, according to the method of Acero et al., (2019) [17]. The specific activity of the enzyme was expressed as unit mg protein$^{-1.}$ The experiment was repeated three times.

2.8. Statistical Analyses

All experiments were conducted in triplicate, as independent experiments. Data were measured using the Statistical Package for the Social Sciences (SPSS, Version 14.0, IBM Corp., Armonk, NY, USA). The significance of the differences between variables was tested using a one-way ANOVA (between groups) and repeated measures of ANOVA (within group). The means were compared using Duncan's multiple range test. Statistical significance was determined at $p < 0.05$.

3. Results and Discussion

3.1. Colour and Firmness

Appearance, color and firmness are the most important quality attributes because consumers usually judge the quality of fruits on the basis of appearance [21]. Thus, in this study, we first investigated the effect of chitosan with different concentrations on the appearance of sweet cherries in order to search for an effective and safe means to control physiological problems such as browning and softening.

As shown in Figure 1A, the chroma index value at harvest was 27.46 and did not significantly change ($p > 0.05$) during the first 12 days of chitosan treatment. The surface color saturation rapidly decreased with increasing storage time. However, this change was slowed down for sweet cherry treated with 0.5% w/v chitosan. After 18 days of storage, the fruit coated with 0.5% w/v chitosan maintained significantly higher chroma values (19.2 ± 0.72) than those with other chitosan concentrations and the control (10.59 ± 6.36). The differences were even much higher between 0.5% w/v chitosan and other treatments ($p < 0.05$) when observed on the 24th day. Instead, no significant difference ($p > 0.05$) was observed between control and chitosan concentration-coated fruit (0.1% and 0.3% w/v). Interestingly, the addition of 0.75% chitosan led to a greater reduction in chroma index as compared to cherries treated with 0.5% w/v chitosan, and no significant change was observed between cherries treated with the highest chitosan concentration and those treated with the low concentrations (0.1% and 0.3%). The reason is probably that the chitosan with a remarkably high concentration leads to higher intermolecular and interparticle forces, and thereby decreases the liquidity of the films, increases their stiffness and enhances their elongation at break, thus leading to the crack of films and disabling the anti-oxidant protection for the sweet cherries. Therefore, we propose that chitosan with 0.5% concentration is most sufficient in controlling browning and maintaining the commercial value of fresh products.

In addition, firmness is the parameter of greatest concern in sweet cherry storage and marketing, because flesh softening is associated with senescence and fruit injuries. the firmness of control and chitosan-treated (0.1% w/v) sweet cherries exhibited similar trends of softening, and decreased during the first 6 days of storage, showing that such low concentrations of chitosan could not protect sweet cherries from oxidant effects (Figure 1B). On the other hand, chitosan with high concentrations (0.5% and 0.75% w/v) efficiently delay softening trends. However, the firmness values of fruit treated at the two concentrations diverged markedly after 12 days. The final values were 25.63 ± 0.57 N/cm^2 for 0.5% w/v and 11.83 ± 0.76 N/cm^2 for 0.75% w/v at 24 days. This result indicates that the highest concentration is not the most efficient concentration, probably because it induces the crisp of the film. Since firmness at 25 N/cm^2 or higher is an indication of fruit not softening

fully [22], it is reasonable to indicate that 0.5% chitosan markedly slows down fruit softening, and thus is suitable for maintaining the firmness of sweet cherries during storage.

