

Article

Effect of Chitosan/Nano-TiO₂ Composite Coating on the Postharvest Quality of Blueberry Fruit

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Abstract: Blueberries are a rich source of health-promoting compounds such as vitamins and anthocyanins and show a high antioxidant capacity. Thus, considerable commercial and scientific interest exists in prolonging its postharvest life to meet the year-round demand for this fruit. In this investigation, the effect of a chitosan-based edible coating, as well as a chitosan-based edible coating containing nanosized titanium dioxide particles (CTS-TiO₂), on the postharvest quality of blueberry fruit quality was evaluated during storage at 0 °C. The blueberries were treated with a chitosan coating (CTS) and a CTS-TiO₂ composite, respectively. The most suitable chitosan and nano-TiO₂ fraction concentrations to be incorporated in the coating formulation were prepared based on the wettability of the corresponding coating solutions. Changes in firmness, total soluble solids (TSS), titratable acidity (TA), ascorbic acid (VC), malondialdehyde (MDA), polyphenol oxidase (PPO), and peroxidase (POD) activities, anthocyanins, flavonoids, total phenolic content, and microbiological analysis were measured and compared. This combined treatment prevented product corruption. Compared with CTS, the CTS-TiO₂ composite coating application effectively slowed down the decrease in firmness, TSS, VC, and TA in the blueberries. Additionally, changes in the total polyphenol, anthocyanin, and flavonoid contents and the antioxidant capacity of CTS-TiO₂ composite coating blueberry fruits were delayed. Therefore, these results indicated that the chitosan/nano-TiO₂ composite coating could maintain the nutrient composition of blueberries while playing a significant role in preserving the quality of fruit at 0 °C.

Keywords: Nano-TiO₂; chitosan; composite film; physicochemical indexes; storage quality



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1. Introduction

Blueberries (*Vaccinium* spp.) are popular among consumers due to their rich nutritional composition and distinctive flavor and are praised as “the king of berries.” They are currently considered among the most valuable fruits worldwide due to their organoleptic and nutritional properties, and particularly their composition in ascorbic acid (VC), flavonoids, phenolics, and anthocyanins. Blueberries also contain essential enzymes, such as polyphenol oxidase (PPO) and peroxidase (POD). Many studies have indicated that blueberries can inhibit the cell proliferation of cancer, diabetes, and obesity [1,2]. They are exceedingly sensitive to storage conditions and last only 2–5 days without preservatives or cold storage, depending on the cultivar, but blueberry fruit can be stored for 28 days at 0 °C. However, postharvest blueberries are highly perishable and have a short storage life, which may be attributed to their high moisture content, high metabolism, and a lack of

protection from hard exocarps. Therefore, it is essential to maintain the microbiological safety and quality of postharvest blueberries technologically.

In recent years, edible films and coatings have been considered one of the technologies with significant potential to improve food safety while protecting it from the influence of external environmental factors, therefore increasing its shelf life [3]. One of the leading food applications of edible coatings involves the surfaces of fruit, such as strawberries, grapes, and tropical fruits. Jing et al. [4] indicated that blue mold rot in sweet cherries was reduced and controlled by dipping the fruits in chitosan. Mahunu [5] demonstrated that treating citrus fruit with a chitosan coating (CTS) controlled the decaying process during storage. Totad et al. [6] indicated that the freshness of fruits in blueberries was increased and controlled by treating them with edible coatings, and it was effective in extension of the shelf life of blueberry fruits. Rokayya et al. [7] also indicated that chitosan/nano-titanium dioxide (tween-thymol) (BC-NT-TT) coating treatment was proved to prolong storage life and quality parameters. Furthermore, Pobiega et al. [8] demonstrated that the pullulan coating with propolis extract has the potential as microbiological protection for blueberry fruit.

Chitosan is an excellent carrier of other functional substances, such as antimicrobials and antioxidants [9]. Furthermore, to improve the efficiency and stability of edible coatings/films, it is essential to use adequate materials.

Nanosized titanium dioxide particles (nano-TiO₂), which are white or transparent, are non-toxic and exhibit excellent viral sterilization and inhibition activity [10]. Nano-TiO₂ has been widely used in the food, pharmaceutical, cosmetic, and chemical industries due to its high resistance, nontoxicity, antibacterial activity, and unique photocatalytic property. Fang et al. [11] showed that chitosan/nano-TiO₂ (CTS-TiO₂) and chitosan/nano-SiO₂ composite coatings could effectively maintain postharvest qualities (decay rate, shrinkage rate, and firmness) by inhibiting the growth of pathogens. Moreover, chitosan containing nanosized TiO₂ film exhibited ethylene photodegradation activity when exposed to ultraviolet (UV) light, delaying the ripening process and inducing changes in the quality of the product [12,13]. The nanoparticles in the coating formulas played an essential role in preserving the quality, improving antimicrobial activity, and extending the storage period.

Coatings/films can be produced using a wide variety of products, such as polysaccharides, proteins, lipids, or resins, alone or, more often, in combination. Other ingredients, such as preservatives, antioxidants, and firming agents, can be added to coatings to improve the stability, appearance, and texture of the coated product [14]. Moreover, according to Xing et al. [15], the properties of the coatings can be enhanced by incorporating functional ingredients, such as anti-browning and antimicrobial agents, volatile nutraceutical precursors, and colors [16]. Several studies have reported that gauze treated with a CTS-TiO₂ composite emulsion displayed exceptional antibacterial activity against *Escherichia coli*, *Aspergillus niger*, and *Candida albicans* [17,18]. The purpose is to create a more efficient food system, aiming to reduce the degradation of the qualitative aspects during the postharvest period and lower loss rates to extend the shelf-life [19].

Therefore, this study aims to evaluate the effect of the combined application of a chitosan-based edible coating containing 2% chitosan + modified nano-TiO₂ on the quality and physiological characteristics of blueberries.

2. Materials and methods

2.1. Materials

Commercially mature “jiaao” blueberries (*Vaccinium* spp.) were obtained from a hundred orchard fruit supermarkets in Chengdu City, Sichuan, China, transported to the laboratory within 3 h, and removed from the branches. Healthy blueberries uniform in 2 g size, color, maturity, and shape were selected, washed with distilled water, and pre-cooled at 4 °C for 24 h. The chitosan was provided by the Shengpeimei Biological Engineering Co. Ltd. (Qingdao, China). Nano-TiO₂ (20–30 nm) was obtained from the Beijing Deke Island Gold Technology Co. Ltd. (Beijing, China). Sodium laurate,

glacial acetic acid, propylene glycol, potassium permanganate, calcium carbonate, sodium hydroxide, phenolphthalein, ethanol (50%), potassium acid phthalate, hydrochloric acid, potassium iodide, potassium iodate, soluble starch, trichloroacetic acid, 2-Thiobarbituric acid, methanol, glacial acetic acid, anhydrous sodium acetate, polyethylene glycol 6000, polyvinylpyrrolidone, triton X-100, catechol, guaiacol, hydrogen peroxide solution, sodium chloride, and L-ascorbic acid were purchased from the Chengdu Kelon Chemical Reagent Factory (Chengdu, China).

