

COATING OF CHITOSAN AND SALICYLIC ACID CAN MAINTAIN QUALITY CHARACTERISTICS OF TABLE GRAPES

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ABSTRACT

International fresh table grape trade has expanded tremendously over the last few decades. However, fresh table grapes decay quickly due to pathogen infestation and water loss, which make it difficult to preserve without an effective treatment. Therefore, this study was conducted to investigate the effects of CT, with SA on maintaining quality characteristics of 'Red Globe' grapes during storage at 0.5°C and 90-95% RH in pomology laboratories of the faculty in 2018-2019. The experiment was conducted using a completely randomized design with treatments [1% CT and two concentrations of SA (0, 1 and 2 mM)] for a 60 day of storage time. To determine the effects of the treatments at 15-day intervals, weight loss, fruit decay, chroma index, total soluble solids, titratable acidity (TA), pH, fruit flesh firmness and secondary metabolites such as total phenolic contents, total flavonoids and total anthocyanins were determined. Also, antioxidant activity and antioxidant capacity of the berry extracts determined by the DPPH and FRAP assays at each sampling time. Results revealed no effect on total soluble solids and total phenolics. However, there were significant effects on the quality parameters fruit decay, weight loss, TA, pH, chroma index, and fruit flesh firmness. Chitosan coating was also found to be a potentially useful treatment for the phytochemical compounds and total antioxidant activity. The pre-storage IC₅₀ value of the berry extract was 0.53 mg mL⁻¹ and by the end of storage period, the values were 0.49 mg mL⁻¹ in control, 0.50 mg mL⁻¹ in CT, 0.53 mg mL⁻¹ in CT + 1 mM SA and 0.46 mg mL⁻¹ in CT + 2 mM SA. Total antioxidant capacity of grapes was 23.7 mg BHT mL⁻¹ before storage and this increased during storage for both coated and uncoated grapes. Based on the findings, there was a strong relationship between total phenolic and flavonoid contents of the berries and antioxidant activity. It was concluded that the use of CT + 2 mM SA together with MAP is a potentially useful alternative to the use of SO₂ in grape storage in commercial contexts.

Keywords: cold storage, chitosan, coating, salicylic acid, *Vitis vinifera*

Abbreviations: BHT – butylhydroxytoluene, CE - catechin equivalents, CT – chitosan, DPPH - 2,2-diphenyl-1-picrylhydrazyl, FRAP - ferric reducing antioxidant power, FW - fresh weight, GAE - gallic acid equivalent, MAP - modified atmosphere packing, SA - salicylic acid, TA - titratable acidity, TSS – total soluble solids

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INTRODUCTION

Grape (*Vitis vinifera*) is one of the most popular among the fresh fruits and it has been widely regarded as "fruit queens" since ancient times. Grapes are also the source of many nutritional health boosters such as polyphenolics antioxidants, vitamins and minerals. Every year more than 77 Mt grapes including table, raisin and wine grapes are produced globally (FAO, 2021). Table grapes are highly perishable and non-climacteric fruits. Quality of table grapes is mainly based on their physical characteristics and chemical contents of berries including secondary metabolites.

Postharvest deterioration in grapes may be due to physical, physiological or pathological factors that may occur before or after harvest, and shelf life is reduced due to loss of firmness, berry drop, discoloration of rachis, desiccation and fungal rot (Sousa *et al.*, 2013). The use of SO₂ during the cold storage of table grapes either by fumigation or generators is the most common commercial method for maintaining fruit quality after harvesting (Crisosto *et al.*, 2002). Despite of its effect in controlling decay and preventing rachis browning, the application of SO₂ has been discontinued in many countries. Use of the modified atmosphere packing (MAP) for table grapes is an alternate to SO₂ application (Hernandez *et al.*, 2006). Under small-scale storage conditions, application of a

semi permeable coating with modified atmosphere (high and low CO₂) has proven satisfactory for storing of perishable fruit (Valle *et al.*, 2005). Usage of edible coatings is one of the best methods for maintaining fruit quality characteristics. Edible coatings have been commonly used to improve physical food appearance and maintain the quality because they are not toxic to humans and environment (Lin *et al.*, 2020). They form boundary layer acting as a barrier to moisture and oxygen during transportation and storage (Vu *et al.*, 2011). In addition, they can delay decay by inhibiting the growth of microorganisms due to their natural activity or in combination of antimicrobial compounds (Nasrin *et al.*, 2017). Edible coatings are usually made of proteins and polysaccharides that can also help to reduce moisture loss, and so improve shelf life. They help to preserve perishable products from decay by deferring dehydration, reducing respiration, boosting textural quality, protecting labile flavor compounds and decreasing microbial growth (Debeaufort *et al.*, 1998). Today many studies regarding with coatings focus on fruits and vegetables, or mixture coatings with CT. CT is a deacetylated chitin derived from high molecular weight polysaccharides consisting of D-glucosamine and N-acetyl-D-glucosamine (Pasquariello *et al.*, 2015). CT is the main component of the shells of crustaceans such as crab, shrimp, crayfish and the exoskeleton of shellfish, or the cell wall of fungi or some microorganisms (Romanazzi *et al.*, 2017). CT coatings are the best edible and bioprotective coatings for different types of food, as they are non-toxic, biodegradable, film forming and have antimicrobial properties, and are easily modified by physical and chemical methods (Yang *et al.*, 2012). Additionally, several researchers have found that CT coatings have the potential to prevent deterioration and therefore extend the storage life of fruit and vegetables (Chien *et al.*, 2007). CT coatings have been used to increase the storage life and shelf life of some fruits such as table grapes, sweet cherry, litchi fruit and apples (Wang and Gao, 2013; Bal and Urun, 2021; Nia *et al.*, 2021). CT has also been approved by the United States Food and Drug Administration "Generally Recognized as Safe" (GRAS) food additive (USFDA, 2013).

