Effect of Chitosan Composite Coatings with Salicylic Acid and Titanium Dioxide Nanoparticles on the Storage Quality of Blackcurrant Berries

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Abstract: The use of chitosan and chitosan composite coatings for the preservation of fruits and vegetables during storage is attracting increasing attention. In this study, a chitosan-based edible coating, as well as a second chitosan-based edible coating containing salicylic acid (CTS + SA), a third containing nanosized titanium dioxide particles (CTS + TiO 2 ), and a fourth containing a combination of these two (CTS + SA + TiO 2 ) were evaluated in terms of their effects on the postharvest quality of blackcurrant fruit during storage at 4 °C. The results showed that compared with the other three treatment groups, the blackcurrants treated with CTS + SA + TiO 2 underwent the smallest changes in weight loss, total soluble solids, titratable acidity, vitamin C, and total anthocyanin content, and retained the highest total flavonoid content. This combined treatment significantly inhibited polyphenol oxidase activity during storage, and the CTS + SA + TiO 2 samples also displayed the lowest malondialdehyde content. These results, thus, indicate that the CTS + SA + TiO 2 composite coating could maintain the nutrient composition of blackcurrants, thereby playing a significant role in preserving the quality of this fruit at 4 °C.

Keywords: blackcurrant; chitosan; salicylic acid; nano-TiO 2 ; quality

1. Introduction

Blackcurrant (Ribes nigrum L.) is one of Europe’s most important berry crops [1], renowned for its considerable potential health benefits, particularly its high levels of ascorbic acid and antioxidant activity [2,3]. Blackcurrant berries are widely used to produce jam, juice, wine, liqueur and spirits [4], all products that are increasingly popular among consumers, not only in Europe and the USA but also, more recently, in China. This is because of the fruit’s extremely high bioactive compounds such as vitamin C, which is four times more concentrated than it is in oranges and 50 times more so than in apples [5]. Consuming blackcurrant products in moderation can provide anti-inflammatory, antioxidant, and antimicrobial effects that are beneficial to human health [6–8]. However, fresh fruits deteriorate rapidly after harvesting due to their loss of water and cellular juice, and senescence [9]. Moreover, in conditions of high humidity, fungal infection can result in sensorial and nutritional damage [10]. Recently, efforts to preserve the shelf-life of fruit have focused on replacing chemical and synthetic preservatives with natural alternatives, and several environmentally friendly methods have been suggested to maintain fruit quality, including those using carbohydrate-based polymers, such as chitosan [11,12].
Chitosan is the most common cationic polysaccharide and is a renewable resource, abundantly available as a low-cost biopolymer [13]. It is widely utilized as a protective coating for a variety of fruits and vegetables after harvesting [9,14,15], for example, to control the decay and bacteriostasis of citrus fruits during storage [16], and to prevent dehydration during handling and packaging [17]. Furthermore, chitosan can also be combined with other biopolymers to increase and assure the effectiveness of crop coatings [12,18,19]. The results of Kaya et al. [20], for example, indicated that the use of a chitosan–acetic acid coating might be an effective technology for prolonging the short shelf-life of red kiwifruit berries, while Hernandez et al. [21] demonstrated that a chitosan coating with a postharvest calcium treatment can improve the firmness of strawberries. Moreover, applications of chitosan and essential oils, antibacterial nanoparticles, and other active ingredients have also been reported in studies on jujubes, strawberries, and apples [14,22,23].

Titanium dioxide (TiO$_2$) has attracted a great deal of attention as an inorganic bacteriostatic, and it has become widely used as a self-disinfecting and self-cleaning material in diverse applications [24,25]. The American Food and Drug Administration (FDA) has approved and recommends the use of TiO$_2$ in healthcare, cosmetics and food materials due to its low toxicity [26]. According to expert research, chitosan–TiO$_2$ composite materials exhibit multifunctional performance in many potential applications, such as wastewater treatment [27], as well as antimicrobial activity [24,28]. Moreover, it has been found that the nano metals used as polymer-forming membranes in TiO$_2$ particles act as a reinforcing compound to provide substantial mechanical strength to scaffolds for the support of cell growth [29].