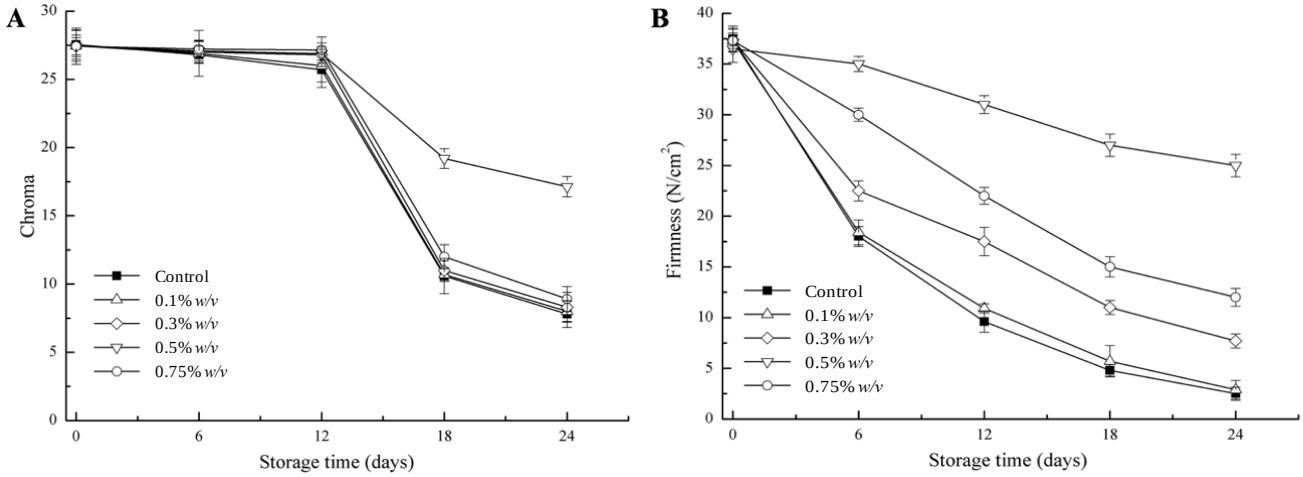

Figure 1. Change of chroma (**A**) and firmness (**B**) in sweet cherry stored at 10 °C during storage time. The data are the means ± standard-deviation (SD).

3.2. Respiration Rate

Respiration rate is a critical index for evaluating the storability of fruit, and it increases with tissue damage and aging [23]. Changes of respiration and storability (delay respiration and consume nutrition) happen during the metamorphic process [24]. In this study, the respiration rate of sweet cherry increases during storage and chitosan treatment can delay the increase to some extent (Figure 2). The respiration rate of high concentrations (0.5% and 0.75% w/v) of chitosan-treated sweet cherries had significant decreases compared with the control and low concentrations (0.1% and 0.3% w/v) of chitosan-treated fruit during the storage ($p < 0.05$), which is consistent with the results of Dong et al. [25]. From the 12th day of storage, the respiration rate of sweet cherry in the 0.5% chitosan treatment group increased more slowly than that in the 0.75% chitosan group. The results clearly indicate that 0.5% w/v chitosan exhibits the maximal response in the inhibition of respiratory intensity in sweet cherries.

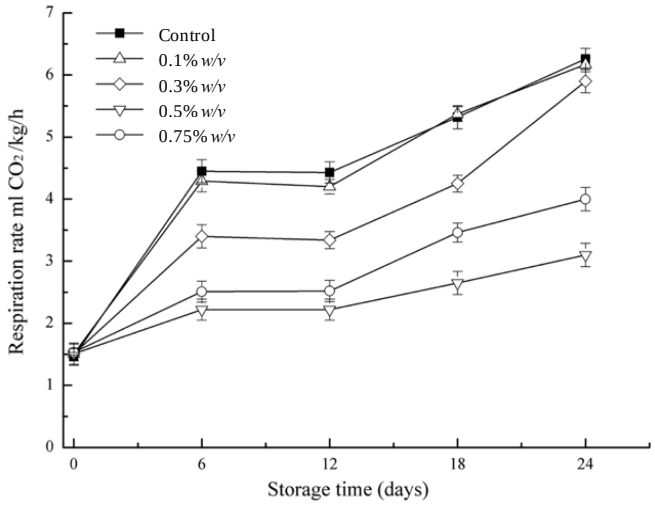

Figure 2. Change of respiration rate in sweet cherry stored at 10 °C during storage time. The data are the means ± standard-deviation (SD).