Also, SA is an endogenous molecule that is important in regulating plant growth and development, and has considerable potential for reducing postharvest losses of horticultural crops (Klessig and Malamy, 1994). Srivastava and Dwivedi (2000) reported that SA delays fruit ripening by restricting ethylene biosynthesis and preserving postharvest quality. SA significantly protects cell walls by reducing degrading enzymes, and as a result, SA prevents the cells from dramatically increasing TSS level. In recent years, consumers have been interested in nutraceuticals or functional foods, which are active food ingredients or specific foods (Harsler, 1998). Secondary metabolite compounds have a wide

distribution in plants and are therefore a valuable part of the human diet. Phenolic compounds might act as an antioxidant and maintain foods from oxidative deterioration. Studying of antioxidant activity of secondary metabolite compounds have increased recently because of the possible role of reactive oxygen species against degenerative diseases such as cancer. In grapes, phenolic compounds contribute desired flavor astringency, color and vitamins (Shiri *et al.*, 2013).

The purpose of this study was to examine the effect of CT coating and SA applications to clusters of 'Red Globe' grapes to increase the postharvest storage period and preserve quality characteristics. In addition, the effectiveness of CT on the total phenolic content of grapes and, accordingly, the total antioxidant activity during storage was determined.

MATERIALS AND METHODS

Plant material: 'Red Globe' is one of the most well-known table grapes internationally due to its ability to withstand long transportation and storage. 'Red Globe' grapes were harvested from a highly productive commercial vineyards located on Manisa, Turkey, in 2018-2019 growing seasons (Figure 1A-B). Then, immediately transported to the laboratory of Agricultural and Sciences and Technologies Faculty, Nigde Omer Halisdemir University. Grape clusters were selected based on absence of defects or disease and randomly distributed into four treatment groups.

CT and SA applications, packing and preserving in the cold storage: For experimental use, 1% CT was prepared by dissolving in 0.5% (v/v) glacial acetic acid under continuous stirring. When dissolved, the pH of the CT solution was adjusted to 5.2 using 1 M NaOH. After that surface sterilized grapes, were distributed into four groups. Each group had at least six clusters per treatment. Each treatment was repeated at least three times. The treatments were: (1) control; (2) CT; (3) CT + 1 mM SA; and (4) CT + 2 mM SA. Firstly, grape clusters were dipped in sterile distilled water for 5 min as a control, and the other treatments were also applied by dipping for 5 min. After treatment, all samples were dried at room temperature for 2 h. Then, the treated clusters were packaged in MAP bags with 0.016 mm pore size to maintain RH and to create low O₂ and high CO₂ atmospheric conditions; they were stored at 0.5°C at 90-95% RH for 60 days. At 15-day intervals (i.e., days 15, 30, 45, 60 after treatment), including initial treatment, samples were removed from storage and kept at room temperature for 24 h to stimulate the shelf life. Then, physical and chemical measurements or secondary metabolite contents of the samples were determined as described below.



Figure 1. 'Red Globe' grapevines from the grower's vineyard (A), harvested 'Red Globe' clusters (B)

Physical characteristics: Berry decay (%), weight loss (g), berry firmness (N), and berry skin color chroma index were determined as physical properties. Berry decay rate of grapes in each treatment group during storage was determined and calculated as percentage of decay berries from total berries. During the storage period of grapes, weight loss in each treatment was measured by monitoring fruit weight changes. Weight loss was calculated as percentage loss of initial weight as: $\text{weight loss (\%)} = \{[\text{initial weight (g)} - \text{weight (g)}] / \text{initial weight}\} \times 100$. Firmness of berries were measured by using the method of Aday and Caner (2010) with a texture analyzer (TA.HD.Plus, Godalming, UK) as the force (N) required for penetration using a 3 mm probe at a speed of 1 mm s^{-1} and a penetration distance of 6 mm. Berry skin color was measured by the method of McGuire (1992) using a Konica Minolta CR-200B. A single measurement was recorded for each berry and 20 replicate berries were measured for each treatment. The value of L^* describes the degree of darkness or lightness. Before analysis, a^* and b^* coordinates were transformed into the equation $C^* = (a^{*2} + b^{*2})^{0.5}$. Richness of color was represented by C^* value.

Chemical measurements and analyses: For the chemical measurements or analyses, grape clusters representing with the vine were harvested according to Rankine *et al.* (1962). Before analyses, berries from the clusters of the treated groups were selected randomly for the berry extraction and homogenized in an ice-cold blender after removal of seeds. A 25 g of the homogenate were macerated in 100 ml of ethanol containing 0.1% HCl and set aside overnight in darkness. After that, the extracts were filtered and the extracts were centrifuged at 6,000 rpm at 4°C for 15 min. Some of the extracts were separated to determine the anthocyanin content and the remaining portions were concentrated by rotary evaporator at 50°C and used to determine the total

phenolic, flavonoid contents and antioxidant potential of the samples.