Salicylic acid (SA), a phenolic compound, is used to enhance the local and systemic resistance of fruits against pathogens [30], thus playing an important role in aspects of fruit quality, such as firmness, taste, aroma and color [31]. In one study, SA was applied to improve the quality of sweet cherries at harvest time by expanding the fruit’s phenolic and anthocyanin synthesis, and aggregating cell antioxidant activities [32]. In addition, SA treatment has shown its effects on promoting the quality of peaches [33], resulting in a higher flavonoid content and increased phenylalanine ammonia-lyase (PAL) activity [34], and inhibiting the production of ethylene, respiration and senescence [35].

The application of chitosan composited with active functional substances has attracted increasing interest, due to their superior antimicrobial activity to maintain the quality attributes of fruit [28,36–38]. The results of Tian et al. [39] suggested that composite coatings of chitosan/nano-TiO$_2$ can effectively maintain the quality of Ginkgo biloba seeds (reducing decay and shrinkage rate, and prolonging firmness), and they were also found to extend the shelf-life of ginkgo seeds and mango fruits [40]. Moreover, the combination of chitosan and TiO$_2$ film is reported to be highly effective at controlling mold and yeast population growth [29], phenol enzymes, total soluble sugars and malondialdehyde in ready-to-eat cantaloupe [37]. According to Cui et al. [38], the combination of chitosan and SA in pre-harvest treatments significantly maintained the postharvest quality of Xiaobai apricots during storage. Other experimental data suggest that chitosan coatings containing SA effectively reduce bacterial and fungal counts in fresh pistachio fruit [41]. In summary, many researchers have reported that chitosan and chitosan composites with active substances can effectively protect the quality attributes of fresh fruits and vegetables and prolong their shelf life.

However, from the literature review, it is apparent that not many studies have investigated the effects of chitosan and chitosan composite coatings on the storage quality of blackcurrants. This study, therefore, aims to examine the effects of chitosan acetate coatings alone, in combination with SA, in combination with TiO$_2$, and in combination with both these active substances, on the quality attributes of blackcurrants during storage. Assessments of these treatments are based on the fruit qualities of weight loss, total soluble solids (TSS), titratable acidity (TA), vitamin C (VC) and malondialdehyde (MDA) content, polyphenol oxidase (PPO) activity, total anthocyanin content (TAC), total flavonoid content (TFC), and microbiological qualities.
2. Materials and Methods

2.1. Materials

Commercially mature blackcurrant (*Ribes nigrum* L.) berries were obtained from the Fresh store in Chengdu City, Sichuan, China. Fruits were selected based on uniform color, size, hardness, and lack of visible physical damage or fungal infection. The blackcurrants were transferred immediately to the laboratory, where, after having been washed three times in distilled water, they were surface-disinfected via immersion in a 5% sodium hypochlorite aqueous solution and dried at room temperature. The chitosan powder (deacetylated ≤ 95%) was provided by Jinan Haidebei Marine Bioengineering Co. Ltd. (Jinan, China). Nano-TiO$_2$ (20–30 nm) was obtained from the Beijing Deke Island Gold Technology Co. Ltd. (Beijing, China). SA, sodium laurate, phosphate buffer, glacial acetic acid, glycerol, sodium hydroxide, sodium hypochlorite, phenolphthalein, ethanol, hydrochloric acid, trichloroacetic acid, thiobarbituric acid, methanol, sodium nitrite, aluminum nitrate, anhydrous sodium acetate, polyvinylpyrrolidone, catechol, sodium chloride, Rutin standard product and L-ascorbic acid were purchased from the Chengdu Kelon Chemical Reagent Factory (Chengdu, China).

2.2. Preparation of Chitosan and Chitosan-Based Coatings

The chitosan solution (CTS) was prepared according to the method of Han et al. [42], with some modifications. Briefly, 1 g of chitosan powder was dissolved in 100 mL aqueous solution of 1% (v/v) glacial acetic acid with 1 g glycerin placed on the magnetic heating agitator (WS-2A, Changzhou Yuexin Instrument Manufacturing Co. Ltd., Changzhou, China) at 90°C and 500 rpm/min for 25 min. The mixture was then filtered through eight layers of cheesecloth and exposed to ultrasound for 30 min to finally produce the CTS.

The nano-TiO$_2$ modification was performed according to the method developed by Xing et al. [43]. Briefly, 1 g nano-TiO$_2$ 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. The solution was then filtered, rinsed, and dried in an oven for 1 h, finally resulting in the modified sodium laurate nano-TiO$_2$.