2.2. Coating Preparation and Sample Treatment

Nano-TiO₂ modification: The method to prepare the coating solutions was developed by Xing et al. [20]. Briefly, 1.0 g Nano-TiO₂ was dispersed in 100 mL of 0.050 mol/L sodium laurate solution, the pH was adjusted to 5.0, and the mixture was stirred at 40 ± 2 °C for 30 min. It was then filtered, rinsed, and dried in an oven for 1 h, after which the modified sodium laurate nano-TiO₂ was obtained.

The chitosan membrane was prepared according to the method described by Krishna et al. [21] with slight modifications. Here, 1.0 g chitosan and 0.5 g propylene glycol were slowly dissolved in a 100 mL glacial acetic acid solution at a volume fraction of 0.6% and stirred in a magnetic stirrer at 40 °C for 1–1.5 h, followed by ultrasonic degassing for 15–30 min. This process was repeated three times and labeled as the CTS.

Preparation of CTS-TiO₂ composite membrane: The modified nano-TiO₂ particles were dissolved in 2.0 g glycerin and a 100 mL glacial acetic acid solution, added at a volume concentration of 0.6%. Then, 1.0 g of chitosan was added slowly, after which the mixture was stirred at 40 °C for 2–3 h using a magnetic stirrer and ultrasonically degassed for about 30 min. Then, 1 g of modified nano-TiO₂ dioxide was prepared via dissolution in 2.0 g glycerin, followed by the addition of 100 mL glacial acetic acid (6% v/v), after which 1 g of chitosan was added and stirred for a further 2–3 h. The chitosan-based solution was ultrasonically degassed for about 30 min, left to stand for 1 h, and labeled as the CTS-TiO₂.

The samples were held over a plastic sieve for 30 min and then placed in polystyrene trays. All the treated samples were sealed with polypropylene film, while the films were prepared via evaporative casting. Briefly, a solution was deposited onto an acrylic plate and allowed to dry in controlled ambient conditions (25 ± 1 °C) for at least 2 days. It was then peeled off and pre-conditioned in a desiccator in controlled ambient conditions for at least 2 days before use. Furthermore, three treatments were conducted in this investigation: (1) no coating with control (CK), (2) CTS, (3) CTS-TiO₂. The pre-cooled blueberries were soaked in the coating solution for 2 min and placed in a cool, ventilated place for natural air-drying. The blueberries were then placed into 0.045 mm polyethylene bags with six air holes in each bag, sealed at the mouth, and stored at 0 °C.

2.3. Measurement of Firmness, TSS, TA, and Ascorbic Acid Content

The firmness was measured using a GY-III firmness tester with a 3.5 mm probe diameter (Shanghai Jingsheng Scientific Instruments Corporation, China). Six fruits were randomly measured, and the results were expressed as N/m² [22].

Tissue (5 g) from one fruit was homogenized and centrifuged at $10,000 \times g$ for 20 min. The supernatant was collected to measure the TSS using a digital refractometer (J1-3A, Shanghai Scientific Instruments, Shanghai, China).

The TA was determined according to a method described in previous research with slight modifications [23]. Ten grams of grated blueberry tissue was diluted in distilled water to reach a quantity of 100 mL. Each gram of sample was decolorized by adding 0.4 g of kaolin. Then, it is homogenized and filtered in a blender. The solution was transferred into a 250 mL beaker and placed over a magnetic stirrer to provide continuous motion to the sample solution. The juice was then titrated using standardized 0.1 mol/L NaOH at the phenolphthalein endpoint ($\text{pH} = 8.2 \pm 0.1$). The TA was expressed as grams per 100 g of acid.

The ascorbic acid content was measured via KIO_3 titration (GB/T601-2002, Code of National Standard of China). Briefly, blueberry tissue (50 g) was immediately homogenized in 50 mL of a 0.02 g/mL oxalic acid solution and then centrifuged at $15,000 \times g$ and 4°C for 15 min. Then, 10 mL of supernatant was titrated to a permanent pink color via 0.1% KIO_3 titration. The ascorbic acid content concentration was calculated according to the titration volume of the KIO_3 and expressed as mg/100 g fresh weight [24].

2.4. PPO and POD Activity

The PPO activity was assayed spectrophotometrically using a modified method by Xing et al. [25]. Fruit tissue (5.0 g) was homogenized in an ice bath with 5.0 mL extraction buffer (1 mmol PEG, 4% PVPP, and 1% TritonX-100) for 4 min. The obtained solution was centrifuged at 4°C and $13,000 \times g$ for 30 min. The reaction solution consisted of 4.0 mL substrate solution (0.05 mol/L catechol in 0.05 mol/L acetic acid-sodium acetate, pH 5.5) and 0.5 mL crude extract. The catechol oxidation rate was evaluated at 420 nm for 2 min at room temperature, and the activity unit was defined as an increase of 0.0001 in absorbance for 1 min.

The POD activity was evaluated according to the method described by Zeng et al. [26]. Here, 2.5 g of fruit tissue was homogenized in 10 mL PBS (25 mmol/L, pH 7.8, containing 1 mmol/L EDTA and 0.8 g/L PVPP) and then centrifuged at 4°C and $13,000 \times g$ for 30 min. Furthermore, 0.5 mL of enzyme extract was incubated in 2 mL buffered substrate (pH 6.4, 100 mmol/L sodium phosphate, and 25 mmol/L guaiacol) for 5 min, and the absorbance was measured at 470 nm every 30 s for 120 s after adding 0.2 mL of H_2O_2 at a concentration of 0.5 mmol/L.

2.5. MDA Content

Tissue (0.2 g) from the 2 g fresh blueberry fruit slices were homogenized with 3 mL of 10% TDA and centrifuged for 10 min at $10,000 \times g$. This process was followed by mixing 2 mL of the supernatant with 2 mL of 6.7 g/L TBA (previously dissolved in 10%). The obtained reaction solution was heat-treated for 30 min at 95°C , rapidly cooled in an ice bath, and centrifuged at $10,000 \times g$ for 10 min to clarify the precipitation. The absorbance of the supernatant was measured at 450, 532, and 600 nm, respectively, using a spectrophotometer (UV/VIS756-PC, T6, PG General, Beijing, China). The MDA content of each sample was determined using the following equation:

$$\text{MDA content } (\mu\text{mol/g} \cdot \text{mL}) = \frac{[6.452 \times (\text{OD}_{532} - \text{OD}_{600}) - 0.559 \times \text{OD}_{450}] \times V_t}{M \times V_s} \quad (1)$$

where V_t is the volume of the extract solution (mL), V_s is the volume of the extract solution contained in the reaction mixture (mL), and M is the mass of the fresh sample (g) [27].