3.3. Effect on Phenolic and Anthocyanin Compounds

Phenolic compounds can improve fruit quality and nutritional value by improving fruit color, taste, aroma and flavor. Anthocyanins are responsible for the red, blue and orange colors of fruits [26,27]. Anthocyanins and phenolics compounds have attracted much attention as food ingredients [28,29]. Hence it is necessary to screen the phenolic properties of sweet cherry to further evaluate the coating efficiency of chitosan [30,31]. As for phenolic compounds, the content of phenolic compounds was established at harvest (70.2 mg100 g^{-1}) in uncoated fruits until day 12 of storage, then decreased significantly until the end of storage (52.00 mg 100 g^{-1}) (Figure 3A). However, total phenolics increased continuously in sweet cherries treated with the chitosan coating. Different concentrations have different effects on the total phenols of sweet cherry. The sweet cherry in a 0.3% chitosan coating showed the highest phenolic compounds with 66.50 ± 2.37 mg 100 g^{-1} at 24 days. Hence, the quality of sweet cherries coated with chitosan was better than that of uncoated sweet cherries [28]. The main phenolic compounds of sweet cherry are hydroxylcinnamic acids caffeoyl tartaric acid and 3-p-coumaroyl-quinic acid, which contribute to the flavor quality of sweet cherry [32]. Chitosan can form a transparent coating on the surface of the fruit; this coating allows the passage of O_2, not CO_2 and H_2O. Thereby, this high CO_2 low O_2 environment can inhibit the respiration of strawberries and delay the degradation of respiratory substrate, thus delaying the accumulation of secondary metabolites, such as anthocyanins and phenolic substances, to achieve the purpose of keeping fresh.

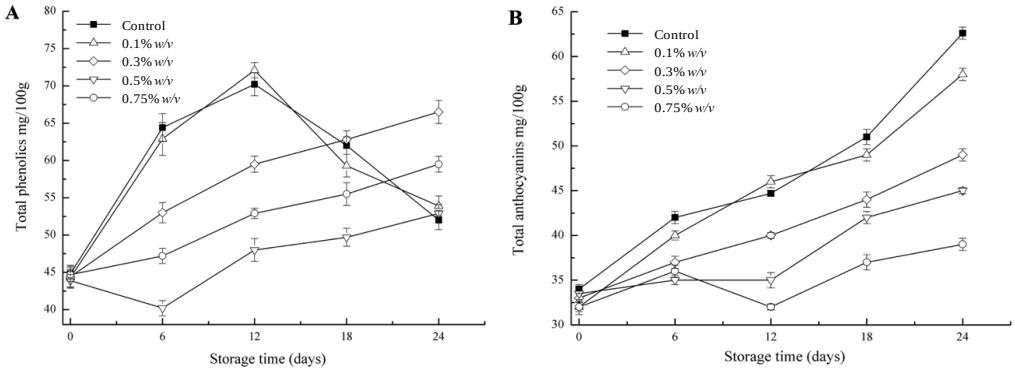

Figure 3. Change of total phenolic contents (**A**), total anthocyanin contents (**B**) in sweet cherry stored at 10 °C during storage time. The data are the means ± standard-deviation (SD).

Anthocyanin content is easily affected and degraded by light and temperature in fruit. Genotype characteristics, irrigation, plant density and fertilization also affect anthocyanin content in fruits. In this study, the anthocyanin amount of sweet cherry treated with chitosan edible coating is lower than that of the control. The concentration increased during the storage period (Figure 3B), and this increase was delayed in sweet cherry coated with chitosan in a concentration-dependent manner. In the fruits treated with chitosan at 0.75%, anthocyanin concentration was 39.00 mg 100 g^{-1} after 24 days, while these values were significant different with 58.00 mg 100 g^{-1}, 49.00 mg 100 g^{-1} and 45.00 mg 100 g^{-1} for fruits coated with chitosan at 0.1%, 0.3% and 0.5% w/v at day 24, respectively ($p < 0.05$). Some studies have also demonstrated that the chitosan edible coating decelerated anthocyanin synthesis in treated strawberries and the effects increased with higher chitosan concentration [33,34]. Anthocyanin concentration can increase after harvest during cold storage in pomegranate, sweet cherry, strawberry and raspberry [35–39]. In addition, Kim et al. have reported that anthocyanin contents ranged from 1–432 mg 100g^{-1} in different sweet cherry genotypes [1], showing the reliability of our data.