The CTS + SA solution was prepared according to the method of Zhang et al. [44], with some modifications. Briefly, 0.1% (w/v) SA and 1% (w/v) CTS were dispersed in an aqueous solution of glacial acetic acid (1%, v/v), and the subsequent process was the same as that employed for the preparation of the pure chitosan film.

The CTS + TiO$_2$ solution was prepared using the method of Xing et al. [40], with some modifications. The 0.03 g sodium laurate modified nano-TiO$_2$ was dissolved in 1 g glycerin, followed by the sequential addition of 100 mL 1% aqueous acetic solution and 1 g chitosan powder. The subsequent steps were the same as those employed during the preparation of the pure chitosan film.

For the CTS + SA + TiO$_2$ solution, the formulation ration was as follows: CTS: SA: TiO$_2$ = 1: 0.1: 0.03 (w/w/w). The preparation procedure was the same as that employed to prepare the pure chitosan film.

2.3. Sample Processing

Blackcurrants were randomly distributed into five groups, one of which provided an untreated control (CK), while the other four were assigned to the following treatments: a chitosan coating group (CTS), a chitosan + salicylic acid coating group (CTS + SA), a chitosan + nano-TiO$_2$ coating group (CTS + TiO$_2$), and chitosan + salicylic acid nano-TiO$_2$ coating group (CT + SA + TiO$_2$). Each group contained 1.5 kg of fruit. The berries were immersed in the respective membrane solutions, prepared as described above, for 2 min, with the control samples dipped in distilled water. They were then dried at room temperature and sealed in micro-perforated bags (PLA) bags to maintain aerobic conditions and limit fruit dehydration, after which they were placed in polyethylene terephthalate (PET) plastic trays and stored at 4°C and 75% relative humidity for 30 days.
2.4. Weight Loss

To determine the weight loss of blackcurrant fruit during postharvest storage, both treated and control fruits were weighed at different sampling intervals. Then, weight loss was calculated as the difference between the initial fruit weight and the fruit weight at the time of measurement, and expressed as a percentage (%) [45].

2.5. Determination of Total Soluble Solids, Titratable Acidity, Vitamin C

TSS content in the fruit pulp juice was measured using an Abbe refractometer (2WAJ, Shanghai Optical Instrument Co. Ltd., Shanghai, China). Briefly, a 10-g blackcurrant sample was ground in a mortar and filtered, and the resultant juice was analyzed, with the values expressed as percentages. TA content, expressed as a percentage of citric acid, was determined by titration using a standard solution of sodium hydroxide (0.1 M) [46]. VC was also assayed by the titration, using a solution of 2,6-dichlorophenolindophenol as described by Gao et al. [47], with some modifications. Briefly, a mixture of 10 g blackcurrant fruit and 10 mL oxalic acid was ground into juice on a mortar, followed by the addition of 5 g of porcellanite, and it was left to stand for 20 min before being filtered. Values were expressed in mg of ascorbic acid per 100 g of fresh blackcurrant.

2.6. Determination of Malondialdehyde Content

The MDA content in the blackcurrant groups was determined via thiobarbituric acid (TBA) reaction, as described by Xu et al. [24], with slight modifications. Briefly, 4 g pulp was dissolved in 20 mL trichloroacetic acid and the mixture was centrifuged for 15 min at 4000 rpm. A 2 mL sample of the supernatant was mixed with 2 mL of 6.7 g L\(^{-1}\) TBA, heated at 100 °C for 30 min, then quickly cooled in an ice bath and further centrifuged at 4000 rpm for 15 min. Supernatant absorbances were measured at 450, 532 and 600 nm, respectively. The MDA content was calculated according to the following formula: 

\[
C (\mu mol L^{-1})_{MDA} = 6.451 \times (A_{532} - A_{600}) - 0.56 \times A_{450},
\]

where \(A_{450}, A_{532}\) and \(A_{600}\) are the absorbencies of the solution at 450, 532 and 600 nm, respectively.