2.6. Measurement of the Anthocyanins, Flavonoids, and Total Phenolics Content

The total anthocyanin content was measured using the pH differential method suggested by Kahramanoğlu [28]. The absorbance was measured spectrophotometrically at 510 and 700 nm in buffers of pH 1.0 and 4.5. The results were expressed as mg/100 g.

The methanol extracts for the different bioactive compound assays were obtained according to the method of Milena [22] with some modifications. Samples of 5 g of fresh weight were extracted with 25 mL of methanol (50% *v/v*). The homogenized samples from the methanol supernatants were subsequently centrifuged at $12,000 \times g$ for 15 min at 4°C . The resulting supernatants were mixed, filtered, and subsequently used for the following assays.

The flavonoid content was determined using the aluminum chloride colorimetric method with catechin as a standard. The total flavonoid content was expressed as milligrams of catechin equivalent (CE) per 100 g fresh weight (FW).

The total phenol content in the blueberry fruits was determined using the Folin-Ciocalteu method, and the results are expressed as milligrams of gallic acid equivalents (GAE) per 100 g fresh weight (FW) using gallic acid as a standard.

2.7. Measurement of the Aerobic Mesophilic Bacteria

The method used for the microbiological analysis was described by Bico et al. [29]. The sample slice (10 g) and a sterile solution (90 mL) were blended for 60 s using a stomacher. The sample solution (1 mL) at an appropriate dilution was pour-plated into PCA and incubated at 37 °C for 24 h to identify the aerobic mesophilic bacteria. Colonies' growth of blueberries was reported as log CFU (colony-forming unit)/grams.

2.8. Statistical Analysis

All analyses were carried out in triplicate, and data were expressed as means $\pm$ standard deviation. A one-way analysis of variance (ANOVA) was performed to calculate the significant differences in the treatment means, while multiple comparisons between the means were performed using the least significant difference (LSD) test. Correlations among the evaluated parameters were analyzed using Pearson's correlations. The experimental data were analyzed using SPSS version 20.

3. Results and Discussion

3.1. Firmness, TSS, TA, and Ascorbic Acid

One of the main indices used to determine fruit quality and postharvest shelf life is the rate and extent of firmness loss during storage. Firmness is an important physical parameter used to assess the quality of fruits during ripeness, storage, and distribution during harvest. Texture loss is the most noticeable change occurring in fruits during prolonged storage, and it is related to metabolic changes and water content [30–32]. According to the research by Sturm et al. [33], fruit softening occurs due to the degradation of cell wall components. Figure 1a showed that the firmness of all the blueberries at different treatments continuously decreased within 32 days of storage at 0 °C. The firmness decreased throughout the cold storage period in chitosan-coated and uncoated blueberry fruit with significant ($p < 0.01$) differences among and within the strawberry cultivars. The blueberry group treated with CTS-TiO₂ presented the highest firmness (1.99 N) after 32 days of storage at 0 °C. This treatment was also more effective in maintaining the firmness of strawberries compared to the CTS coating, although the difference between these two treatments was not significant ($p > 0.05$). After 32 days of storage, the loss in the firmness of the fruit in the CK groups was 30%, while strawberry cultivars coated with CTS and CTS-TiO₂ showed a reduction in firmness values of 16% and 12%, respectively.

The results indicated that the chitosan coating controlled the firmness loss of fruits more effectively during storage. The fruit samples treated with the CTS-TiO₂ coating presented a lower weight loss than CK ($p < 0.01$). CTS serves as a protective layer against water loss during the storage of the postharvest fruits [34–36]. Previous studies have reported the beneficial effects of several coating applications [37], such as cactus mucilage, chitosan-oleic acid, and chitosan combined with calcium dips and chitosan-beeswax, on the texture of strawberries [38,39].

The effect of different coatings on the postharvest TSS of the blueberries is shown in Figure 1b. The TSS represents a group of soluble compounds, such as sugar, acids, and vitamins, forming the primary substrates of respiration that are typically used to indicate the postharvest quality and maturity of fruit [40]. Figure 1b shows that when the storage time was increased, the TSS in fresh blueberries displayed a decline in all relevant samples. Additionally, Figure 1b indicates that the pulp TSS content of the CK group decreased rapidly after 8 days of storage. The fruit in the CTS coating group and CTS-TiO₂ coating group exhibited a rapid increase from day 4 to day 16 of storage, followed by an appreciable decrease until day 32 ($p < 0.05$). At the end of the storage period, the blueberries that were subjected to the CTS-TiO₂ coating exhibited a 36.9% higher TSS content than those in the

CK group ($p < 0.05$). In addition, the soluble solid content in the CK group decreased the most at 33.09%, while the decline in the CTS- TiO_2 and CTS coating groups only decreased by 9.26% and 11.99% ($p < 0.05$), respectively.

