Folia Hort. 36(1) (2024): 149–160

DOI: 10.2478/fhort-2024-0009

FOLIA HORTICULTURAE

Published by the Polish Society for Horticultural Science since 1989

ORIGINAL ARTICLE | Open access | https://sciendo.com/journal/FHORT

Chitosan–putrescine nanoparticle coating attenuates postharvest decay and maintains ROS scavenging system activity of strawberry cv. 'Camarosa' during cold storage

Reza Bahmani[1], Farhang Razavi[1], Seyed Najmmaddin Mortazavi[1], Antonio Juárez-Maldonado[2], Gholamreza Gohari[3,]*

[1] Department of Horticulture, Faculty of Agriculture, University of Zanjan, Zanjan, Iran
[2] Departamento de Botánica, Universidad Autónoma Agraria Antonio Narro, Saltillo 25315, Coahuila, Mexico
[3] Department of Horticultural Science, Faculty of Agriculture, University of Maragheh, Maragheh, Iran

ABSTRACT

The application of some natural compounds and cold storage can reduce postharvest loss and improve fruit quality and storability, which increase the fruit marketing period and exportation to distant markets. This study investigated the effectiveness of using coatings based on natural polymers to prolong the shelf life of fruits and protect their tissues from damage. Specifically, the researchers examined the impact of putrescine (PUT), chitosan (CTS) and chitosan–putrescine nanocomposites (CTS-PUT NPs) on preserving the quality and reducing decay in strawberry. Strawberries were treated with either 1 mM or 2 mM of PUT, 0.1% CTS, 0.1% CTS-PUT NPs or distilled water at a temperature of 20°C for 5 min and then stored at 4°C for 12 days. At the end of the storage period, the lowest decay percentage was related to the treatments of CTS-PUT NPs and CTS, and the highest was observed in the control. The results showed that all coating treatments effectively prevented the excessive loss of ascorbic acid, fruit firmness, total soluble solids (TSS), and acidity (TA) compared to control at the end of the storage period. Additionally, strawberries treated with CTS-PUT NPs and CTS demonstrated the highest levels of total anthocyanin and total flavonoid. Antioxidant enzyme activities containing CAT and SOD were preserved at higher levels in treated strawberries than in the control during cold storage. These results confirm that the application of CTS and CTS-PUT NPs can effectively preserve fruit quality and prolong the longevity of strawberries during cold storage by enhancing their antioxidant capacity and scavenging free radicals.

Keywords: antioxidants, biopolymer, nanotechnology, polyamine, postharvest

Abbreviations: CTS, chitosan; PUT, putrescine; NPs, nanoparticles; MDA, malondialdehyde; H_2O_2, hydrogen peroxide; CAT, catalase; SOD, superoxide dismutase.

INTRODUCTION

Strawberry (*Fragaria* × *ananassa*) is a non-climacteric fruit of the widely grown hybrid species of the genus *Fragaria*. The quality of strawberries depends on their appearance, texture, aroma, taste and nutritional value. Sugars, organic acids and aromatic compounds play an important role in strawberry flavour (Buendia et al., 2010). Strawberries have a short shelf life due to their high respiration rate, abundant water content (about 91%), high metabolic activity and susceptibility to fungal rot (Liu et al., 2018). The exogenous postharvest treatments (spraying, coating or dipping) contribute to the maintenance of the fruit quality during storage (Buendia et al., 2010). Nowadays, much effort has been made to increase the shelf life of strawberry to preserve

*Corresponding author.
e-mail: gohari.gh@maragheh.ac.ir (Gholamreza Gohari)

Open Access. © 2024 Bahmani et al., published by Sciendo. This work is licensed under the Creative Commons Attribution alone 3.0 License.

its nutritional value during storage by using different methods (e.g. modified atmosphere packaging, moisture and ethylene absorber, and chemical and radiation treatments) (Wszelaki and Mitcham, 2003; Jesus Filho et al., 2018; Baranyai et al., 2020; Nguyen et al., 2020). Fruit edible coating by bio-polymer is one of the suitable methods to increase the shelf life of fruits and prevent tissue damage.

Chitosan (CTS) is a natural coating that has been used successfully in postharvest physiology; it is obtained from chitin shells of shrimp and other crustaceans after deacetylation. CTS films are relatively permeable to water vapour and are a good barrier to oxygen gas. Moreover, CTS is used in food packaging due to its semipermeable and antimicrobial properties (Bautista-Banos et al., 2006). CTS coatings have been shown to reduce weight loss, firmness and decay and increase antioxidant activity and the soluble solids content in fresh fruits (Wang and Gao, 2013; Adiletta et al., 2019). Edible coatings with nanostructures have been studied as an alternative to traditional coating methods in maintaining crop quality, increasing shelf life and reducing microbial spoilage (He and Hwang, 2016).

Polyamines are small aliphatic amines having low molecular weight, are ubiquitous in living organisms and play a role in a wide range of physiological processes, including embryogenesis, root formation, pollen grain formation, flowering, fruit development and stress conditions (Alcazar et al., 2020). Putrescine (PUT) is the main product of polyamine biosynthesis and a synthetic precursor of spermidine and spermine (Zokaei Khosroshahi et al., 2007). The application of PUT, along with other polyamines, can be used to extend the shelf life of fruits by delaying the ripening process (Zokaei Khosroshahi et al., 2007). The application of PUT in four plum cultivars delayed the senescence of fruits and increased their shelf life at 26°C. In addition, it increased total soluble solids (TSS), titratable acidity (TA) content and firmness of the fruit tissue (Serrano et al., 2003). Postharvest application of PUT has been reported to delay fruit ripening and extend shelf life in some fruits such as strawberries (Zokaei Khosroshahi et al., 2007), peaches (Abbasi et al., 2019) and Murcott mandarins (Ennab et al., 2020).