Titrateable acidity (TA as % tartaric acid), TSS ($^\circ\text{Brix}$) and pH of the grape samples were measured in the fruit juice by standard methods immediately after crushing of the samples. A subsample of 10 g of berry was tissue homogenized in ddH₂O and the final liquid level was adjusted to 100 ml for measuring pH and TA. TSS were measured by digital refractometer (Kruss, AR2008, Germany). NaOH solution (0.1 M) was added to the homogenized berry juice until to the endpoint of pH 8.2. TA acidity results were expressed as the percentage of a tartaric acid reference. Total anthocyanin content (TAC) of the grape berry extracts were determined by the pH differential method (Giusti and Wrolstad, 2003). All values were expressed as malvidin-3-glucoside using a molar extinction coefficient of $28,000 \text{ kg}^{-1} \text{ FW}$ (Wrolstad, 1976). TP in ethanolic berry extracts were estimated by the method of Slinkard and Singleton (1977) with minor modifications. A 20 μL of berry extract was added to a 3 mL of centrifuge tube then 2.48 mL of ddH₂O added. After that, 0.2 mL folin reagent was added and incubated for 8 min. At the end 0.3 mL Na₂CO₃ was added to the mixture and stood for 60 min at room temperature and then the absorbance values of the samples were read at 750 nm using a spectrometer. Total phenolic content of samples were obtained from the calibration curve prepared with gallic acid (GAE, 20-250 mg L⁻¹) and expressed as mg GAE per kg⁻¹ of FW. TP content of the berry extracts were determined by the modified colorimetric method (Zhishen *et al.*, 1999). TF content obtained from the calibration curve prepared with catechin (20-250 mg L⁻¹) and expressed as mg of CE kg⁻¹ FW.

Antioxidant potential of berry extracts: Total antioxidant potential of ethanolic berry extracts consisted of antioxidant activity and antioxidant capacity. Total

antioxidant activity of samples was determined by DPPH free radical scavenging activity and total antioxidant capacity was determined by FRAP assay. The DPPH free radical scavenging activity of the ethanolic berry extracts were measured according to DPPH standard method (Blois, 1958). Fifty percent inhibitory concentration (IC_{50}) values were calculated after constructing the percent inhibition versus log curve. The total antioxidant capacity of samples was determined by the FRAP assay (Oyaizu, 1986). The absorbance was measured at 700 nm. BHT (20-250 mg mL⁻¹) was used as standard for construction of the calibration curve and reducing power was expressed as BHT equivalents per mg mL⁻¹ of extract.

Statistical analyses: All experiments were repeated three times and reported as the mean of three experiments. Analysis of variance was used to analyze the data. Statistically significant differences among the coating applications and storage time were determined by using $p \leq 0.05$ values in SAS (SAS Institute, 1995). Significant differences in numbers are presented in letters. Different letters are indicated on the column as significant.

RESULTS

Physical parameters: Physical grape quality characteristics including fungal berry decay, weight loss, berry skin firmness and chroma index of the berries are presented in Figure 3A-D. Decay rate increased with increasing storage time. CT treatment significantly decreased berry decay rate. Berry decay rate was 0% in the first 15 days, in second 15 days fruit decay had started and after 60 days there was more than 3.2% fruit decay in the control group. In treatment groups, the least decay after 60 days (0.9%) was with CT + 2 mM SA (Figure 3A). Weight loss of grape berries during the storage is shown in Figure 3B. CT coating treatment significantly decreased weight loss in grape berries. CT + 2 mM SA was the most effective for decreasing weight loss during the storage. The maximum weight loss after 60 days in storage was in control group with 0.14 and CT + 2 mM SA had the least weight loss with 0.1%. Berry firmness results are shown in Figure 3C with changes in the flesh firmness value (N) of the uncoated and coated samples occurring during storage. Control and CT group lost firmness more quickly than CT + 1 mM SA and CT + 2 mM SA. The highest firmness value after 60 days storage was with CT + 1 mM SA group by 4.5 N. C^* values of coated and uncoated berries decreased during storage. At the end of storage, the highest C^* value (10.9) was in fruit in the CT group, and the least chroma value (8.6) was in CT + 2 mM SA. The highest C^* value was measured after 15 days of storage in control group and the lowest C^* value after 30 days storage for CT + 2 mM SA. While the differences in the C^* values of the applications

were significant, the effect of the storage period on the C^* values was also significant.

Chemical properties: Changes in TSS, TA and pH are presented in Figure 4A-C. TSS decreased during storage, from 17.6% and to the lowest of 13.1% after 60 days in CT + 1 mM SA. Although storage time was significant for TSS, the CT treatments were not. TA content is shown in Figure 3B. Changes in the amount of acidity of the uncoated and coated treatments occurred during the cold storage. The highest TA after 60 days was in the control group by 0.73%, which significantly controlled the increasing TA rate in treatment groups. The pH values of the control and coated grape berries decreased from 3.7 to 3.1 respectively. While the effect of coating on the pH values was significant, the effect of storage period was also significant.

Secondary metabolite content: TAC, TP and TF content of the grapes are presented in Figure 5A-C. TAC in grape samples before storage was 171 mg malvidin-3-glucoside kg⁻¹ FW. During storage, the amount of anthocyanins changed sporadically. At the early stage of storage, the total anthocyanin decreased and after storage period of 30 days again they increased then at the last stage decreased again. At the end of storage, total anthocyanin were 127 mg malvidin-3-glucoside kg⁻¹ FW (CT + 2 mM SA), 105 mg malvidin-3-glucoside kg⁻¹ FW (CT), 97.2 mg malvidin-3-glucoside kg⁻¹ FW (control) and 96.4 mg malvidin-3-glucoside kg⁻¹ FW (CT + 1 mM SA). Applications were significantly controlled the decreasing in total anthocyanins during storage times compared to the control. TP in all treatments generally tended to increase during storage time. The highest phenolic content was with CT application (268 mg GAE kg⁻¹ FW) and the lowest with CT + 1 mM SA (195 mg GAE kg⁻¹ FW) after 60 days. The TF contents of all treated and untreated grapes during the storage increased in the early stage then decreased at the end of the storage period. The highest TF content was determined with CT + 1 mM SA (80.5 mg CE kg⁻¹ FW) after 15 days of storage and at the end of storage period the highest level was with CT (71.8 mg CE kg⁻¹ FW) and the lowest was in the control (64.5 mg CE kg⁻¹ FW).