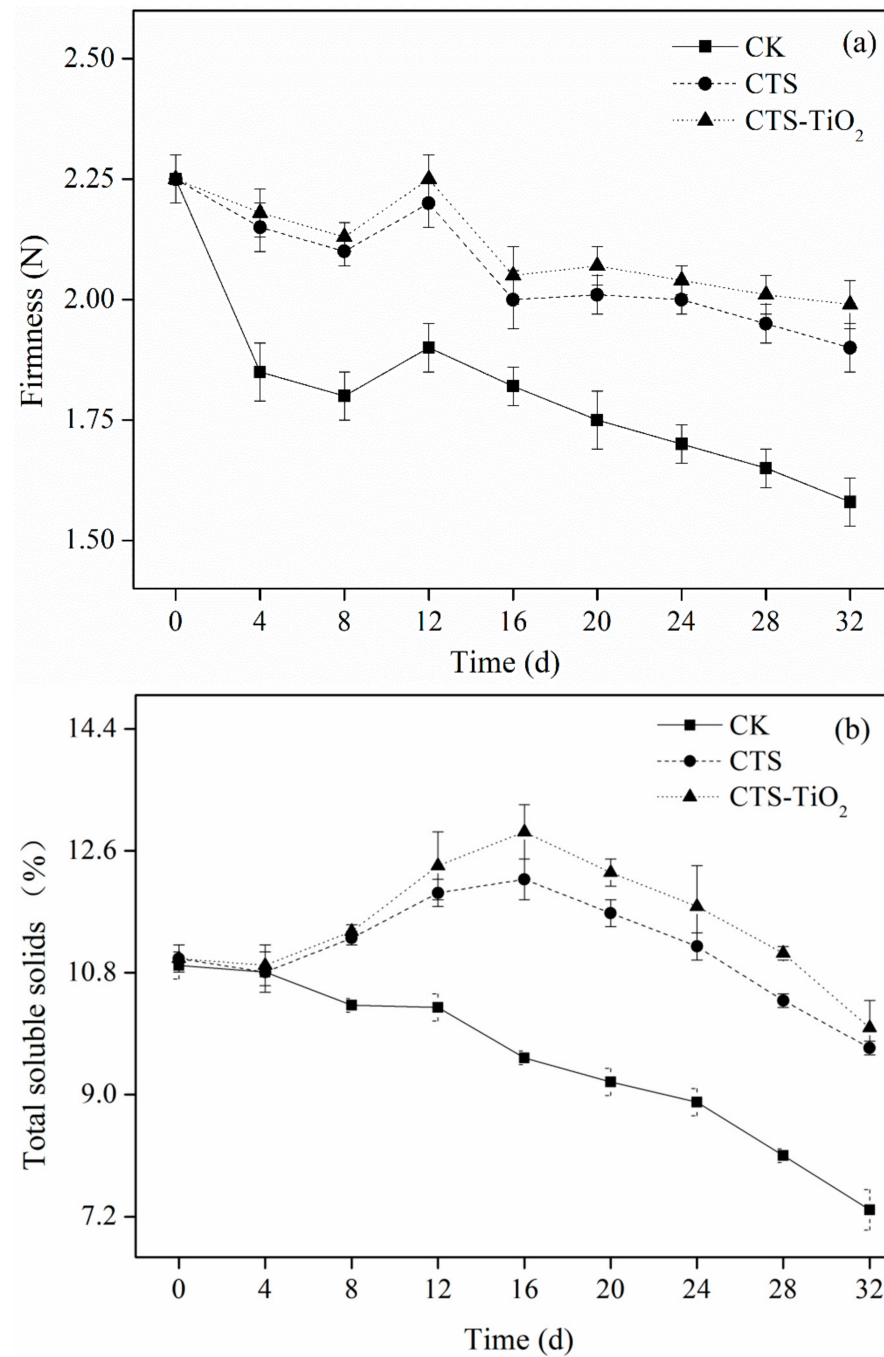


Figure 1. Cont.

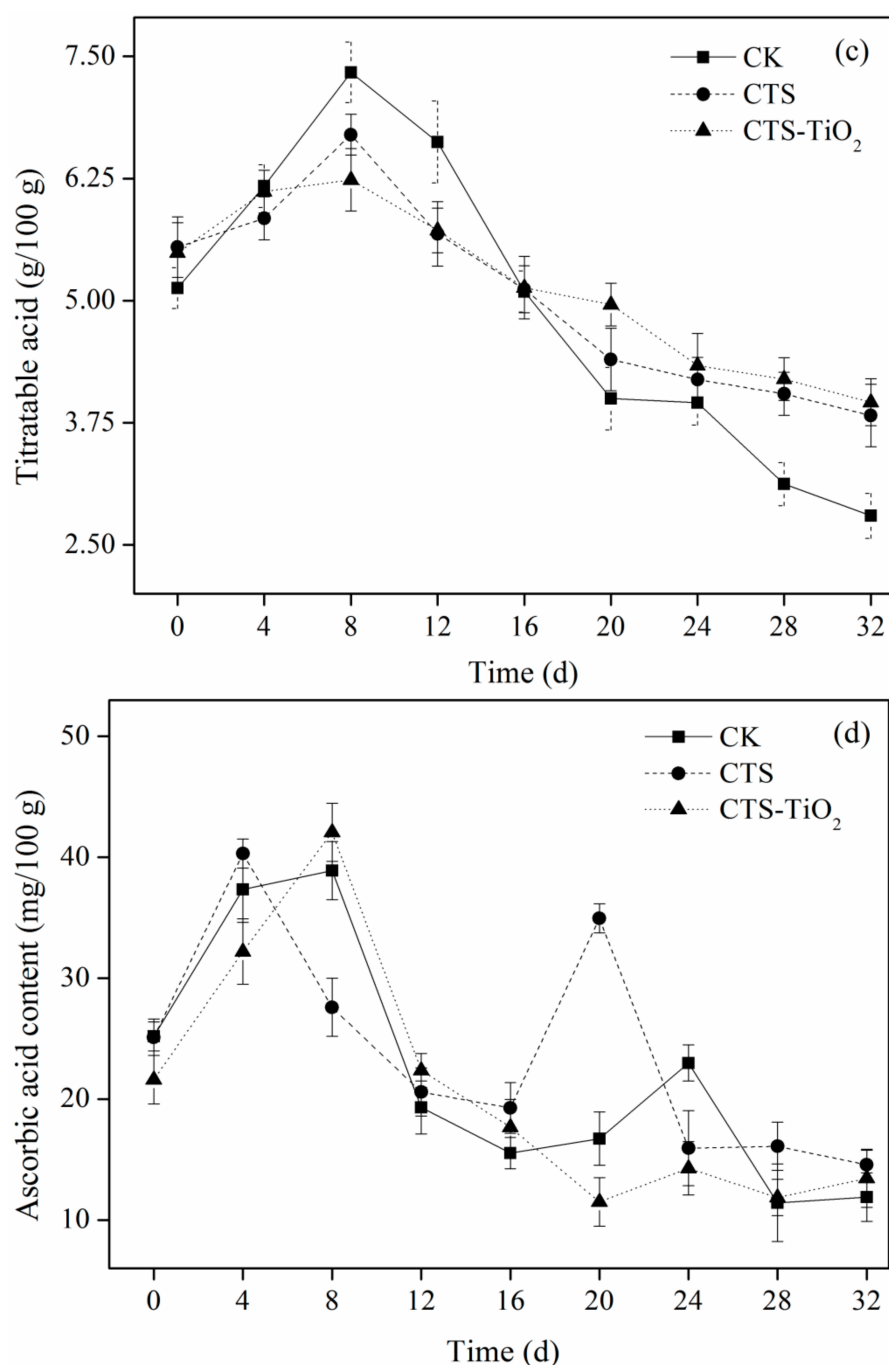


Figure 1. Effects of different treatments on the firmness (a), TSS (b), TA (c), and ascorbic acid content (d) of blueberry fruit during 32 days of storage at 0 °C (CK stands for no coating; CTS stands for chitosan coating; CTS-TiO₂ stands for chitosan/nano-TiO₂).

The fluctuation in the pulp TSS content may be caused by respiratory consumption or the degradation of macromolecular carbohydrates into TSS. Compared to the CK group, the CTS group, especially the CTS-TiO₂ group, displayed a higher TSS level in the blueberry pulp during postharvest storage ($p < 0.05$). This might be attributed to the fact that the CTS-TiO₂ composite film reduces water loss, effectively maintaining the TSS content inside the fruit. These results are consistent with those of other studies concerning the effects of CTS treatment on different commodities, such as mangoes [41], guavas [21], bananas [42], and sweet cherries [43]. Xing et al. [20] reported that samples treated with CTS and MAP exhibited the highest overall visual quality scores in fresh-cut lotus root at the end of

storage. In conclusion, CTS-TiO₂ at appropriate doses is considered a safe alternative for maintaining fruit quality.