In recent years, the application of CTS-based nanocomposites has been found to be very useful in maintaining the quality and shelf life of fruits and vegetables (Xing et al., 2019; Van et al., 2020; Nasr et al., 2021; Bahmani et al., 2022; Mahmoudi et al., 2022). The CTS treatment in combination with nano-silicon reduced the quality attributes of jujube fruits such as red colour, respiration ratio, decay percentage and weight loss compared to the control samples after storage for 32 days at room temperature (Yu et al., 2012). Hernández-López et al. (2020) indicated that using CTS nanoparticles with α-pinene markedly preserved the physicochemical quality, delayed weight loss, inhibited *Altenaria alternata* and increased the content of total carotene in bell peppers.

The existing literature does not provide any findings regarding the effects of chitosan coated with putrescine nanoparticles (CTS-PUT NPs) on postharvest quality attributes of strawberries. Therefore, the objective of this study was to explore the potential of PUT, CTS, and CTS-PUT NPs in preventing decay, extending the shelf life, enhancing antioxidant enzyme activities and improving the overall quality of strawberries during a 12-day storage period at 4°C.

MATERIALS AND METHODS

Plant materials and experimental site and time

The current research was conducted on strawberry cv. 'Camarosa' fruits in a greenhouse in Zanjan, Iran, in 2020. The fruits were harvested at the commercial maturity stage (>80% of the surface red colour) and were selected in terms of uniformity in shape and size and absence of visual damage. Then, they were immediately transferred to the postharvest physiology laboratory of the Department of Horticulture, University of Zanjan, to be applied with the treatments.

Treatments, preparation and application

Chitosan-coated putrescine nanoparticles (CTS-PUT NPs) were synthesised at the Nanochemistry Laboratory of the University of Maragheh in Iran, following the method described by Mahmoudi et al. (2022) with some modifications. To obtain a clear solution of CTS, 0.1 g of low-molecular weight CTS powder was added to 25 mL of 1% acetic acid solution (w/v) and mixed at a rate of 300 rpm at 70°C for 2 hr. Subsequently, 0.1 g of PUT was dissolved in 15 mL of distilled water and slowly added to the CTS solution. The ratio of tripolyphosphate (TPP) to CTS was 1:2.5, so 0.4 g of TPP was dissolved in 5 mL of distilled water and gradually added to the CTS and PUT solution, resulting in the formation of CTS nanoparticles through crosslinking. Strawberries were then immersed in solutions containing 1 mM or 2 mM of PUT, 0.1% (w/v) CTS, 0.1% (w/v) CTS-PUT NPs or deionised water (control) for 5 min at 20°C. After dipping treatment, the strawberries were placed on a kraft paper and allowed to dry for 1 h at room temperature (20°C). Following drying, the fruits were sealed in polyethylene film bags of 0.03 mm in thickness with 4–5 holes (7–8 mm) to maintain the composition of air within the container and stored in a cold storage facility at 4°C with a relative humidity of 85%–90% for 12 days. They were randomly allocated into five groups of 240 for each treatment in three replications (80 per replication). Three boxes from each treatment served as three replications. The samples were taken out of storage on days 3, 6, 9 and 12, and their shelf life was evaluated after 24 hr at 20°C. After that, samples of fruit from each treatment/replicate were collected for quality measurements. Also, the additional samples of fruits were frozen in liquid nitrogen and

stored at −80°C immediately. The frozen samples were used to determine the content of ascorbic acid, malondialdehyde (MDA), hydrogen peroxide (H_2O_2), flavonoids, total anthocyanin, and total phenolics; total antioxidant capacity; and the antioxidant activity of catalase (CAT) and superoxide dismutase (SOD).

Determination of decay percentage

The decay percentage was assessed by counting the number of rotten fruits at each sampling step and determining the difference between the number of initial healthy fruits and rotten fruits in each box. It was expressed in percent (Meng et al., 2008).

Determination of firmness, TSS, TA and ascorbic acid

Tissue firmness was measured on both sides of fruits using a texture analyser (FT011, Facchini srl., Alfonsine [RA], Italy) of a probe diameter of 5 mm. It was expressed in newtons (N) (Meng et al., 2008).

The TSS content of the fruits was determined using a digital refractometer (PAL-1, Atago Co, Tokyo, Japan). To measure TA, 10 mL of fruit juice was mixed with 45 mL of distilled water and two drops of phenolphthalein as an indicator. Then, it was titrated with sodium hydroxide 0.1 N until its pH reached 8.1. The amount of NaOH consumed was used to calculate acidity in terms of the dominant acid (citric acid) (Hernández-Muñoz et al., 2006).

Ascorbic acid was measured by the method of titration with potassium iodide for which 10 mL of the fruit juice was poured into a beaker, and 2 mL of starch 1% was added was added to it. The resulting solution was titrated against the iodine solution and the titration process was continued until the formation of dark blue colour. The amount of ascorbic acid was expressed in mg · 100 g^{-1} FW (Saini et al., 2006).

Determination of total anthocyanin, phenol and flavonoid contents and antioxidant capacity

To measure total anthocyanin, phenol and flavonoid contents and antioxidant capacity, 1 g of the strawberry fruit tissue was mixed with methanol 80%. Then, its volume was adjusted to 10 mL, and the extract was centrifuged at 12000 × *g* for 10 min.

The total anthocyanin content (TAC) was measured by using the pH differential method. First, 200 µL of the fruit supernatant was added to 1800 µL of the potassium chloride buffer (pH = 1) and 1800 µL of the sodium acetate buffer (pH = 4.5). The diluted solutions were allowed to stand for 15 min to equilibrate. Finally, the absorbance was recorded at 510 nm and 700 nm, and the anthocyanin content was calculated in mg · 100 g^{-1} FW (Pineli et al., 2011).

To measure the total flavonoid, 250 µL of the extract was added to 75 µL of sodium nitrite 5%, 150 µL of aluminium chloride 10% and 500 µL of sodium hydroxide 1 M, and its volume was adjusted to 2.5 mL by adding distilled water. After 5 min, the absorbance of the solution was read at 507 nm using a spectrophotometer (Specorp 250 Jena-History, Germany) (Pineli et al., 2011). The standard curve was drawn based on the absorbance at certain concentrations of quercetin.