Antioxidant potential: IC_{50} values of the samples, derived from DPPH free radical scavenging activity, are presented in Figure 6A. IC_{50} values of all treatment samples decreased during storage. However, the lowest IC_{50} value was recorded after 45 days with CT + 1 mM SA by 0.34 mg mL⁻¹. The initial IC_{50} value was 0.53 mg mL⁻¹ but after storage the values for the control, CT, CT + 1 mM SA and CT + 2 mM SA were 0.49, 0.50, 0.53 and 0.47 mg mL⁻¹, respectively. Figure 6B presents the total antioxidant capacity obtained by FRAP assay. The initial total antioxidant capacity of grapes was 23.8 mg BHT mL⁻¹, and total antioxidant capacity of coated and

uncoated grapes increased during storage. The highest antioxidant capacity after a storage for 60 days was for

CT by 49.9 mg BHT mL⁻¹ and the lowest for CT + 2 mM SA by 39.1 mg BHT mL⁻¹.

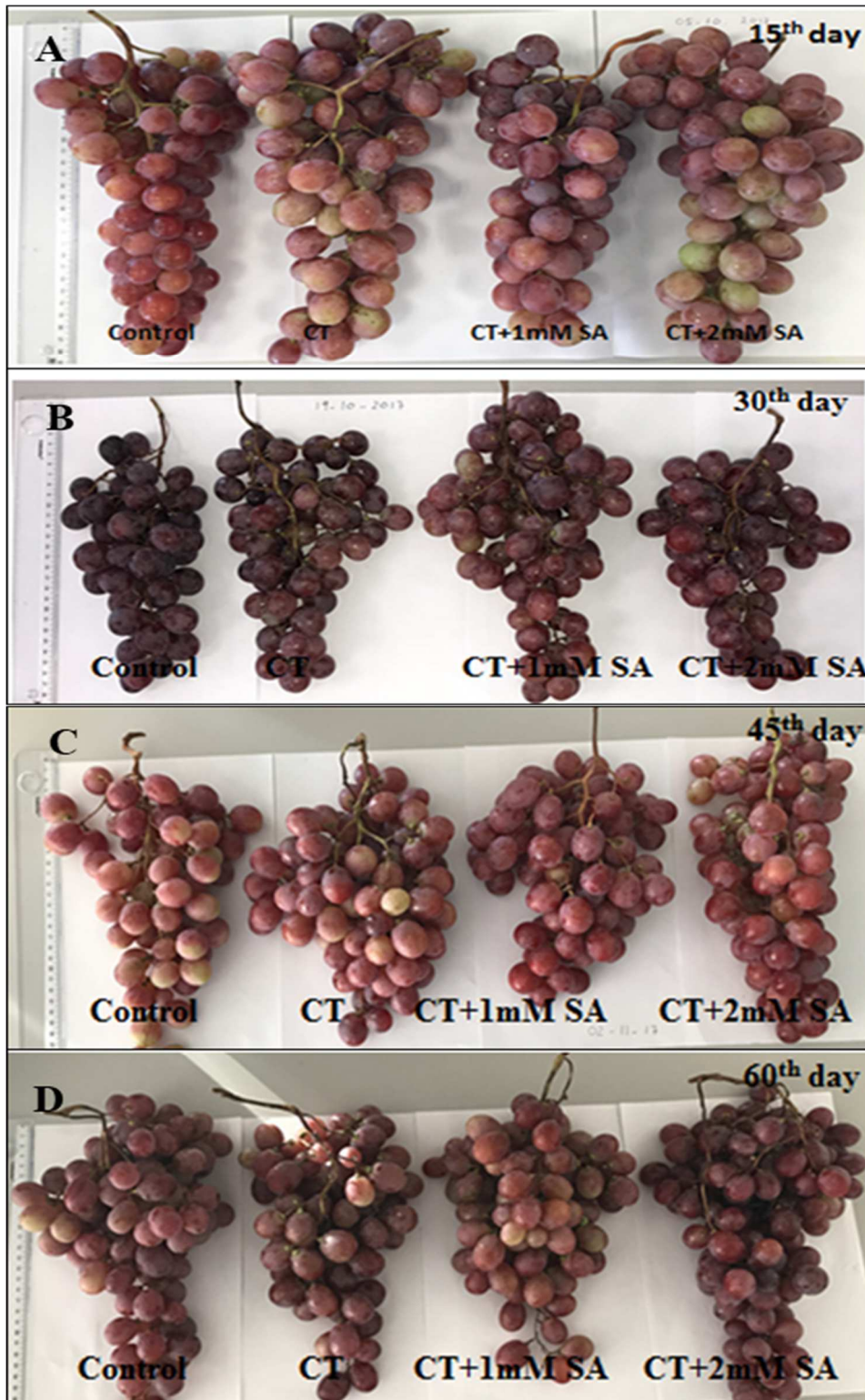


Figure 2A-D. 'Red Globe' clusters treated with 1) treated with sterile water (control); 2) 1% of CT; 3) 1% of CT + 1 mM SA; 4) 1% of CT + 2 mM SA and stored in normal atmosphere cold storage rooms. Pictures taken on days 15 (A), 30 (B), 45 (C) and 60 (D).