3.4. Antioxidant Enzymes

In plants, antioxidant enzymes, mainly including SOD, CAT and APX, catalyze reactions to neutralize free radicals and ROS, thereby forming the body's endogenous defense

mechanisms to help protect against free radical-induced cell damage [40]. In this study, SOD, CAT and APX were detected in sweet cherries to further estimate the effect of chitosan film on antioxidant enzymatic activities.

SOD as a major antioxidant enzyme in cells catalyzes the breakdown of such an anion into oxygen and hydrogen peroxide [41,42]. As shown in Figure 4A, 0.3%, 0.5% and 0.75% chitosan treatment have an obvious enhancement of SOD activity in sweet cherry after 12 days of storage ($p < 0.05$). However, the effect of chitosan on SOD activity after 12 days of storage was significantly, obviously higher than before 12 days of storage. This result indicated that the effect of chitosan film on SOD activity was significantly influenced by storage time, probably because the superoxide anion content at a later storage was higher and then stimulated the SOD activity [19]. In addition, the result showed that the SOD activity of sweet cherry treated with chitosan edible coating (0.5%) is higher than those in chitosan edible coating (0.75%). This might be because the higher concentration chitosan edible coating (0.75%) prevents more oxygen transmittance and inhibits the production of reactive oxygen species (ROS) on the surface microenvironment of sweet cherry. Some studies have demonstrated that lower ROS do not effectively regulate SOD enzyme activities by activating the signal molecules of a plant [43,44]. Therefore, the SOD and APX activities of sweet cherry treated with chitosan coating (0.75%) were lower than those in the 0.5% treatment group. The chitosan coating treatment could be an effective method for enhancing SOD activity in postharvest fruits and vegetables and the influence of chitosan on SOD activity is comparable to other treatments such as methyl-jasmonate and 1-MCP [45,46]. The increase of SOD may reduce free radical accumulation on fruit peel after chitosan treatment, thus leading to increased dismutation of radicals to hydrogen peroxide [47].