The total phenol content was measured by using the Folin–Ciocalteau reagent for which 100 µL of the extract along with 2 mL of carbonate sodium 2% was poured into a test tube, and after 5 min, it was added to 100 µL of the diluted Folin–Ciocalteau (50%) reagent. The absorbance was read at 720 nm using a spectrophotometer (Specorp 250 Jena-History). To draw the standard curve, gallic acid was prepared at different rates, and their absorbance was recorded. The total phenol content was reported in mg · 100 g^{-1} FW (Pineli et al., 2011).

The total antioxidant capacity was determined by using the DPPH method. First, a solution of 0.1 mM DPPH was prepared. Then, 50 µL of the fruit extract was added to 1950 µL of the DPPH solution (0.1 mM), and the volume was adjusted to 2 mL. The absorbance was read at 517 nm after 10 min using a spectrophotometer (Specorp 250 Jena-History, Germany). The absorbance of the samples was compared with the DPPH solution. The total antioxidant activity was calculated by the following equation in percent (Pineli et al., 2011).

DPPH scavenging capacity (%): Ac − As/Ac × 10,

where Ac is the absorbance of the control and As is the absorbance of the sample.

Determination of MDA and hydrogen peroxide (H_2O_2)

To measure MDA, 1 g of the fruit tissue was added to 5 mL of trichloroacetic acid 10% solution and centrifuged at 10000 × *g* for 5 min. Then, 2 mL of the supernatant was mixed with 2 mL of trichloroacetic acid 1% and 0.6 g of thiobarbituric acid. The mixture was kept in a hot water bath at 100°C for 20 min, immediately cooled down in ice, and re-centrifuged at 10000 × *g* for 5 min. Finally, MDA absorbance was read at 532 and 600 nm, and the result was expressed in nmol · g^{-1} FW.

MDA = (A532 − A600) × *W* × *V*/155 × 1000,

in which A532 is the absorbance at 532 nm, A600 is the absorbance at 600 nm, *W* is the sample weight and *V* is the TCA volume.

To measure H_2O_2, 1 g of the strawberry fruit tissue was ground in 5 mL of trichloroacetic acid 1%. The resulting extract was centrifuged at 10000 × *g* at 4°C for 5 min. In the next step, 750 µL of the resulting supernatant was mixed with 250 µL of 10 mM potassium phosphate buffer (pH = 7) and 1 mL of potassium iodide 1 M. Then, the absorbance was read at 390 nm using a spectrophotometer. The H_2O_2 content was then determined using a standard curve in nmol g^{-1} FW of fruit fresh tissue (Ma et al., 2016).

Determination of antioxidant enzymes assay

To measure the activity of antioxidant enzymes including SOD and CAT, one gram of fruit tissue was ground in 3 mL of 50 mM potassium phosphate buffer (KH_2PO_4) (pH = 7.8) containing 0.2 mM Na_2EDTA and 2% (w/v) polyvinylpyrrolidone (PVP). Then, the homogenous compound was centrifuged at 12000 × *g* at 4°C for 20 min, and the supernatant was used as the enzymatic extract.

SOD

To measure SOD, 50 µL of the enzymatic extract was first mixed with 2 mL of 50 mM phosphate buffer, 100 mL of ethylenediaminetetraacetic acid (EDTA) and 200 µL of nitroblue tetrazolium (NBT). It was then exposed to 40-*W* fluorescent light for 10 min. In the next step, 50 µL of 0.15 mM riboflavin was added to the mixture and was exposed to the same light for 12 min. Finally, the absorbance was read at 560 nm. One unit of the SOD activity expressed as $U \cdot g^{-1}$ FW is based on the amount of enzyme that can inhibit NBT oxidation by 50% (Chen et al., 2011).

CAT

To determine the CAT activity, 100 µL of the enzymatic extract was added to 50 µL of H_2O_2 and 200 µL of 50 mM phosphate buffer with an acidity of 7. The CAT activity was recorded with a spectrophotometer based on the decline in absorbance at 240 nm in 2 min. The results were expressed in $U \cdot g^{-1}$ FW (Chen et al., 2011).

Data analysis

The study was conducted as a factorial experiment in a completely randomised design with three replications. The first factor was assigned to PUT treatment at two levels (1 mM and 2 mM), CTS (0.1% w/v) and CTS-PUT NPs (0.1% w/v), as well as a control. The second factor included the storage time (3 days, 6 days, 9 days and 12 days). Two-way analysis of variance (ANOVA) was performed using the SPSS statistical software package program version 18.0 (SPSS Inc., Chicago, IL, USA), and significant differences between mean values were compared using Duncan's multiple range test at a level of 5%. Data were expressed as the mean ± standard error (SE).

RESULTS AND DISCUSSION

Quality characteristics

According to Table 1, the treatments positively influenced the reduction of the fruit decay percentage over 12 days of storage. The decay percentage was zero in both control and treated fruits on the 3rd day of storage. PUT reduced the decay of strawberries during 9 days and 12 days of storage effectively. At the end of the storage period, the lowest decay was 16.66% related to the treatment of CTS-PUT NPs and 15.83% related to 0.1% CTS, and the highest was 57.5% observed in the control fruits (Table 1).

Strawberries contain a lot of water and have susceptible tissue, so they are easily exposed to fungal pathogens during the postharvest period. In this regard, grey mould (*Botrytis cinerea*), which can grow at low temperatures, inflicts significant losses (Harker et al., 2000). Presently, it is highly important to propose safe methods for extending the shelf life of strawberries. One method is to use edible coatings, e.g., CTS and nanocomposites, which can increase the longevity and preserve the quality of strawberries (Ribeiro et al., 2007). In this research, the fruits treated with CTS-PUT NPs were infected less than the control fruits, reflecting the effective role of these treatments in the control of fungal infections. Similar results were observed in a study by Bal and Ürün (2021) in which the decay incidence of strawberries coated with CTS and CTS + PUT was lower than that of control and PUT-treated fruits during storage. It has been stated that an increase in internal polyamines, such as PUT, can be effective in controlling fungal diseases including grey mould, and this shows the role of these compounds in defensive responses of plants to pathogens (Walters, 2003). By increasing the activity of biomolecules and strengthening the defensive system against pathogens, the PUT treatment reduced decay in peaches (Abbasi et al., 2019), mandarins (Ennab et al., 2020) and strawberries (Zokaei Khosroshahi et al., 2007). CTS has natural antimicrobial activity and elicits defensive responses (Terry and Joyce, 2004). This coating is a poly-cation that consumes negative charges on the cell surface and changes cell permeability, thereby causing the leakage of intracellular electrolytes and proteins (Avadi et al., 2004). It also penetrates fungal cells and suppresses and slows down the synthesis of mRNA and proteins (Li et al., 2015). CTS has been used to control postharvest diseases of many fruits, such as pears (Yu et al., 2008), jujubes (Xing et al., 2015) and grapes (Meng et al., 2008) with results similar to our findings. Nanoparticle coatings have tiny particles, so they reduce the diameter of respiratory pores on the fruit surface. Consequently, their permeability to oxygen and water decreases, and fruit decay is retarded (Xing et al., 2019).