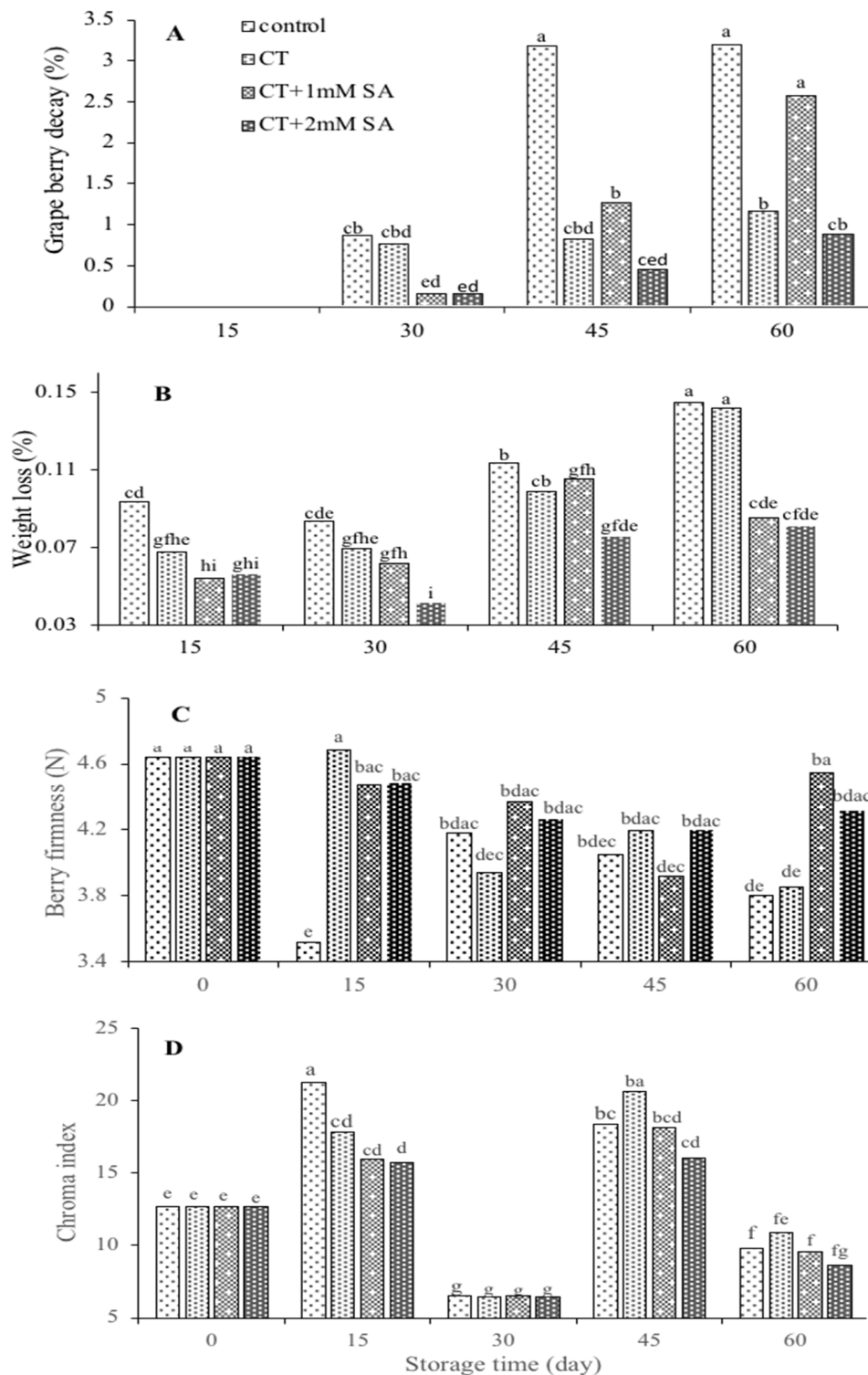


Figure 3A-D. Effects of CT and SA applications and storage times on physical quality characteristics of ‘Red Globe’ grape cultivar. Grape clusters were treated with;1) sterile water (control); 2) 1% of CT;3)1% of CT + 1 mM SA; 4) 1% of CT + 2 mM SA and stored in normal atmosphere cold storage rooms for 60 days.At 15-day intervals,A) grape berry decay (%), B) weight loss (%), C) berry firmness (N), D) chroma index of the samples were measured. The letters on the top of the bars, which are the same, indicate that there is no statistically significant difference.