2.7. Polyphenol Oxidase Activity Analysis

PPO activity was measured using the methods described by Xiao et al. [25], with some modifications. Briefly, 5 g blackcurrant samples were ground at ice-cold temperatures in a mortar and pestle containing 50 mL of 0.05 M potassium dihydrogen phosphate buffer (pH 6.8). After rapid homogenization, the mixture was centrifuged at 8000 rpm for 15 min at 4 °C in a Heraeus Multifuge X1R refrigerated centrifuge (Thermo Fisher Scientific, Waltham, MA, USA). The clear supernatant was used to determine enzyme activity. The enzyme solution (0.2 mL) was added to a mixture of 3 mL of 0.05 M phosphate buffer (pH 6.8), and 1 mL of 0.04 M catechol as substrate. PPO activity was measured in a spectrophotometer (WFJ7200, UNIC (Shanghai) Equipment Co. Ltd., Shanghai, China) at 398 nm. The units of enzyme activity were defined as a change of 0.01 in the absorbance value per minute under the conditions of the assay. Each determination was run in triplicate.

2.8. Determination of Total Flavonoid Content and Total Anthocyanin Content

Briefly, 5 g blackcurrant pulp was placed into a precooled 50 mL centrifuge tube containing 1% HCL-methanol solution (approximately 20 mL), and homogenized in an ice bath. The homogenate was then transferred to a 50 mL volumetric flask, the volume was fixed with 1% HCL-methanol solution, shaken well, and then placed in the dark at 4 °C for 20 min [48].

TFC was estimated as described by Xu et al. [49], with slight modification. A 10 mL glass tube containing 2 mL of the diluted extract was prepared, to which 1 mL of 5% NaNO\(_2\) solution was added, and it was left to stand for 6 min. 1 mL of 10% Al (NO\(_3\))\(_3\) and 3 mL of 4% NaOH solution were added, and then 3 mL of 70% ethanol was added to the reaction mixture. After being left to stand for a further 10 min, the absorbance was measured at 510 nm using a spectrophotometer (WFJ7200, UNIC (Shanghai) Equipment Co. Ltd.,
Shanghai, China). A blank was prepared by replacing the sample with 1% HCL-methanol solution. The TFC was represented as milligrams of rutin equivalents per gram of fresh weight (mg RE/g FW). All analyses were performed in triplicate.

The pH-differential method was used to determine the TAC of the blackcurrants, according to the details of this analysis described by Johnson et al. [50]. Aqueous buffer solutions at pH 1 and pH 4.5 were prepared from 0.025 M potassium chloride and 0.4 M sodium acetate, respectively, with the pH adjusted using concentrated HCl. A sample extract (2 mL) was then mixed with 8 mL buffer and, after equilibration at room temperature in darkness for 40 min, the absorbances at 510 nm and 700 nm were read using a UV-visible spectrophotometer (WFJ7200, UNIC (Shanghai) Equipment Co. Ltd., Shanghai, China), with 1% HCL-methanol solution used as the blank. The monomeric anthocyanin concentration was calculated using the following formula:

$$\text{mg cyd-3-glu L}^{-1} = \left( A \times MW \times DF \times 1000 \right) / \left( \varepsilon \times 1 \right)$$  \hspace{1cm} (1)

where $A = (\text{pH1: } A_{510} - A_{700}) - (\text{pH4.5: } A_{510} - A_{700})$, $DF$ is the dilution factor (10 following the procedure given), $MW$ is the molecular weight = 449.2 g/mol for cyaniding-3-glucoside, $\varepsilon$ is the molar extinction coefficient = 26,900 L/cm, and 1 is the path length (1 cm). The reactions were performed in triplicate and the results were calculated as milligram cyaniding-3-O-glucoside equivalents per 100 g of fresh weight (FW).

2.9. Measurement of the Aerobic Mesophilic Bacteria

Microbiological analysis was performed as described by Bico et al. [51]. A 10 g sample slice was blended with 90 mL sterile solution for 60 s using a Stomacher. The sample solution (1 mL) at an appropriate dilution was pour-plated into plate count agar (PCA), and then incubated at 37 °C for 24 h, after which the aerobic mesophilic bacteria were identified. The results were expressed in log CFU/g.

2.10. Statistical Analysis

Completely randomized designs were used in triplicate, both in measurements and analyses in this study, and this investigation was carried out in triplicate. Test results were analyzed using SPSS 20.0 statistics software (IBM USA) and expressed as mean ± standard deviation, while the figures were generated using Origin 2017 (OriginLab Corporation, Northampton, MA, USA).