According to Table 1, fruit tissue firmness was reduced in both control and treated fruits over the storage period. The highest firmness was observed in the treatments of CTS and CTS-PUT NPs. At the end of the storage period, the lowest firmness was 0.9 N observed in the control, and the highest was 2.9 N observed in fruits treated with CTS-PUT NPs (Table 1). The applications of CTS-PUT NPs and CTS effectively preserved the tissue firmness of strawberries.

The loss of firmness is accompanied by changes in the metabolism of cell wall carbohydrates and the structural components of cell walls. These changes are induced by the activity of cell wall-degrading enzymes (Valero et al., 2002). PUT binds to pectin of cell walls,

Table 1. The effect of PUT coated with CTS nanoparticles (CTS-PUT NPs), PUT and CTS on decay, weight loss, firmness, TSS, TA and ascorbic acid of strawberries stored at 4°C for 12 days.

Treatments	Storage period (days)	Decay (%)	Firmness (N)	TSS (Brix)	TA ($g \cdot 100\ g^{-1}$)	Ascorbic acid ($mg \cdot 100\ g^{-1}$)
Control	3	0	3.42 ± 0.14 bc	3.3 ± 0.17 g	0.97 ± 0.02 fg	72 ± 2.56 a
	6	8.83 ± 0.83 ef	3.5 ± 0.15 bc	7.26 ± 0.27 a	1.24 ± 0.02 ab	54.83 ± 2.19 c–e
	9	30.83 ± 1.9 c	2.91 ± 0.13 de	7.33 ± 0.21 a	0.91 ± 0.04 gh	44.33 ± 2.4 h
	12	57.5 ± 2.25 a	0.96 ± 0.14 g	4.46 ± 0.21 f	0.7 ± 0.01 i	18.16 ± 1.51 j
PUT 1 mM	3	0	3.01 ± 0.15 b	4.8 ± 0.35	0.82 ± 0.02 h	60.33 ± 2 b
	6	8.53 ± 1.15 de	2.91 ± 0.21 de	6.8 ± 0.27 ab	1.14 ± 0.06 cd	54.1 ± 1.99 cd
	9	20 ± 1.73 de	2.79 ± 0.16 de	5.7 ± 0.26 a–c	1.07 ± 0.01 ab	47.83 ± 2.3 e–h
	12	44.16 ± 2.48 b	2.36 ± 0.12 f	4.9 ± 0.19 cd	0.97 ± 0.01 gh	29.06 ± 0.98 i
PUT 2 mM	3	0	3.64 ± 0.18 de	5.7 ± 0.25	0.95 ± 0.03 gh	61 ± 1.44 bc
	6	5.5 ± 1.13	3.1 ± 0.11 cd	6.9 ± 0.18 ab	1.15 ± 0.05 de	56.66 ± 1.58 d–g
	9	16.83 ± 0.91 d	2.97 ± 0.15 bc	6.3 ± 0.32 de	0.97 ± 0.03 fg	52.4 ± 0.57 d–g
	12	29.5 ± 1.12 b	2.36 ± 0.13 f	4.9 ± 0.41 f	0.92 ± 0.02 gh	35.4 ± 1.82 j
CTS-PUT NPs 0.1%	3	0	4.21 ± 0.15 ab	5.9 ± 0.22 f	0.96 ± 0.03 ef	63.66 ± 2.72 d–h
	6	0	3.66 ± 0.12 ab	7.3 ± 0.24 a	1.37 ± 0.02 de	58.56 ± 2.18 f–h
	9	11 ± 1.29 f	3.38 ± 0.16 de	6.6 ± 0.36 b–d	1.05 ± 0.03 bc	55 ± 2 gh
	12	16.66 ± 1.11 e	2.91 ± 0.18 e	5.2 ± 0.27 ef	0.96 ± 0.01 f–h	36.16 ± 0.95 i
CTS-0.1%	3	0	4.06 ± 0.11 a	5.86 ± 0.15 de	0.94 ± 0.04 f–h	47.66 ± 1.44 f–h
	6	0	3.4 ± 0.15 bc	7.56 ± 0.31 a	1.33 ± 0.03 a	51.33 ± 2 d–f
	9	15.83 ± 1.71 e	3.53 ± 0.08 bc	5.8 ± 0.25 de	0.93 ± 0.01 f–h	47.7 ± 2.3 f–h
	12	17.5 ± 1.17 de	2.83 ± 0.06 de	5.13 ± 0.2 ef	0.89 ± 0.03 gh	31 ± 2.3 i

The data are the means ± SE of three replications. Means in the same column with different letters are significantly different ($p < 0.05$) according to Duncan's multiple range tests.
CTS, chitosan; PUT, putrescine; SE, standard error; TA, titratable acidity; TSS, total soluble solids.

thereby suppressing the cell wall-degrading enzymes, such as polygalacturonase and pectin methylesterase, which are responsible for postharvest softening of fruits (Martínez-Romero et al., 2002). The application of polyamines, such as PUT, to apricots (Martínez-Romero et al., 2002), peaches (Abbasi et al., 2019) and papayas (Hanif et al., 2020) contributed to preserving fruit firmness. Similar to our findings, CTS reduced the activity of cell wall-degrading enzymes, thereby leading to the preservation of fruit firmness of mangoes (Chien et al., 2007), tomatoes (Liu et al., 2007) and cornelian cherries (Duan et al., 2011).