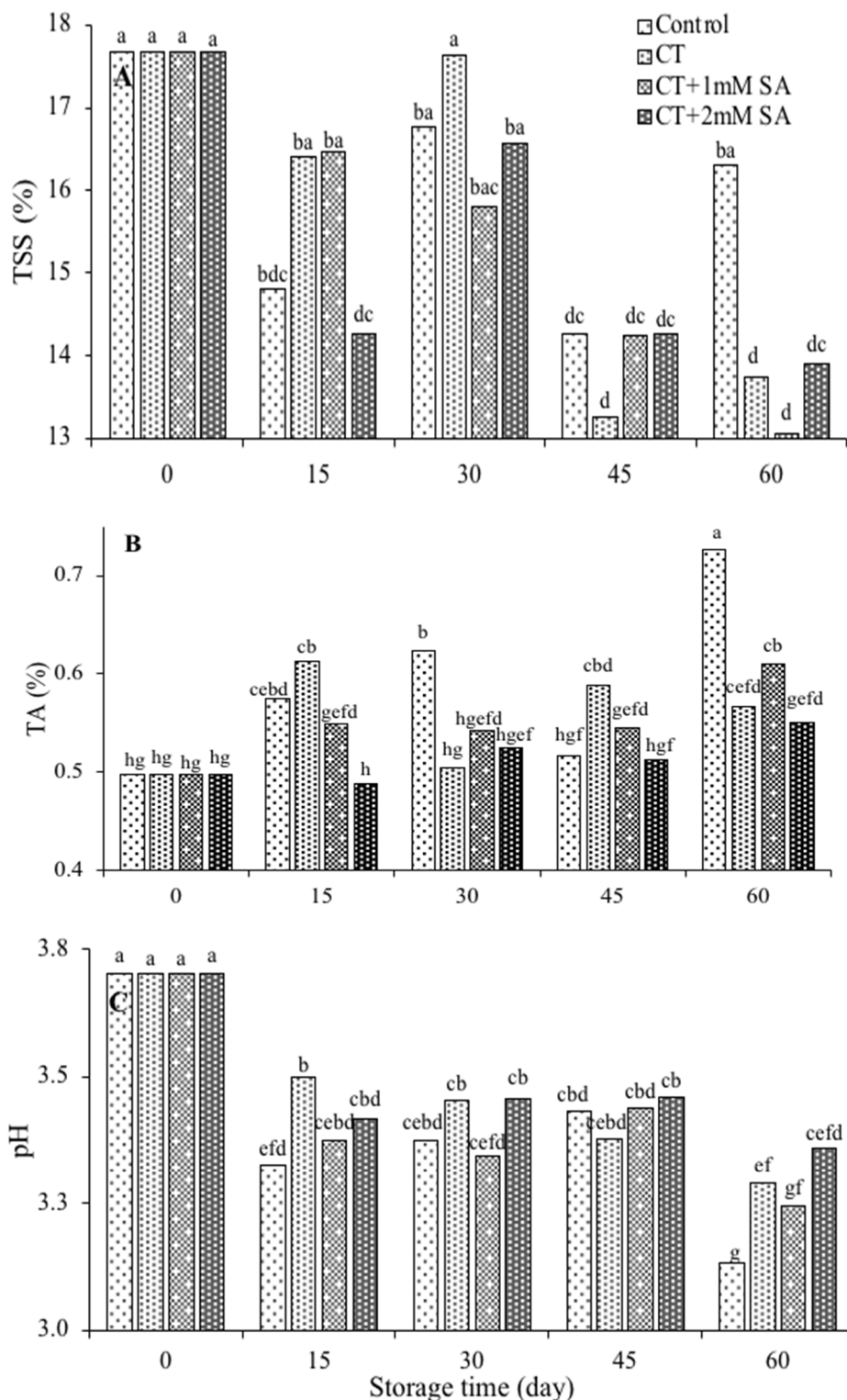


Figure 4A-C. Effects of CT and SA applications and storage times on chemical quality characteristics of ‘Red Globe’ grape cultivar. Grape clusters were treated with; 1) sterile water (control); 2) 1% of CT;3)1% of CT + 1 mM SA; 4) 1% of CT + 2 mM SA and stored in normal atmosphere cold storage rooms for 60 days. At 15-day intervals, A) TSS (%), B) TA (%), C) pH of the samples were measured. The letters on the top of the bars, which are the same, indicate that there is no statistically significant difference.