3. Results

3.1. Weight Loss

In this study, all the treatments using chitosan, both alone and in combination, were found to be highly effective in minimizing the weight loss of blackcurrant fruit during storage at 4 °C, compared to CK (Figure 1). Irrespective of treatments, however, weight loss increased progressively with the advancement of storage to 30 days. Of the five different treatments, the maximum weight loss (11.49%) was recorded in CK fruits, significantly higher than other treatment groups ($p < 0.05$), followed by the CTS (9.90%), CTS + TiO$_2$ (8.24%), and CTS + SA (8.10%) groups of blackcurrants. The fruit treated with CTS + SA + TiO$_2$ showed the least weight loss (7.47%) at the end of the full storage period ($p < 0.05$).

Weight loss in post-harvest blackcurrant fruit occurs primarily due to the rapid loss of moisture from the pericarp tissues [45]. This moisture loss leads to the development of micro-cracks in the fruit pericarp, which move progressively inward up to the parenchymatous mesocarp [52], inducing further moisture loss, pathogen attack, and the rapid browning of the pericarp [45]. In this study, chitosan alone, and in combination with SA and nano-TiO$_2$, reduced weight loss in the blackcurrants comparatively more than the CK. SA has been reported to reduce moisture loss by closing the stomata in fruits like mandarins [53], while CTS combined with TiO$_2$ has also been reported to effectively prevent water loss [40]. Chitosan treatment provides an additional barrier against the diffusion
of gases and water vapor. Thus, the chitosan combination treatment with SA and TiO$_2$ might have exerted a synergistic effect in preventing the loss of moisture and gases in the blackcurrants, consequently reducing weight loss during storage.

![Figure 1](image-url)

**Figure 1.** Effect of chitosan and chitosan composite coatings on weight loss (%) of blackcurrant fruit during storage.

### 3.2. TSS, TA and VC

Changes in the contents of TSS, TA and VC in the blackcurrants during storage were investigated in this study and shown in Figure 2, and were found to be different depending on the fruit coating treatment. In all groups, the TSS content increased during storage (Figure 2a), however, in the CK group, TSS increased most rapidly and continuously, reaching 18.13 Brix°, followed by the CTS, CTS + TiO$_2$, CTS + SA, and CTS + SA + TiO$_2$ groups, in which the TSS content values reached 17.80 Brix°, 17.57 Brix°, 17.33 Brix° and 17.02 Brix°, respectively, at the end of the storage period. These results demonstrate that the coating treatment can effectively slow down the rate of TSS ($p < 0.05$). As shown in Figure 2b, the TA content of all groups also followed the same trend, showing an increase in the first five days; however, the CTS + SA + TiO$_2$ group showed the fastest increase, reaching 1.98% in the remaining 25 days, after which its TA content showed a continuous decline. On the last day of storage, the groups with the highest to lowest levels of TA were CTS + SA + TiO$_2$ (1.73%), CTS + TiO$_2$ (1.63%), CTS + SA (1.60%), CTS (1.52%), and CK (1.43%). These results show that the coating treatment can significantly inhibit the decrease of TA content ($p < 0.05$). The VC content in all groups gradually decreased during storage (Figure 2c), with the decrease in the CK fruit most remarkable ($p < 0.05$) and the CTS + SA + TiO$_2$ group showing the slowest decline ($p < 0.05$). The initial value of VC content in the fresh blackcurrants was 128 mg/100 g, but by the end of storage time, the VC contents of the CTS + SA + TiO$_2$, CTS + SA, CTS + TiO$_2$, CTS and CK groups had declined to 99.10 mg/100 g, 91.87 mg/100 g, 89.12 mg/100 g, 87.68 mg/100 g, and 83.99 mg/100 g, respectively.
The main quality indexes of blackcurrant fruit include the TSS, TA and VC content, and these have been shown to change dynamically during postharvest storage [32]. In particular, TSS represents the degree of ripening in fruits [54], and comprises sugar, a small amount of acid, vitamins, minerals and some soluble pectin [55]. In this study, the TSS content increased in all treatment groups during the storage, probably due to ripening and loss of water [36]. All the groups treated with chitosan and chitosan composite coatings were found to have a lower TSS content than the CK group. This is consistent with the result reported by Xing et al. [40], in which a slow rise in TSS content was recorded in mango treated with chitosan composited with nano-TiO₂. The capacity of chitosan to inhibit the rapid increase of TSS content could be attributed to a decline in respiration and metabolic activity, which consequently delays the ripening process. The lowest TSS content was observed in the group treated with the CTS + SA + TiO₂ coating, possibly due to the presence of SA and nano-TiO₂. SA treatment is reported to inhibit the ripening progress of fruit by directly interfering with signaling pathways [56] and ethylene biosynthesis [57].