Based on the results, the treatments inhibited the loss of TSS during storage effectively (Table 1). In the 6 days of storage, the highest TSS content was exhibited by the fruits treated with CTS-PUT NPs (7.3°Brix), those treated with CTS (7.5°Brix) and the control (7.2°Brix). At the end of storage, no significant differences were observed in TSS among the treatments. The lowest TSS content was 4.4°Brix related to the control (Table 1).

In the 6 days of storage, the highest TA was observed in the treatments of CTS-PUT NPs and CTS and the lowest in the treatments of 1 mM and 2 mM PUT. The differences among the fruits stored for 12 days were not significant. The lowest TA at the end of the storage period was observed in the control. All treatments were effective in preserving the TA of the strawberries at the end of the storage period (Table 1).

The concentration of TSS continuously increases over the process of fruit maturing due to the hydrolysis of polysaccharides and organic acids (Piero et al., 2005). Respiration contributed to the consumption of organic acids and the reduction of TA in fruits (Zokaei Khosroshahi et al., 2007). The effect of PUT on TSS and TA can be associated with the reduction of respiration and the retardation of the ripening process. Similar results have been reported for plums (Davarynejad et al., 2015), apricots (Martínez-Romero et al., 2002) and mangoes (Wannabussapawich and Seraypheap, 2018). CTS and its nanocomposite coatings create a semipermeable layer around fruits and a controlled internal atmosphere, thereby reducing respiration and ethylene synthesis, preventing the effect of ethylene and extending fruits' storage life. Consequently, TSS consumption and reduction are hindered (Bautista-Banos et al., 2006; Xing et al., 2020). According to our results, the CTS treatment prevented the decline in TSS in papayas (Bautista-Banos et al., 2003), mangoes (Xing et al., 2020) and tomatoes (Meena et al., 2020).

The ascorbic acid content was decreased in both control and treated fruits during the storage. The comparison of the means revealed that no treatment

had any effects on preventing the loss of ascorbic acid on the 3rd day of storage and the highest ascorbic acid content was observed in the control. On the 9th and 12th days of storage, the highest ascorbic acid content was associated with the treatments of CTS-PUT NPs and 2 mM PUT. At the end of the storage period, all treatments effectively prevented the excessive loss of ascorbic acid in the strawberries. The lowest ascorbic acid (18.16 mg · 100 g^{-1} FW) was related to the control (Table 1).

Ascorbic acid is a major factor in strawberry quality and is very sensitive to oxidation. It rapidly oxidises during storage. PUT helps preserve ascorbic acid by reducing the activity of ascorbate peroxidase (APX) (Ennab et al., 2020). The application of PUT in mangoes (Razzaq et al., 2014), mandarins (Ennab et al., 2020) and apricots (Davarynejad et al., 2013) contributed to preserving the amount of ascorbic acid. CTS reduces the oxidation of ascorbic acid by reducing the amount of oxygen, thereby preventing the decline ascorbic acid (Wang and Gao, 2013). CTS and CTS nanocomposites have effectively been used to prevent the decline in ascorbic acid in kiwifruits (Vivek and Subbarao, 2016), litchis (Sun et al., 2010) and mangoes (Chien et al., 2007).

Total anthocyanin, phenol and flavonoid contents and antioxidant capacity

In the present study, the TAC of the strawberries first increased but then started to decrease during the storage period. The results showed no significant difference between the coating treatments and the control in total anthocyanin on the 3rd day of storage. The anthocyanin content was increased in all fruits on the 6th day. The highest anthocyanin content was related to the CTS-PUT NPs and 2 mM PUT treatments. At the end of the storage period, the lowest TAC was observed in the control fruits and the highest was related to the treatments of CTS-PUT NPs and CTS (Figure 1A).

Strawberry is rich in anthocyanins. The dominant anthocyanin in this fruit is pelargonidin-3-glucoside (Petriccione et al., 2015). The amount of anthocyanin during the postharvest period increases due to its biosynthesis, but it decreases at the end of storage due to the senescence process (Hu et al., 2011). During stress, the activity of the PAL enzyme, which plays a key role in the biosynthesis pathway of phenol compounds, increases, resulting in an increase in anthocyanin synthesis (Keutgen and Pawelzik, 2007). PUT increases anthocyanin by inducing the enzymes involved in its synthesis, e.g., phenylalanine ammonia-lyase and chalcone isomerase. It also binds to anthocyanin and inhibits its oxidation and degradation (Barman et al., 2014). The application of PUT to pomegranates (Barman et al., 2014) and strawberries (Bal and Ürün, 2021) prevented the postharvest decline in anthocyanin.

CTS inhibits postharvest anthocyanin loss by controlling the ripening process, lowering oxygen levels, and suppressing the activity of peroxidase and polyphenol oxidase enzymes. CTS and nanocomposite