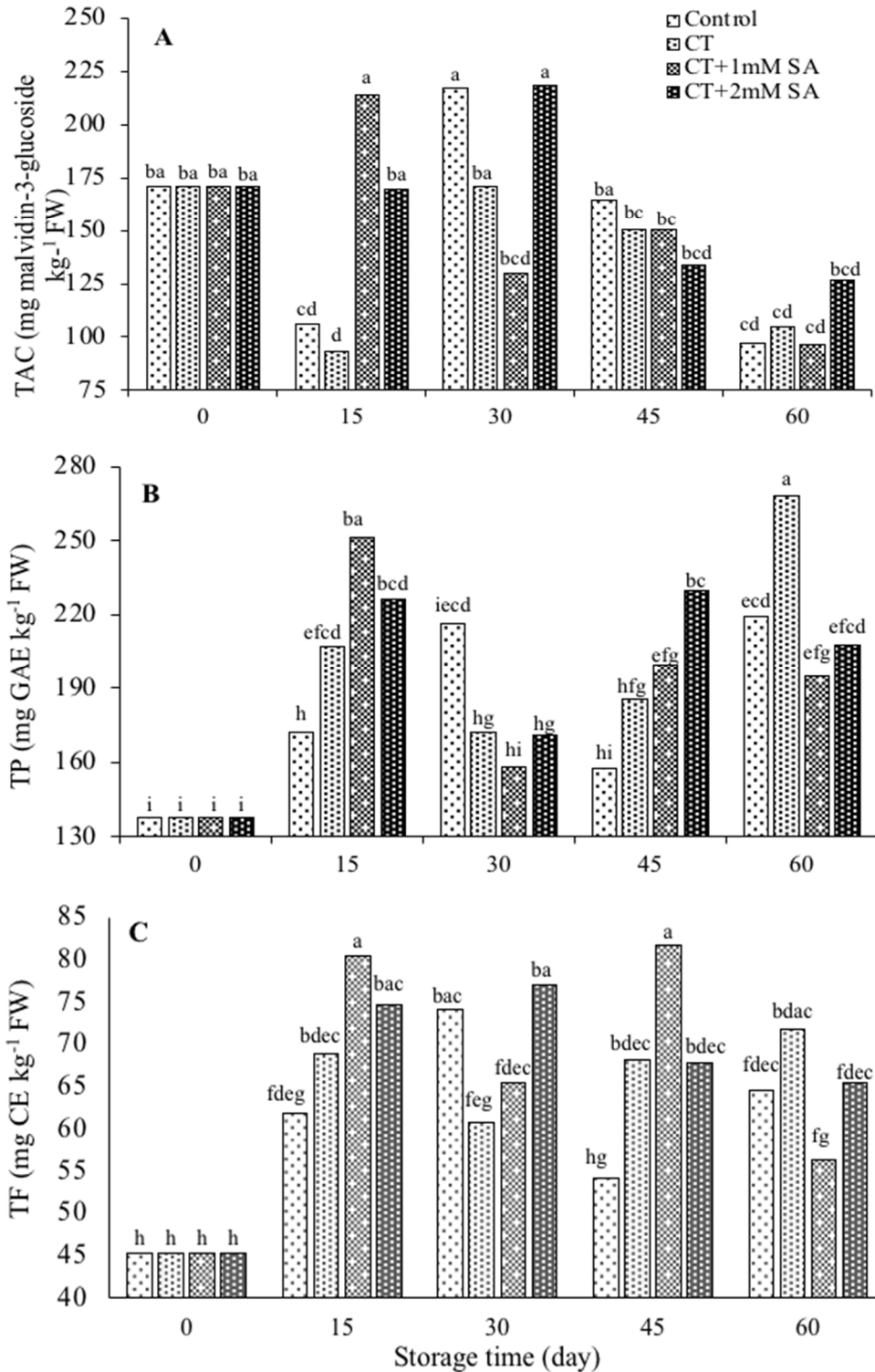


Figure 5A-C. Effects of CT and SA applications and storage times on secondary metabolite contents of ‘Red Globe’ grape cultivar. Grape clusters were treated with; 1) sterile water (control); 2) 1% of CT; 3) 1% of CT + 1 mM SA; 4) 1% of CT + 2 mM SA and stored in normal atmosphere cold storage rooms for 60 days. At 15-day intervals, A) TAC (mg malvidin-3-glucoside kg⁻¹ FW), B) TP (mg GAE kg⁻¹ FW), C) TF (mg CE kg⁻¹ FW) content of berry ethanolic extracts were measured. The letters on the top of the bars, which are the same, indicate that there is no statistically significant difference.

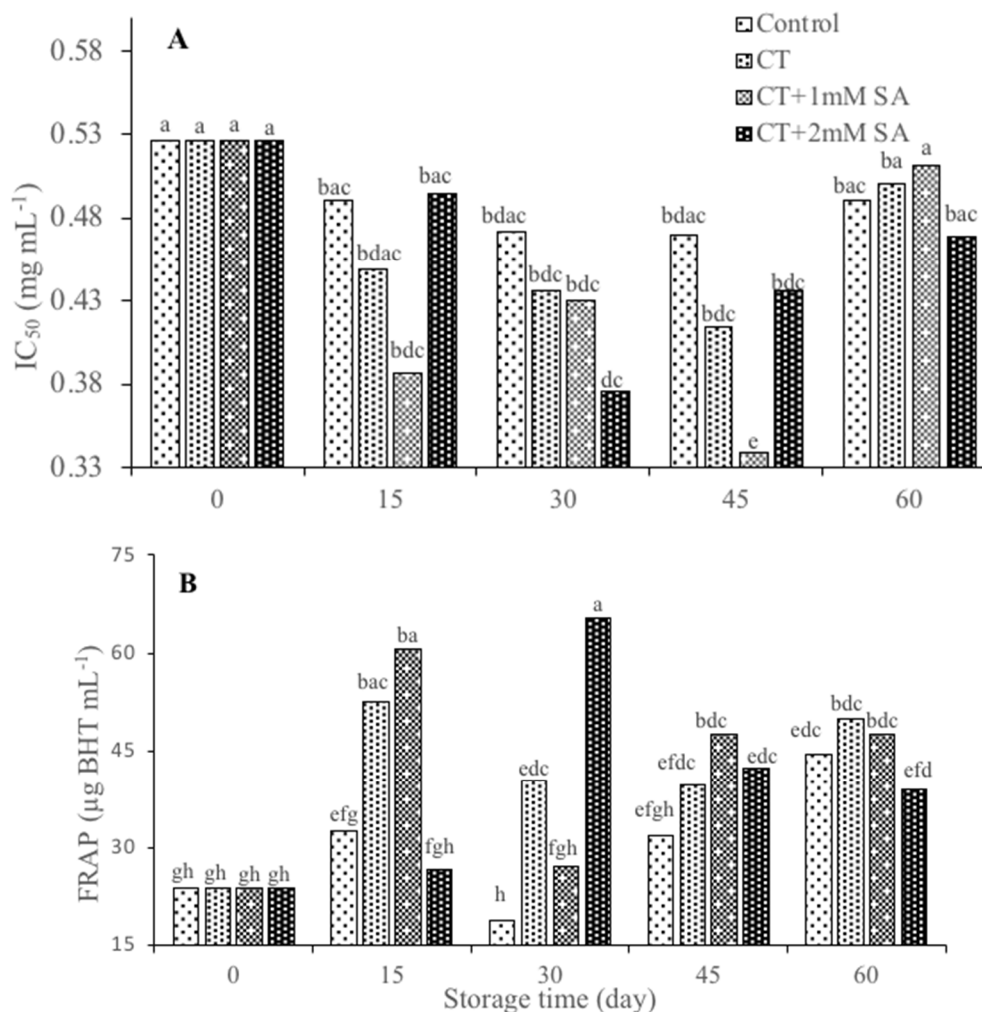


Figure 6A-B. Effects of CT and SA applications and storage times on secondary metabolite contents of ‘Red Globe’ grape cultivar. Grape clusters were treated with; 1) sterile water (control); 2) 1% of CT; 3) 1% of CT + 1 mM SA; 4) 1% of CT + 2 mM SA and stored in normal atmosphere cold storage rooms for 60 days. At 15-day intervals, A) IC₅₀ (mg mL⁻¹), and B) FRAP (µg BHT mL⁻¹) capacity of berry ethanolic extracts were measured. The letters on the top of the bars, which are the same, indicate that there is no statistically significant difference.