A high TA content could be attributed to the chitosan coating’s control over the permeability of CO₂ and O₂, which could slow the ripening rate of fruits [60] and the substrates for respiration responses, such as organic acids [13]. A reduced rate of respiration in fruits may be reflected in fewer changes in their TA. In this study, the CTS + SA + TiO₂ treatment more effectively inhibited the losses of TA in comparison with the other groups, which supports the findings of previous studies that chitosan combined with SA can greatly retain the content of TSS and TA [38]. The link between TA and ripening rate thus warrants closer investigation in future research.

Blackcurrants are an important source of VC, however, much of this essential vitamin is typically lost during storage. According to the investigation of Xing et al. [22] and Xiao et al. [25], the loss of VC is promoted by the presence of O₂ and CO₂, but chitosan coatings can control the permeability of CO₂ and O₂ content in the microenvironment and further slow the rate of maturation. This explains the rapid decline of VC content in this study’s CK group. As a water-soluble antioxidant, VC directly decreases the damage from...
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Respiration, a major postharvest metabolism, is based on the amount of stored sugar and organic acids [58]. A lower level of respiration may, thus, contribute to the retardation of TSS [59]. In addition, nano-TiO2 can effectively resist the invasion of bacteria [29], thereby delaying the deterioration of blackcurrant quality.

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3.3. MDA Content

MDA, an important product of membrane lipid peroxidation, has been found to aggravate membrane damage in fruits and vegetables, so its content can indirectly reflect the degree of damage to their tissue and membrane systems [32]. As can be seen in Figure 3, the MDA content in all groups showed a trend of continuous increase during the storage period; however, the rates of increase differed, with the CK group showing the fastest increase and reaching 9.25 µmol/g by the 30th day (p < 0.05). By contrast, the CTS + SA + TiO2 group showed the slowest growth of only 6.19 µmol/g MDA content in the same period (p < 0.05). The final levels of the other three treatment groups were 8.47 µmol/g (CTS), 8.02 µmol/g (CTS + TiO2), and 6.83 µmol/g (CTS + SA), indicating that both chitosan and chitosan composite coatings can inhibit the production of MDA (p < 0.05).
Figure 3. Effect of different coatings on the content of MDA in blackcurrant during 30-day storage at 4 °C.

Postharvest fruit senescence involves a burst in the production of ROS, with cell damage such as lipid peroxidation resulting in the accumulation of MDA [17,56,67]. Here, as storage time increased, the blackcurrants gradually senesced, and their content of MDA increased. Compared with the CK group, those coated by chitosan and chitosan composited with SA and TiO₂ exhibited a slow increase in MDA content (p < 0.05). Many studies have reported that chitosan treatment can increase enzymatic and non-enzymatic antioxidant systems to reduce ROS accumulation, thereby improving the quality and delaying the senescence of postharvest fruits [46,68]. In this work, the lowest MDA content was observed in the CTS + SA + TiO₂ group on the last day (p < 0.05), indicating that this composited coating effectively inhibited the accumulation of MDA, thereby reducing the degree of membrane lipid peroxidation [32]. Low MDA content was also found in the CTS group, at only 98.88 U/g (p < 0.05). The combination of chitosan and SA can effectively inhibit the deterioration of fruit quality. Nonetheless, as reported by Rasouli et al. [61], cold storage may lead to chilling injury, causing fruit cell membrane damage and, as the blackcurrants’ storage in this study was at 4 °C for 30 days, this condition may have significantly influenced the changes to their MDA content.