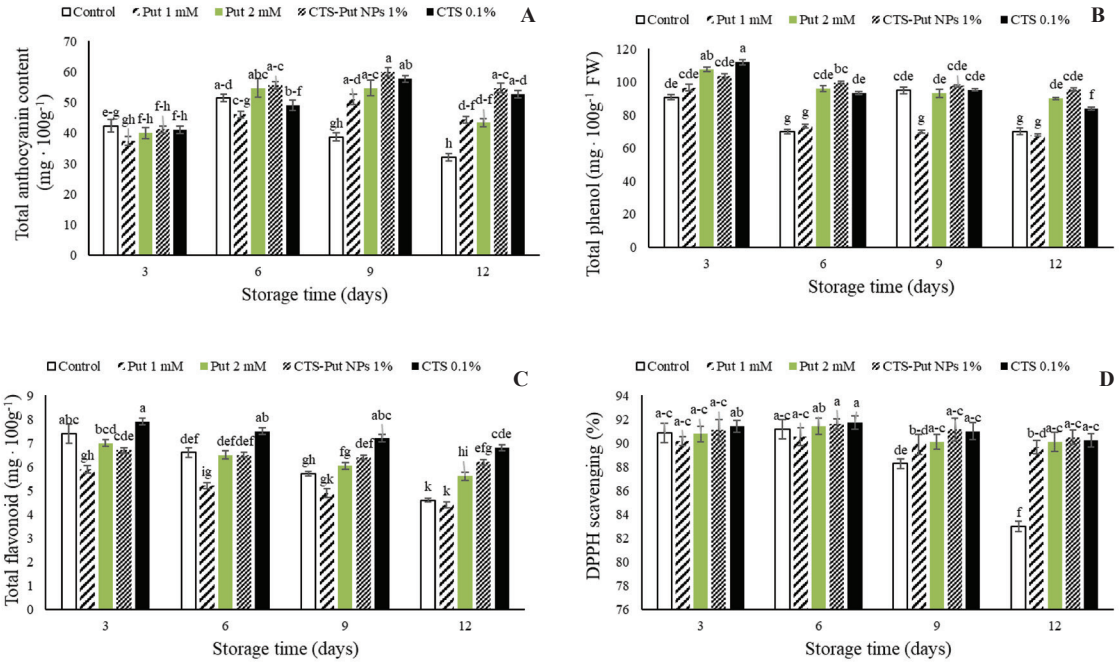


Figure 1. The effect of CTS nanoparticles (CTS-PUT NPs), PUT and CTS on TAC (A), total phenol content (B), total flavonoid (C) and total antioxidant capacity (D) of strawberries stored at 4°C for 12 days. Different letters of each bar indicate a significant difference between treatment groups within the same analysis day at $p < 0.05$. CTS, chitosan; PUT, putrescine; TAC, total anthocyanin content.

coating effectively preserved anthocyanin in strawberries (Resende et al., 2018) and plums (Chang et al., 2019), which agrees with our findings.

It was found that increasing the storage time decreased the total phenol content in all treatments. On the 3rd day of storage, the highest amount of total phenols was related to the treatments of CTS and 2 mM PUT, showing significant differences from the other treatments (Figure 1B). Since the 6th day of storage, the total phenol content was decreased over time so that the lowest amount at the end of the storage period was related to the control and 1 mM PUT and the highest was related to the treatments of CTS-PUT NPs and 2 mM PUT. These treatments differed from the other treatments significantly.

The total flavonoid content was decreased with increasing the storage time. The fruits treated with CTS had the highest total flavonoid content over the whole storage period. On the 12th day of storage, the lowest amount of flavonoid was observed in the control and 1 mM PUT treatments and the highest in the treatments of CTS and CTS-PUT NPs, exhibiting significant differences from the other treatments (Figure 1C).

The results for the antioxidant capacity revealed a decrease in this capacity with the increase in the storage period, but there was not a significant difference between the coating treatments and the control until the 9th day (Figure 1D). The antioxidant capacity of the control was dropped from the 9th day to the end of the storage period and showed strongly significant differences with the treated fruits. However, the fruits treated with the edible coatings did not differ from one another in the total antioxidant activity significantly. In general, antioxidant contents increased and then decreased during storage. Determining antioxidant capacity is a method for expressing the biological properties of fruits, which play a key role in preserving fruit quality during the storage period (Wannabussapawich and Seraypheap, 2018). Wang and Gao (2013) and Bal and Ürün (2021) reported a positive effect of CTS- and CTS + PUT-based edible coatings on the antioxidant activity of strawberry. High values of the antioxidant content observed in berries treated with CTS, CTS-PUT NPs and 2 mM PUT could be attributed to the high level of phenolic compounds. Previous studies indicate a linear correlation between the phenolic content and antioxidant capacity of fruits (Razzaq et al., 2014; Bal and Ürün, 2021). The decline in phenolic content during extended storage periods may result from the breakdown of cell structure, leading to the release of phenolics that are subsequently exposed to enzymatic oxidation. Research on pomegranates (Mirdehghan and Rahemi, 2007), plums (Davarynejad et al., 2015), kiwifruits (Jhalegar et al., 2012) and zucchinis (Palma et al., 2015) showed that PUT can effectively inhibit the loss of phenol compounds at the postharvest period. PUT prevents the postharvest loss of phenol compounds by increasing the activity of PAL and preventing the oxidation of these compounds (Hosseini et al., 2018). CTS is used to increase the synthesis of most phenol compounds in tissues of plants. For example, it increases the total phenol compounds in strawberries (Resende et al., 2018). Furthermore, the plants treated with CTS and CTS-coated titanium dioxide preserved higher levels of phenols, flavonoids and flavonoids in mangoes (Xing et al., 2020). Previous studies have shown that the treatment of CTS and nanocomposite coating increased phenols in blueberries (Xing et al., 2021) and grapes (Gohari et al., 2021).

MDA and hydrogen peroxide contents and antioxidant enzyme activities

Based on the results, the MDA content increased during the storage period, but the treatments were effective in inhibiting the increase in the MDA content. The lowest MDA content on the 3rd day of storage was related to the treatment of CTS-PUT NPs, which differed from the other treatments and the control significantly. The highest MDA content at the end of the storage time was related to the control, differing from the other treatments significantly, whereas no significant difference was observed among the coating treatments (Figure 2A).

As the storage period was extended, the H_2O_2 content was increased in both treated and control fruits. On the 6th day of storage, the lowest H_2O_2 content was observed in the fruits treated with CTS-PUT NPs, differing from the other treatments and the control significantly. At the end of the storage period, the control fruits exhibited the highest H_2O_2 content and differed from the other coating treatments significantly. But the coating treatments did not differ from one another significantly (Figure 2B). The CTS-PUT NPs, CTS and PUT treatments effectively inhibited the increase in H_2O_2 in the strawberries during the storage period.