DISCUSSION

The results showed that the application of CT alone or in combination with SA, significantly reduced the decay rate in grape berries compared with control during storage. Similar findings were reported for fruit decay in grapes caused mostly by *Botrytis cinerea*, which decreased with CT coating (Shen and Yang, 2017). Liu *et al.*, (2007) found that CT significantly reduced spore germination and mycelial growth of *B. cinerea* by damaging plasma membrane of the spores *in vitro*. In another study (Meng *et al.* 2008), preharvest and postharvest CT coating gave good control of fungal decay of table grapes. These results indicate that CT coating can effectively decrease decay of grapes. However, CT + 2

mM SA coating was even more effective in reducing the decay than CT alone. Shen and Yang (2017) also showed that CT with SA was more effective than CT alone for inducing grape berries resistance to fungal diseases, because SA also forms a film on the surface of the berries that acts as a physical barrier to infection. Overall, CT + 2 mM SA performed best among the treatments. Weight loss in grapes is mainly due to water loss through respiratory processes. Weight loss in all treatments increased over the storage time. After 60 days, weight loss in the control was 0.14% whereas CT + 2 mM SA reached just 0.08%. Previous studies found that the CT coating functions as self-controlling atmosphere and selectively leaches O₂, CO₂ and C₂H₄, inside and outside the fruit, thus reducing fruit respiratory metabolism

(Hagenmaier, 2005). In the present study, it was found that all CT treatments gave less weight loss than the control. Similar findings for CT coated grapes were reported by Gao *et al.* (2013). However, CT + 1 mM SA and CT + 2 mM SA were more effective than other treatments at reducing weight loss due to sustained release of SA, consistent with the results of Shen and Yang (2017). Fruit color and firmness are important quality parameters for grapes, and there were significant differences between the treatments for C^* values based on storage time and treatment in the present study. The highest saturated value was with CT alone and the least with CT + 2 mM SA. Perdones *et al.* (2012) reported that the vivid colors of strawberries decreased during storage and found the highest value in CT coating, as was found in the present study. During the storage there was a significant decline in firmness of the control samples compared to treated samples. Firmness changes of fruit flesh during storage is attributed to disruption of primary cell wall and middle lamella structures (Yang *et al.*, 2011). In the present study, CT, CT + 1 mM SA and CT + 2 mM SA might have delayed berry softening by inhibiting cell wall-degrading enzymes activities. Guerra *et al.* (2016) also have reported a similar delays in softening of grapes with CT coating.

For the chemical quality parameters of the grapes, TSS, TA and pH changed with treatments and storage time. TSS decreased with longer storage. These data were consistent with the results of Sanchez-Gonzalez *et al.* (2010), who reported a sharp decrease in TSS in grape samples after 8 days with CT application. Also, TA of grapes in the present study increased during storage. Which the initial 0.50% and after storage the values for the control, CT, CT + 1 mM SA and CT + 2 mM SA were 0.73, 0.56, 0.61 and 0.55%, respectively. CT coating significantly limited the increase in TA during storage but CT + 2 mM SA was more effective than other treatments. This result is consistent with similar studies in which the application of CT coating and SA applied after harvest were examined with strawberry, guava fruit and banana (Asghari and Aghdam, 2010; Shafiee *et al.*, 2010; Maqbool *et al.*, 2011; Hong *et al.*, 2012). The pH of the grapes also decreased during storage, from the initial pH of 3.7 and after 60 days the pH of the control, CT, CT + 1 mM SA and CT + 2 mM SA had dropped to 3.1, 3.3, 3.2 and 3.4, respectively. A similar result was reported by Sanchez-Gonzalez *et al.* (2010) with decreased pH of grape fruit and attributed to the natural variability of the product. TAC, which is effective in the coloration of the fruit, are synthesized in the fruit skin and fruit flesh. Therefore, it is an important parameter for fruit quality due to its effect on fruit appearance and antioxidant properties. TAC in the grapes before storage was 171 mg malvidin-3-glucoside kg^{-1} FW. During storage, TAC changed and at the end of storage period it decreased to 127 mg malvidin-3-

glucoside kg^{-1} FW with CT + 2 mM SA. CT films form an effective gas barrier, probably due to the dense structure of the film (Wong *et al.*, 1992). Therefore, a possible internal change in the coated samples due to CT coating could explain this behavior. Although, TP content of the grapes in all treatments increased early time, they decreased with further storage. After 60 days, the highest TP was with CT alone and a similar result was reported in grapes (Shen and Yang, 2017). In all treatments, TF increased comparably with harvest time and decreased at the final storage stage. The initial TF was 45.3 mg CE kg^{-1} FW. After 30 days, amount of flavonoids increased in all treatments. This is considered to be that flavonoid synthesis, one of the secondary metabolites that might increase under low temperature stress (Quan *et al.*, 2008).

During storage, the antioxidant activity in grape extracts decreased in uncoated grapes compared to coated fruit extracts. The decline in antioxidant activity in untreated grape extract at the end of storage might be due to senescence and decay. This indicated that CT coating might not only extend the shelf life, but also it can also enhance health by promoting natural antioxidant activity in grapes (Al-Qurashi and Awad., 2015). Total antioxidant capacity of the grapes in all treatments increased during storage. Tavarini *et al.* (2007) found the increasing total antioxidant capacity, determined by FRAP method, in kiwifruit and reported that antioxidant capacity increased during storage. Shivashankara *et al.* (2004) reported that an increase in antioxidant capacity during low temperature storage might be possible in fruits which the contribution of total phenolic contents of the samples. CT coating enhanced antioxidant capacity and it could be used as an antioxidant and anti-agent in the food industry (Devlieghere *et al.*, 2004). In the present study, the highest FRAP value was detected in coated samples. A strong relation was found between secondary metabolite content and antioxidant activity. Especially the TP and TF contents of the samples had a significant effect on the antioxidant power of the grape extracts.

Conclusions: It may be concluded that the application of the natural product, CT, alone or in combination with SA significantly reduced the decay rate in grape berries and maintained the quality characteristics. In particular, CT + 2 mM SA coating more effectively reduced the incidence of rot of grapes compared to CT alone or the other treatments, as SA formed a film on the surface of grape berries that served as a physical barrier to infection. The use of CT + 2 mM SA together with MAP can be a viable alternative to the use of SO_2 in grape storage and may prove to have value in commercial applications.

Authors' Contributions: MO guided the study, analyzed the data and wrote the manuscript; RQ collected the study material, performed the laboratory work. Both authors read and approved the manuscript.

Conflict of Interests: The authors declare that there are no conflicts of interest related to this article.

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