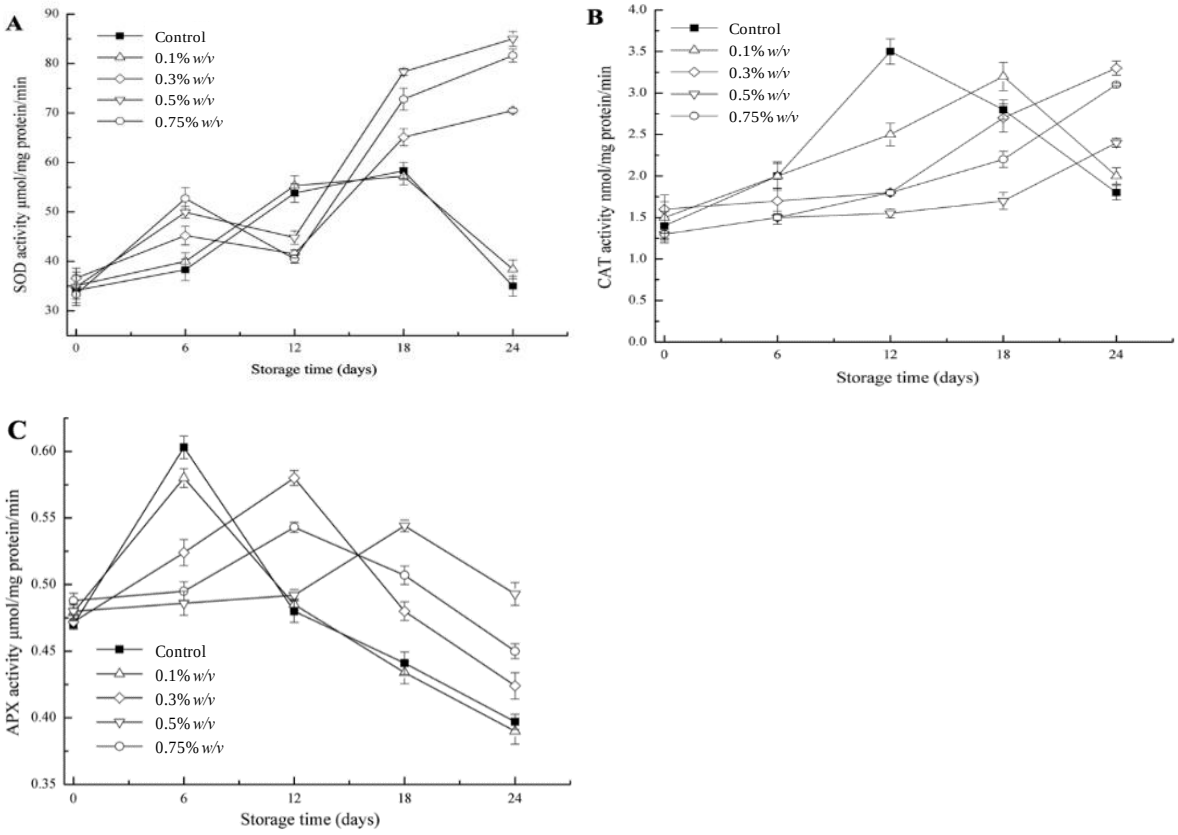

Figure 4. Change of SOD activity (**A**), CAT activity (**B**) and APX activity (**C**) in sweet cherry stored at 10 °C during storage time. The data are the means ± standard-deviation (SD).

CAT is a common enzyme found in nearly all living organisms that are exposed to oxygen, where it functions to catalyze the decomposition of hydrogen peroxide to water and oxygen in order to maintain H_2O_2 homeostasis in cells. As shown in Figure 4B, CAT activity sharply decreased after the 12th day in the control; however, 0.3%, 0.5% and 0.75% w/v chitosan coating retarded this reduction. Chitosan coating treatment has enhanced CAT activity, which only occurred after 18 days storage. Probably because the enhancement of SOD activity leads to H_2O_2 accumulation and then reduces CAT activity. The results show that 0.5% chitosan is most efficient in maintaining high CAT activity in sweet cherry ($p < 0.05$), which may be important in the defense mechanisms against oxidative stress. Except for CAT, APX is another efficient method for reducing power for H_2O_2 detoxification [48].

For APX, this type of enzyme uses ascorbate as a specific electron donor to catalyze the conversion of H_2O_2 into H_2O, and thereby detoxifies ROS in the ascorbate—glutathione cycle in plant cells [49]. In this study, APX activity in the control and 0.1% chitosan sweet cherry increased rapidly and then decreased significantly from the 6th to the 24th day of storage (from 0.59 to 0.40 μmol/mg/min). Exposure to 0.3%, 0.5% and 0.75% chitosan retard the reduction of APX activity, with the latest peak (0.54 ± 0.04 μmol/mg/min) found in the 0.5% w/v treatment at the 18th day. As shown in Figure 4C, the APX activity in 0.3%, 0.5% and 0.75% chitosan treatment groups increased significantly compared with the control; this result was similar to the change trend of SOD. An enhancement in APX activity observed in chitosan coating could be due to the availability of ascorbic acid in sweet cherry during storage [15]. These results suggested that chitosan coating has an obvious improvement on antioxidant enzymatic activity in postharvest sweet cherry during 10 °C storage.

4. Conclusions

The edible chitosan coating treatment is an effective method for delaying the postharvest ripening process of sweet cherry, which is manifested by reduced color changes, firmness losses and respiration rate, as well as a positive effect in maintaining higher concentrations of total phenolics and anthocyanins, and in delaying the increases of CAT, SOD and APX activities. Overall, it is suggested that the 0.5% chitosan coating is the most effective factor in storing the sweet cherries for 24 days at 10 °C.

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