The first cell structures in plants that are damaged by chilling stress are lipids and proteins of the plasma membrane (Liang et al., 2003). The increase in free oxygen radicals damages most cell structures and components, such as fats, proteins and carbohydrates. The treatment of PUT decreased H_2O_2, MDA and electrolyte leakage by increasing the activity of antioxidant enzymes (Palma et al., 2015). Separate studies have reported the effect of PUT on decreasing MDA and H_2O_2 in peaches (Abbasi et al., 2019) and kiwifruits (Yang et al., 2016). CTS and CTS nanocomposites effectively hindered the increase in MDA and H_2O_2 during storage (Xing et al., 2021), which can be ascribed to the CTS and CTS nanocomposites' potential to inducing a defensive system (Badawy and Rabea, 2009; Vieira et al., 2016). The application of CTS and CTS nanocomposite coatings significantly reduces the MDA increase in peppers and cucumbers (Xing et al., 2011; Zhang et al., 2015).

According to the results, the CAT activity was the lowest in the CTS treatment on the 3rd day of storage, differing from the other treatments significantly. On days 6 and 9, the CAT activity increased so that its

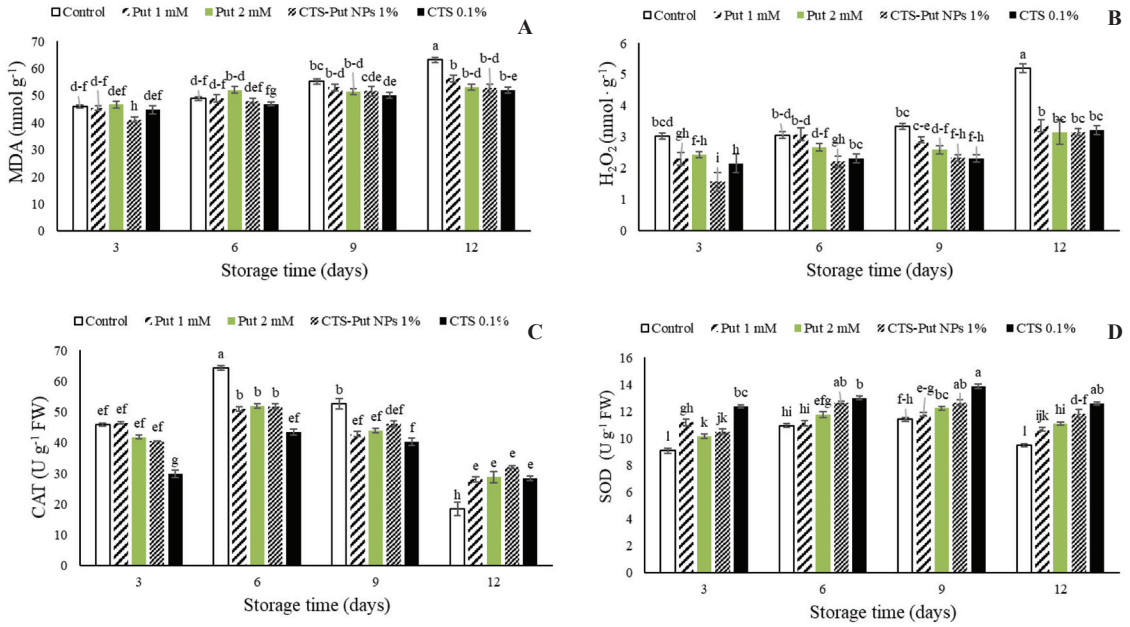


Figure 2. The effect of CTS nanoparticles (CTS-PUT NPs), PUT and CTS on MDA content (A) and H2O2 content (B), CAT activity (C) and SOD activity (D) of strawberries stored at 4°C for 12 days. Different letters of each bar indicate a significant difference between treatment groups within the same analysis day at $p < 0.05$. CAT, catalase; CTS, chitosan; MDA, malondialdehyde; PUT, putrescine; SOD, superoxide dismutase.

highest activity was related to the control and its lowest activity to the treatment of CTS (Figure 2C). At the end of the storage, the CAT activity was decreased so that the lowest was related to the control, which differed from the coating treatments significantly. But, no significant differences were observed among the coating treatments.

The comparison of the means revealed that the SOD activity was gradually increased until the 9th day of storage, but it, then, started to decrease. The highest SOD activity was related to the treatments of CTS and CTS-PUT NPs, which differed from the control and other coating treatments significantly (Figure 2D). At the end of the storage, the highest and the lowest activity of this enzyme were related to the treatment of CTS and the control, respectively. They differed from the other treatments significantly. In general, the treatment of CTS prevented the loss of SOD activity significantly.

During fruit ripening, the synthesis and accumulation of reactive oxygen species (ROS) such as H_2O_2 and superoxide anions increase continuously. So, the continuous increase in oxidation induces stress at the fruit ripening stage (Sudhakar et al., 2001). ROS induces oxidative stress on lipid membranes, nucleic acids and proteins. Plants are equipped with defensive mechanisms to eliminate ROS damage. ROS can be scavenged by the plants' enzymatic defence including SOD, APX and CAT or by its nonenzymatic antioxidants such as ascorbic acid, phenols and glutathione (Gill and Tuteja, 2010). Polyamines are significantly effective in increasing plant tolerance to stress. They act as the deactivators of free oxygen radicals. The antioxidant nature of these compounds may be related to the suppression of NADPH oxidase and the inhibition of the accumulation of superoxide radicals (Martin-Tanguy, 2001).

Superoxide radicals are converted to H_2O_2 by SOD, which is then converted to water and superoxide anions by CAT and peroxidase (Wannabussapawich and Seraypheap, 2018). The strawberries treated with 2 mM PUT and CTS-PUT NPs showed higher CAT activity than the control and other treatments. CTS and nano-silica coatings hindered the reduction of the activity of antioxidant enzymes in loquat fruits at the postharvest stage by suppressing free radicals and preserving the integrity of cell membranes (Song et al., 2016). The CTS coating and cellulose nanocomposite films effectively prevented the reduction of the activity of CAT, SOD and APX in mangoes (Xiao et al., 2021) and eggplants (Attia et al., 2021).

CONCLUSIONS

Nowadays, due to the harmful effects of using chemical substances for humans and the environment, new approaches in the use of substances that do not have harmful effects on humans and the environment are important. The results of the present study indicate that fruits treated with CTS, PUT and CTS-PUT NPs had higher antioxidant and enzymatic activities than untreated fruits. Also, fruits coated with CTS and CTS-PUT NPs had the lowest decay percentage, the highest tissue firmness, the highest TSS and the lowest MDA and hydrogen peroxide (H_2O_2) at the end of storage

period. Therefore, the application of CTS-PUT NPs, CTS and 2 mM PUT could be effective in preserving the nutritional quality and prolonging the postharvest life of strawberries during storage at 4°C for up to 12 days.