3.4. PPO Activity Analysis

The PPO activities in blackcurrant fruits treated by different coatings during storage at 4 °C for 30 days were investigated. The results presented in Figure 4 show the same overall upward trend for all groups, indicating that both CK and coating-treated fruits can increase PPO activity during storage. Of the treatment groups, PPO activity increased fastest in the CK group, reaching 110 U/g by the last day (p < 0.05). The growth rate in the other four groups was relatively gentle, but the CTS group showed the fastest growth, reaching 104.69 U/g by the last day (p < 0.05). At the end of storage, the lowest PPO activity was observed in the CTS + SA + TiO₂ group, at only 98.88 U/g (p < 0.05).
The combination of chitosan and TiO$_2$ could significantly inhibit PPO activity in stored mangos. The oxidation of phenolic substrates by PPO, which occurs during ripening, is believed to be a major cause of browning in many fruits and vegetables [51]. Enzymatic browning of phenolic substrates catalyzed by PPO is a symptom of the loss of membrane integrity during fruit senescence [41]. In concurrence, PPO activity in the chitosan and chitosan composite coated blackcurrants was found to be significantly lower than that of the CK group. The chitosan’s inhibition of PPO activity and other biochemical reactions, which prevented the surface browning of the fresh blackcurrants, can be attributed to the low gas permeability of chitosan film, especially against O$_2$ [70]. Furthermore, the coating can provide the ability to remove metal ions, further promoting the potential inhibition of PPO activity in blackcurrant fruits [22]. In this study, the groups treated with CTS + SA and CTS + SA + TiO$_2$ coatings had the lowest levels of PPO on the last day ($p < 0.05$), which is consistent with the results of Tareen et al. [33]. Moreover, according to Xing et al. [40], the combination of chitosan and TiO$_2$ could significantly inhibit PPO activity in stored mangos.

3.5. TFC and TAC

The TFC and TAC in blackcurrant fruit treated by different coatings at 4 °C for 30 days were investigated, and TFC was found to increases gradually in all groups (Figure 5a). At the end of the storage period, the highest content of TFC was observed in the CTS + SA + TiO$_2$ group, at up to 10.55 mg RE/g FW ($p < 0.05$). The other treatments (CTS $p < 0.05$, followed by the CTS + SA (9.94 mg RE/g FW), CTS + TiO$_2$ (9.81 mg RE/g FW) and CTS (9.54 mg RE/g FW) groups, respectively. TFC content was found to be lowest in the CK group on the last day, at only 9.18 mg RE/g FW ($p < 0.05$). The changes in TAC are presented in Figure 5(b). During the whole storage process, the TAC in the CK group increased continuously at first, reaching a peak of 44.78 C-3-G mg/100 g FW on day 25, and then decreased rapidly to 32.03 C-3-G mg/100 g FW by the final day. The TAC in the other four groups also increased throughout the storage period, and their contents on the last day (highest to lowest) were 43.31 C-3-G mg/100 g FW (CTS + SA + TiO$_2$), 42.59 C-3-G mg/100 g FW (CTS + SA + TiO$_2$), 42.14 C-3-G mg/100 g FW (CTS + TiO$_2$) and 39.55 C-3-G mg/100 g FW (CTS + SA).
Anthocyanins are a group of phenolic compounds responsible for the red-blue color of many fruits and vegetables, which provide beneficial effects for human health [71]. Flavonoids are secondary metabolites and derivatives of phenols, which exhibit antioxidant capacity [69]. Blackcurrants are one of the best sources of antioxidants among fruits and vegetables because of their natural flavonoid compounds and anthocyanin contents [72]. In the current study, the TFC and TAC of all treatment groups showed an upward trend during the storage period (as seen in Figure 5a,b). These findings concur with previous reports, and may be due to the continued biosynthesis of these compounds after harvest [71]. At the end of storage, the highest levels of both TFC and TAC were found in the CTS + SA + TiO$_2$ group ($p < 0.05$), indicating that this composite film could improve the preservation of anthocyanin and flavonoid content. This corresponds with the observations of Xing et al. [40], in which mango coated with chitosan film exhibited higher flavonoid levels after storage.

In this study, the fruits treated with the chitosan and chitosan composite coatings showed higher retention of TFC during the entire period of storage. Furthermore, the film with chitosan, combined with SA and CTS + SA + TiO$_2$, performed best. The CTS + SA
coated blackcurrants retained more flavonoids than other treatments, as the semipermeable coating of chitosan restricted moisture loss, gas exchange and senescence by modifying endogenous \( \text{O}_2 \) and \( \text{CO}_2 \) [69]. A reduced loss of TFC in response to pre-storage SA treatment was similarly reported in litchi [45], and chitosan was also reported to be highly effective in retaining higher TFC in treated grapes than the control [73]. On the other hand, these TFC and TAC might also be due to \( \text{TiO}_2 \) nanoparticles, which are able to delay, retard or prevent the oxidation processes of flavonoids and anthocyanins by reacting with free radicals, chelating metals and acting as oxygen scavengers [40,74].