The pulp TA and ascorbic acid represent the key flavor substances and nutritional ingredients in blueberries [44]. The effects of different coatings on the TA in blueberry fruits are shown in Figure 1c. The results reveal that the pulp TA content of all treatment groups displayed a similar pattern of variation, showing an escalating trend from day 0 to day 8 of storage and a decreasing trend after 8 days of storage. The TA content in the three groups was significantly higher than the initial values after 8 days of storage at 0 °C ($p < 0.05$). The blueberries in the CK group exhibited the highest decrease in TA after 32 days of storage, while those treated with the CTS-TiO₂ coating showed the lowest decrease, and the TA was 3.96 g/100 g on day 32. The TA content of the CK, CTS, and CTS-TiO₂ groups decreased by 45.42%, 31.12%, and 27.87%, respectively. Compared to the CTS group, the CTS-TiO₂ group displayed a higher pulp TA level during the storage process ($p < 0.05$). Furthermore, the effect of the CTS-TiO₂ coating on the ascorbic acid content was also evaluated during storage. Ascorbic acid is not only a significant indicator for assessing the fruit quality and the nutritional value of the pulp but is also an important antioxidant for removing reactive oxygen species from the fruit [45]. Figure 1d illustrates that a significant decrease in the ascorbic acid values of the three groups was observed in conjunction with the storage period. The ascorbic acid content of the blueberries in the three treatment groups showed an increasing trend 4 days before storage, after which a gradual downward trend was evident. It began to rise again on day 16 of storage, after which it leveled out. After storage at 0 °C for 32 days, the ascorbic acid values of the fruits in the CK, CTS, and CTS-TiO₂ groups were 11.9 mg/100 g, 14.57 mg/100 g, and 13.45 mg/100 g, respectively.

These results illustrate that the TA content in the CTS-TiO₂ group was the highest of all the treatments during storage. The CTS and CTS-TiO₂ groups displayed a certain inhibitory effect on the decrease of the TA content of the blueberries during storage, initially increasing, followed by a decline over 32 days of storage. This may be due to the change in the respiration environment; part of the sugar is converted into acid, resulting in an increase in TA content, but with the increase of the storage period, the higher acidity loss might reflect the use of organic acids as substrates for respiratory metabolism. However, the reason for the high TA in the CTS-TiO₂ group can also be attributed to the slower ripening rate of the coated fruit slices [5]. The chitosan coating controlled the permeability of O₂ and CO₂, playing a crucial role in inducing the slower ripening rate of the blueberry samples [46–48]. Therefore, blueberries treated with CTS-TiO₂ retained a higher pulp TA content during the entire storage period, preserving the postharvest fruit quality and nutritional properties of the blueberry pulp [11]. Furthermore, the ascorbic acid content of the CTS-TiO₂ group was the highest on day 8, and the respiration peak was delayed for 4 days in the CTS-TiO₂ coating group, indicating the inhibitory effect of this composite film. These findings could be ascribed to the CTS-TiO₂ characteristics, indicating that the composite film was selective in light transmission after adding nano-TiO₂, while the ascorbic acid was easily damaged by heat, light, and oxygen, rendering the results unstable. However, the CTS film remained relatively stable during light transmission, better maintaining the ascorbic acid content in the blueberries. The CTS, as a permeable membrane, slowed the ripening rate of the blueberries. Furthermore, anti-browning agents in the coating protected the Ascorbic acid content of the blueberries [27].

3.2. PPO Activity and POD Activity

The PPO and POD activities in the blueberries treated with different coatings during storage at 0 °C for 32 days were investigated. The degree of browning on the surface of the blueberries is correlated with PPO activity [6].

The coordinated action of POD, an important oxyradical detoxification enzyme in fruit, can help to reduce the oxidative damage during the regeneration of ascorbate and glutathione metabolites. Therefore, the PPO and POD activity was investigated to deter-

mine its correlation with blueberry browning. Figure 2a illustrates that the PPO content of the blueberries in the CK, CTS, and CTS-TiO₂ groups initially showed a gradual increase, followed by a slow decline. The results indicated that the PPO activity of the CK group presented an increasing trend of up to 10.13 U/g at the end of storage while inducing the accumulation of phenolic compounds in the blueberries. CTS-TiO₂ exhibited the lowest PPO activity in the samples; at only 3.21 U/g after 32 days of storage, CTS-TiO₂ showed values significantly ($p < 0.05$) lower than CK. Moreover, Figure 2b shows that high POD activity was observed in the beginning. However, after 32 days of storage at 0 °C, this activity was 431.3 U/g and 583.4 U/g in samples ($p > 0.05$) and the blueberry fruits treated with the CTS and CTS-TiO₂ coatings, respectively. The POD activity increased in both the CTS and CTS-TiO₂ groups during storage, while the CTS-TiO₂ group displayed higher PPO activity at the end of storage, and CTS-TiO₂ showed values significantly ($p < 0.05$) lower than CK.

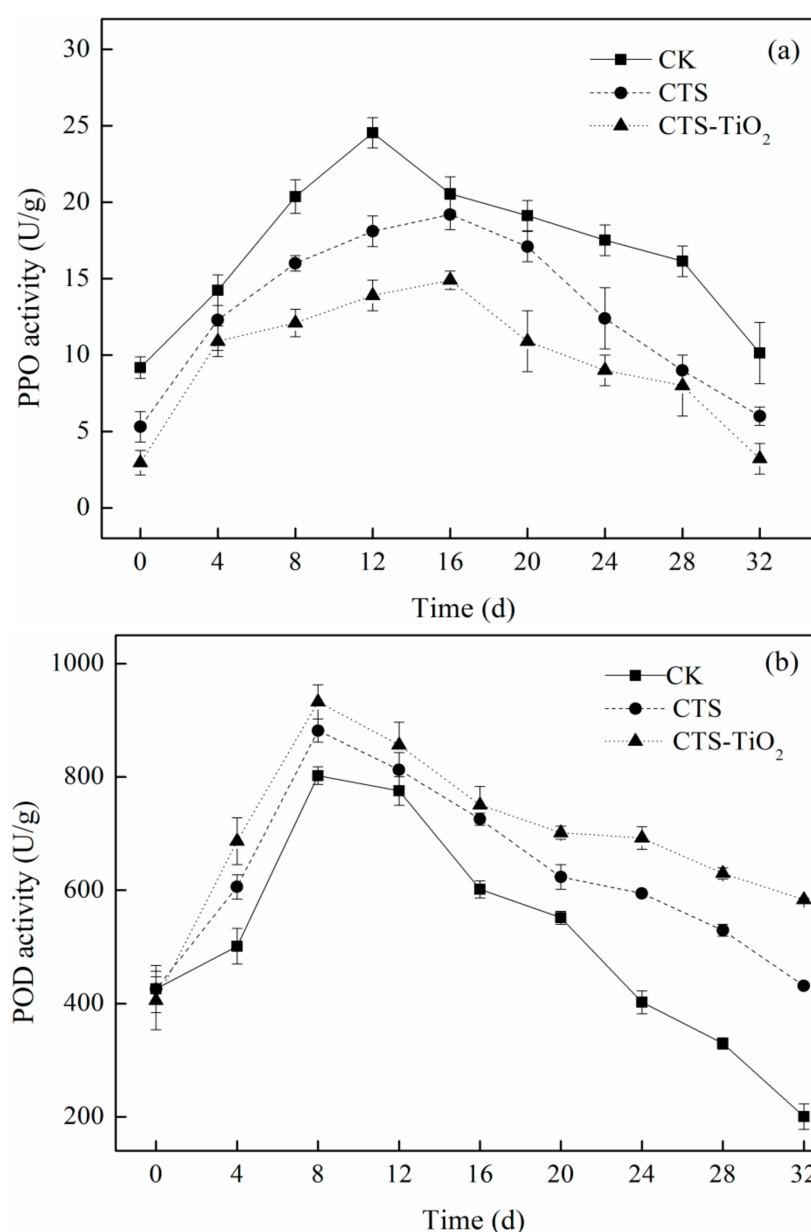


Figure 2. Effects of different treatments on the PPO (a) and PPO (b) of blueberry fruit during 32 days of storage at 0 °C.

The results demonstrated that the PPO and POD activity changes were related to the chilling injuries on the surfaces of the blueberries since it could produce polyphenol compounds and induce browning. This phenomenon was consistent with the observation reported by Badawy et al. [24], who indicated that CTS provided the ability to remove the metal ions, potentially inhibiting the PPO activity in blueberries, while CTS-TiO₂ treatment also affected PPO activity. This phenomenon was consistent with the observation reported by Zhi et al. [12], who showed that CTS-TiO₂ displayed the potential to inhibit PPO activity in blueberries, which could be attributed to incorporating nano-TiO₂ into CTS. Furthermore, Figure 2b indicates that the POD activity increased in both the CTS and CTS-TiO₂ groups during storage. The increased POD activity in the blueberries may be induced by the complex CTS and nano-TiO₂, which could be beneficial in inducing disease resistance in fruit during storage. CTS induced the activities of defense-related enzymes, promoting the protection of fruit [49,50].

3.3. MDA Content

As the final product of lipid peroxidation, MDA is always used as an index for oxidative damage to fruit tissue cells. Therefore, MDA, a secondary product of polyunsaturated fatty acid oxidation, is usually an indicator of the degree of oxidative stress in plants [25]. A continuous increase was evident in the MDA content of the three groups during storage at 0 °C (Figure 3), while the application of CTS-TiO₂ packaging delayed an increase in the MDA of the postharvest blueberries. However, at the end of storage, the MDA content in the CK, CTS, and CTS-TiO₂ groups reached 0.1852, 0.1422, and 0.1101 $\mu\text{mol/g}\cdot\text{mL}$, respectively. During storage, the MDA content in the blueberries treated with CTS-TiO₂ showed a slower increase than the CK and CTS groups and was 0.1101 $\mu\text{mol/g}\cdot\text{mL}$ after 32 days of storage, which was lower than in CK ($p < 0.05$). Furthermore, the accumulation of MDA in CK indicated significant lipid peroxidation. Moreover, combined treatment with CTS-TiO₂ effectively controlled MDA accumulation and reduced oxidative damage during storage.

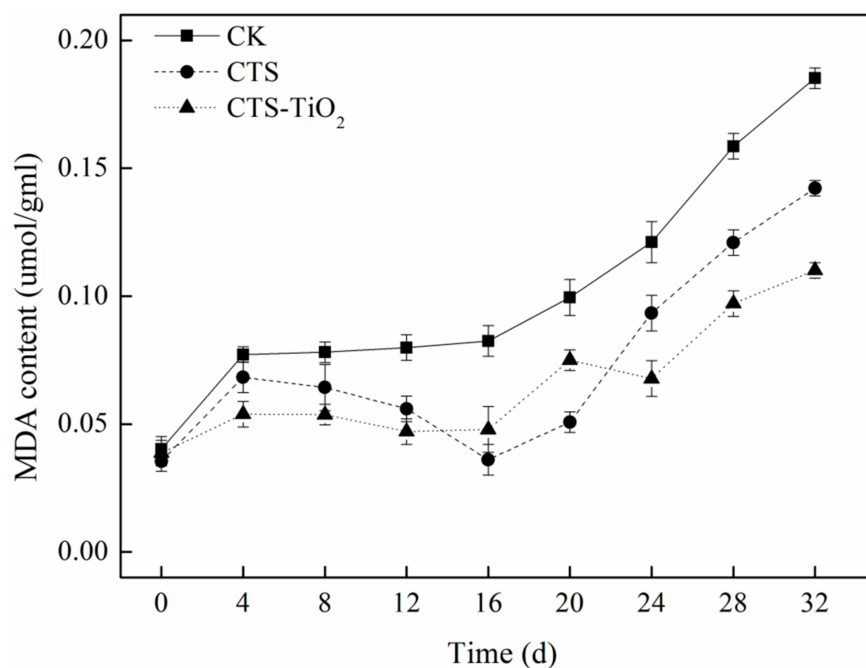


Figure 3. Effects of different treatments on MDA of blueberry fruit during 32 days of storage at 0 °C.

The results indicated that CTS and CTS-TiO₂ treatment effectively inhibited an increase in the MDA content in the fruit during storage. This could be attributed to the potential induction of the chitosan defense system [24]. CTS-TiO₂-treated fruits displayed signif-

icantly lower relative leakage rates than CK, indicating that higher membrane integrity was maintained during storage. Similar results were obtained by Xu et al. [27]. However, the application of a chitosan-oil coating significantly delayed the increase in MDA and electrolyte leakage in sweet peppers during storage. Results reported by Xing et al. [25] also illustrated that the effect of the treatment combining chitosan-based coatings with modified atmosphere packaging controlled MDA accumulation and reduced oxidative damage in fresh-cut lotus root during storage.

3.4. Measurement of the Anthocyanins, Flavonoids, and Total Phenolic Content in the Blueberries

Anthocyanins are considered as functional ingredients. They are the principal components of phenolic pigments such as red, blue, and purple color. However, anthocyanins can easily be oxidized to colorless or brown-color compounds with a decrease in their antioxidant potential. Anthocyanins are susceptible to several influencing factors such as temperature, oxygen, pH, metals, light, oxygen, and enzymes during processing and storage [51,52].