With respect to TAC (Figure 5b), the application of coating films was found to be highly effective in retaining anthocyanin pigments in the fruit pericarp \((p < 0.05)\). All treatment groups exhibited incremental increases in TAC during storage, with the highest seen in the CTS + SA + \( \text{TiO}_2 \) group \((p < 0.05)\). In the CK group, after 25 days, the anthocyanin content had decreased rapidly due to the degradation of anthocyanin pigments, which consequently increased the incidence of pericarp browning. Moreover, the lack of coating treatment in the CK group facilitated a more rapid moisture loss than in the treated groups \((p < 0.05)\), which, in turn, led to plasmolysis and the breakdown of membranes in the pericarp tissue. Consequently, anthocyanins present in the vacuole were released and encountered the enzyme anthocyanase and PPO, which further accelerated the degradation of anthocyanin pigments [45].

3.6. Microbiological Analysis

The quality of blackcurrants during storage is limited by the highly perishable nature of the fruit, including susceptibility to postharvest diseases associated with bacteria, yeasts and fungal infection [8]. The number of pathogenic colonies is, thus, a critical index to the quality of blackcurrants during storage. Figure 6 shows the effects of different chitosan treatments on the total viable counts in blackcurrants 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 \( 2.0 \times 10^1 \) CFU/g on day 0. The colony number in the CK group was higher than those of the other four groups throughout storage, finally measuring \( 3.34 \log \text{CFU/g} \) on the last day \((p < 0.05)\), while the conditions of the other four groups were as follows: CTS \((3.20 \log \text{CFU/g})\), CTS + SA \((3.18 \log \text{CFU/g})\), CTS + \( \text{TiO}_2 \) \((2.90 \log \text{CFU/g})\) and CTS + SA + \( \text{TiO}_2 \) \((2.67 \log \text{CFU/g})\).

![Figure 6. The colony units of each handle group in blackcurrants during 30-day storage at 4 °C.](image)

As shown in Figure 6, the CTS solution, both with or without nano-\( \text{TiO}_2 \) or SA, seemingly inhibited bacterial growth, compared with the CK samples. Furthermore, the
blackcurrants treated with CTS + SA + TiO$_2$ showed the best performance in terms of the total number of colonies ($p < 0.05$). To a certain extent, these results indicate that SA and nano-TiO$_2$ treatment controlled the rapid growth of bacteria. According to Moscoso-Ramirez et al. [75], SA treatment showed antimicrobial activity in oranges, decreasing green and blue molds. It has also been shown that the exogenous application of SA increases the endogenous level of SA [64], thereby increasing antioxidant enzyme activities and the production of pathogenesis-related proteins in some plants [76]. Nano-TiO$_2$ is also effective for inhibiting bacteria. According to the antimicrobial mechanism reported by Maneerat et al. [77], microorganisms carry a negative charge while metal oxides carry a positive charge, which creates an electromagnetic attraction between microbe and treated surface. Moreover, with CTS + SA + TiO$_2$ coating as an outer membrane protective barrier, the entry of bacteria to blackcurrant fruit was prevented or slowed and the SA + nano-TiO$_2$ might also kill or injure the bacterial colonies.

4. Conclusions

This investigation ascertained that different treatments with edible coatings could directly and positively influence the quality of blackcurrants in storage. In conclusion, a CTS + SA + TiO$_2$ coating is eco-friendly and can be used for shelf-life improvement by maintaining quality and controls the development of decay in postharvest blackcurrants. In this study, the fruit treated with CTS + SA + TiO$_2$ presented the smallest changes in weight loss, TSS, TA, VC and TAC, and the highest TFC. This combined treatment also significantly inhibited PPO activity during storage, and these treated samples displayed the lowest MDA content. Microbial analysis indicates that fruit treated with CTS alone, in combination with SA, in combination with TiO$_2$, and in combination with both these active substances, showed bacteriostatic activity; however, CTS + SA + TiO$_2$ treatment has the best antibacterial properties. These findings thus show that the application of chitosan-based coatings and SA + nano-TiO$_2$ may provide an excellent method for maintaining the quality of postharvest blackcurrant fruits.

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