Figure 4a shows that the pericarp anthocyanin content of the CK increased rapidly throughout the storage period, followed by a decline after 16 days. The anthocyanin content of the samples treated with CTS-TiO₂ was significantly higher at 279.5 mg/100 g at the end of storage, while that of CK was 185.1 mg/100 g. A similar decrease in the anthocyanin content was evident in the longan pericarp of the chitosan-treated groups [53]. A notable difference was apparent in the blueberry anthocyanin content of the CTS-TiO₂ and CK samples in the final 32 days of postharvest storage ($p < 0.05$). Furthermore, a distinct increase in the total flavonoid content was evident throughout the storage ($p < 0.05$) period. Figure 4b demonstrated that the pericarp flavonoid content of the CK group blueberry showed an increase during the storage. A similar tendency in the blueberry flavonoid content was observed in the CTS and CTS-TiO₂ groups. There was an uptrend in the flavonoid content during the early stage of storage, which could be ascribed to further flavonoid biosynthesis to cope with external stresses while protecting the fruit [54]. The flavonoid in the CK, CTS, and CTS-TiO₂ groups also recovered after a decline on day 24. The CTS-TiO₂ group showed the most significant recovery, while the CK group recovered the least, which may be caused by fruit ripening. Ultimately, the flavonoid contents in the CK and CTS-TiO₂ groups were 61.66 and 85.84 mg CE/100 g FW, respectively, by the end of the storage period. Compared with CK, the total flavonoid content was significantly higher in the samples treated with CTS-TiO₂ ($p < 0.05$). A continuous increase was observed in the total phenol content of both the CK and CTS coating fruits during storage. Figure 4c shows that the total phenolic content in the three groups increased gradually, while the increase was the most significant in the CTS-TiO₂. However, the total phenolic content decreased in three groups following the initial increase. The total phenolic content of the blueberries showed an increasing trend throughout storage and was 406.76 and 418.48 mg GAE/100 g FW for CTS and CTS-TiO₂, respectively, while it was 370.19 mg GAE/100 g FW in the CK group.

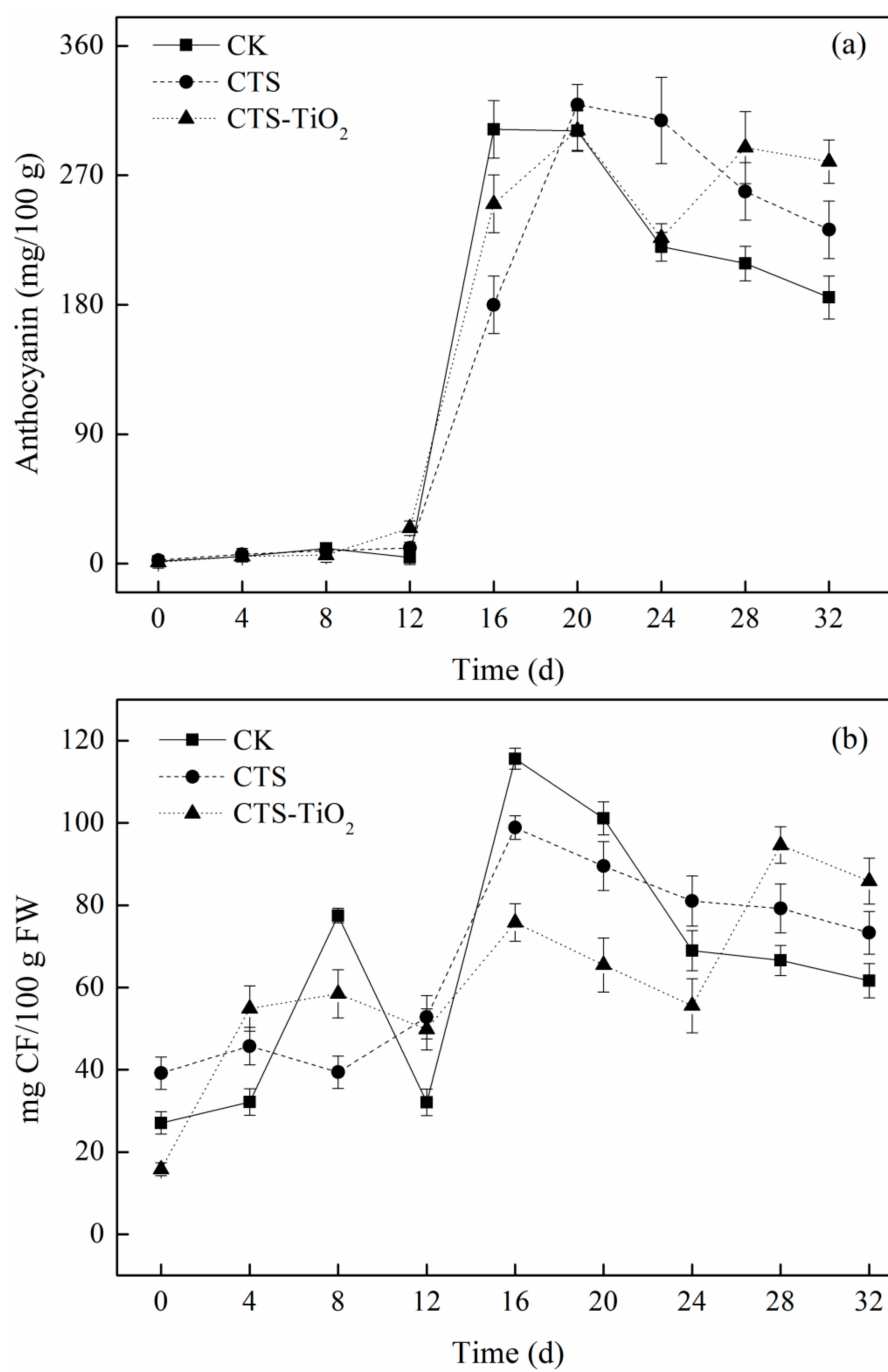


Figure 4. Cont.

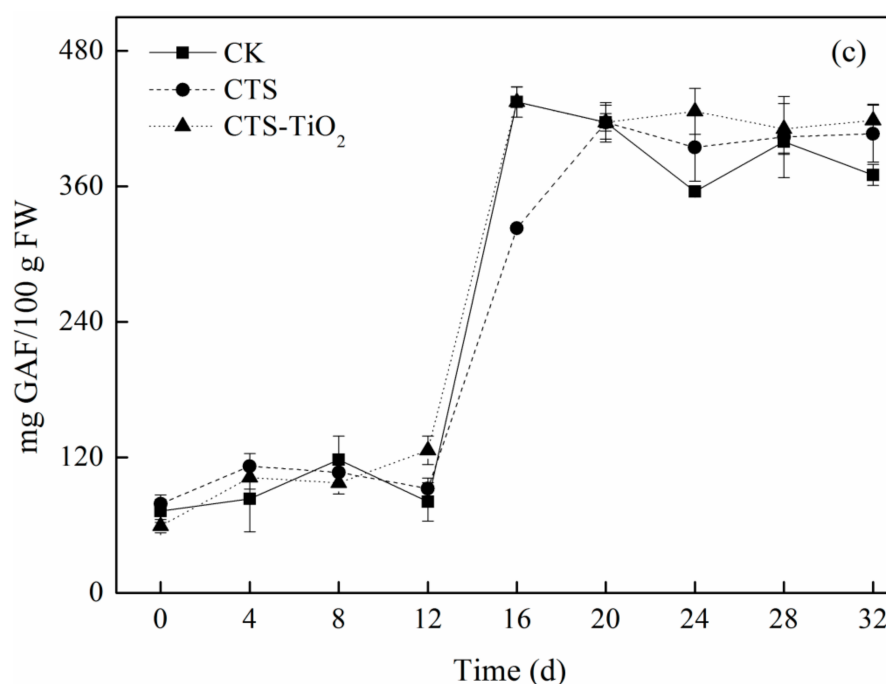


Figure 4. Effects of different treatments on contents of anthocyanin (a), flavonoid (b), and total phenolics (c) in pericarp of blueberry fruit during 32 days of storage at 0 °C.

The results indicate an increase in the anthocyanins, flavonoids, and total soluble phenolic compounds in the chitosan-treated blueberries. Other studies indicate that the production of phenolic compounds in tomato plants was induced by treatment with CTS [25]. Furthermore, the fruit in the CTS and CTS-TiO₂ groups retained higher flavonoid and anthocyanin levels than the CK group at the end of cold storage ($p < 0.05$). However, the CTS-TiO₂ group displayed higher levels of total phenols, flavonoids, and anthocyanins and was, therefore, the optimal coating for treating fruit. These findings are consistent with previous studies demonstrating that chitosan treatment improved the nutraceutical properties of blueberries, maintaining high levels of postharvest phenolics, anthocyanins, and flavonoids, further suggesting that CTS-TiO₂ treatment delays fruit senescence and enhances the phytochemical content during storage [1]. Therefore, CTS-TiO₂ treatment inhibited pigment degradation, leading to higher anthocyanin, flavonoid, and total phenolic levels in blueberries, resulting in better visual and postharvest quality of blueberries [26,27].

3.5. The Aerobic Mesophilic Bacteria

Microbial safety is one of the most important factors to be considered for the preservation of minimally processed fruits and vegetables [54]. Blueberries provide an excellent source of nutrients for microorganism growth because they are high in water, starch, and vitamins [55,56]. Figure 5 shows the effect of different treatments on the total viable counts of blueberries during storage. A total aerobic plate count showed that the total number of aerobic mesophilic microorganisms in all the samples was below the detection limit of 2.0×10^1 cfu/g on day 0. The total bacteria in the postharvest blueberries treated with CTS-TiO₂ reached a level of 1.03×10^2 cfu/g on day 32 of storage, while the CTS and CK groups reached 3.15×10^2 cfu/g and 2.53×10^3 cfu/g, respectively, on day 32.

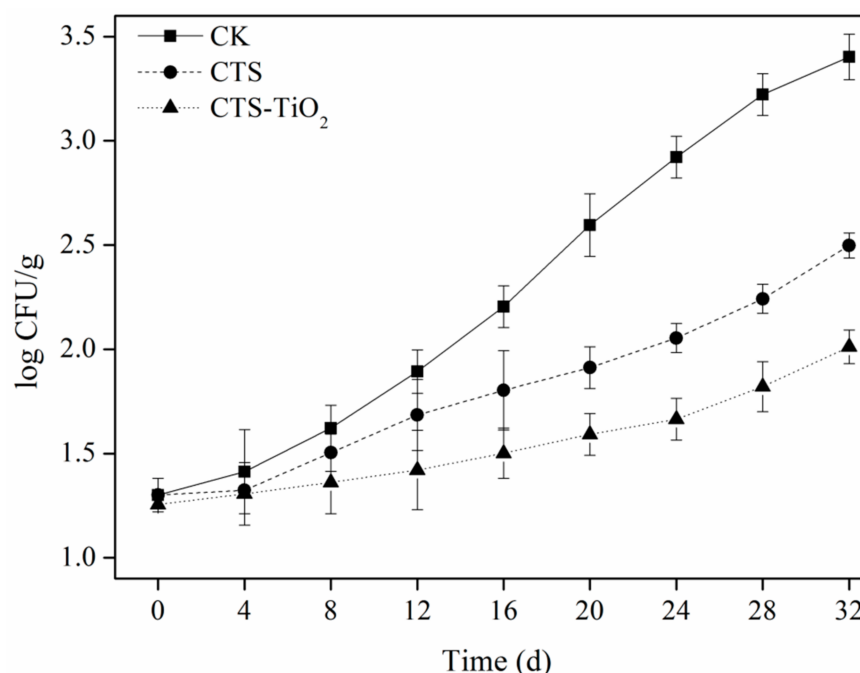


Figure 5. Effects of different treatments on contents aerobic mesophilic bacteria of blueberry fruit during 32 days of storage at 0 °C.

To a certain extent, these results indicate that CTS-TiO₂ treatment controlled the rapid growth of bacteria. Compared with the CK group, the bacteria contents of blueberry fruit were significantly lower in the samples treated with CTS-TiO₂ ($p < 0.05$). The CTS solution with or without nano-TiO₂ seemingly inhibited bacterial growth, compared with the CK samples. Both CTS and CTS-TiO₂ may improve the safety of the blueberries by inhibiting or delaying total viable counts growth. Although CTS and CTS-TiO₂ both effectively extended the shelf life of the blueberries, CTS-TiO₂ and CTS displayed a bacteriostatic effect at the same level. Jorge et al. [23] also showed that chitosan-Aloe could be applied as a postharvest treatment to inhibit microbial spoilage and reduce decay incidence during the storage of blueberry. However, as reported by Xing et al. [20], in refrigerated conditions, the storage time of minimally processed fruits and vegetables were extended after treatment with CTS-TiO₂ as an alternative treatment for controlling diseases in fruits and vegetables. This could be attributed to the fact that nano-TiO₂ exhibited significant antibacterial activity.

4. Conclusions

Chitosan/nano-TiO₂ are eco-friendly and can be used for blueberries shelf life improvement by maintaining quality parameters. CTS-TiO₂ maintains the quality and controls the development of decay in postharvest blueberries. Microbial analysis indicates that treatment with CTS-TiO₂ and CTS showed bacteriostatic activity. The blueberries treated with CTS-TiO₂ present the smallest changes in firmness, TA, TSS, anthocyanins, flavonoids, and total phenolics. This combined treatment significantly inhibits the PPO activity during storage. Furthermore, the CTS-TiO₂ samples displayed the lowest MDA content. This suggests that CTS-TiO₂ not only maintains firmness but also improves the postharvest quality during cold storage and also suggests that CTS-TiO₂ is promising as an edible coating to be used in commercial postharvest applications. In conclusion, the combination of chitosan and Nano-TiO₂ as coating materials has great potential in expanding the shelf life of blueberries.

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