ACKNOWLEDGEMENTS

We are grateful to Prof. Gholam Reza Mahdavinia and Mr. Hessam Jafari for their critical support and help in nanomaterial synthesis and characterisation. All authors read and approved the final manuscript.

FUNDING

This research received no external funding.

AUTHOR CONTRIBUTIONS

R.B.: methodology, formal analysis and data curation. F.R.: conceptualisation, supervision, experiment design and writing – original draft preparation. S.N.M.: conceptualisation and supervision. A.J.M.: methodology and writing – review and editing. G.G.: supervision, validation, experiment design and writing – review and editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Abbasi, N., Ali, I., Hafiz, I., Alenazi, M., and Shafiq, M. (2019). Effects of putrescine application on peach fruit during storage. *Sustainability*, *11*, 7–18.

Adiletta, G., Zampella, L., Coletta, C., and Petriccione, M. (2019). Chitosan coating to preserve the qualitative traits and improve antioxidant system in fresh figs (*Ficus carica* L.). *Agriculture*, *9*, 84.

Alcazar, R., Bueno, M., and Tiburcio, A. F. (2020). Polyamines: Small amines with large effects on plant abiotic stress tolerance. *Cells*, *9*(11), 2373.

Attia, M. S., El-Naggar, H. A., Abdel-Daim, M. M., and El-Sayyad, G. S. (2021). The potential impact of *Octopus cyanea* extracts to improve eggplant resistance against *Fusarium*-wilt disease: *In vivo* and *In vitro* studies. *Environmental Science and Pollution Research*, *28,* 35854–35869.

Avadi, M. R., Sadeghi, A. M. M., Tahzibi, A., Bayati, K. H., Pouladzadeh, M., and Zohuriaan-Mehr, M. J. (2004). Diethylmethyl chitosan as an antimicrobial agent: Synthesis, characterization and antibacterial effects. *European Polymer Journal*, *40*, 1355–1362.

Badawy, M. E. I., and Rabea, E. I. (2009). Potential of the biopolymer chitosan with different molecular weights to control postharvest gray mold of tomato fruit. *Postharvest Biology and Technology*, *51*, 110–117.

Bahmani, R., Razavi, F., Mortazavi, S. N., Gohari, G. H., and Juarez-Maldonado, A. (2022). Evaluation of proline-coated chitosan nanoparticles on decay control and quality preservation of strawberry fruit (cv. Camarosa) during cold storage. *Horticulturae*, *8*, 648.

Bal, E., and Ürün, B. A. (2021). Effects of chitosan coating with putrescine on bioactive compounds and quality of strawberry cv. San Andreas during cold storage. *Erwerbs-Obstbau*, *21*, 7–14.

Baranyai, L., Nguyen, L. L. P., Sao Dam, M., Zsom, T., and Hitka, G. (2020). Evaluation of precooling temperature and 1-MCP treatment on quality of 'golden delicious' apple. *Journal of Applied Botany and Food Quality*, *93*, 130–135.

Barman, K., Asrey, R., Pal, R. K., Kaur, C., and Jha, S. K. (2014). Influence of putrescine and carnauba wax on functional and sensory quality of pomegranate (*Punica granatum* L.) fruits during storage. *Journal of Food Science and Technology*, *51*(1), 111–117.

Bautista-Baños, S., Hernandez-Lauzardo, A. N., Hernandez-Lopez, M., Barka, E. A., Velazquez-Del Valle, M. G., Bosquez-Molina, E., and Wilson, C. L. (2006). Chitosan as a potential natural compound to control pre and postharvest diseases of horticultural commodities. *Crop Protection*, *25*, 108–118.

Bautista-Baños, S., Hernández-López, M., Bosquez-Molina, E., and Wilson, C. L. (2003). Effects of chitosan and plant extracts on growth of *Colletotrichum gloeosporioides*, anthracnose levels and quality of papaya fruit. *Crop Protection*, *22*, 1087–1092.

Buendia, B., Gil, M., Tudela, J. A., and Gady, A. L. (2010). HPLC-MS analysis of Pro anthocyanidin oligomers and other phenolics in 15 strawberry cultivars. *Journal of. Agricultural and Food Chemistry*, *58*(7), 3916–3926.

Chang, X., Lu, Y., Li, Q., Lin, Z. H., Qiu, J., Peng, C. H., Brennan, C. S., and Guo, X. (2019). The combination of hot air and chitosan treatments on phytochemical changes during postharvest storage of 'Sanhua' plum fruits. *Foods*, *8*, 338.

Chen, L., Han, Y., Jiang, H., Korpelainen, H., and Li, C. (2011). Nitrogen nutrient status induces sexual differences in responses to cadmium in *Populus yunnanensis*. *Journal of Experimental Botany*, *62*, 5037–5050.

Chien, P., Sheu, F., and Yang, F. (2007). Effects of edible chitosan coating on quality and shelf life of sliced mango fruit. *Journal of Food Engineering*, *78*, 225–229.

Davarynejad, G. H., Zarei, M., Ardakani, E., and Nasrabadi, M. E. (2013). Influence of putrescine application on storability, postharvest quality and antioxidant activity of two Iranian apricot (*Prunus armeniaca* L.) Cultivars. *Notulae Scientia Biologicae*, *33*, 5–19.

Davarynejad, G. H., Zarei, M., Nasrabadi, M. E., and Ardakani, E. (2015). Effects of salicylic acid and putrescine on storability, quality attributes and antioxidant activity of plum cv. 'Santa Rosa'. *Journal of Food Science and Technology*, *52*(4), 2053–2062.

Duan, X. W., Liu, T., Zhang, D., Su, X. G., Lin, H. T., and Jiang, Y. M. (2011). Effect of pureoxygen atmosphere on antioxidant enzyme and antioxidant activity of harvested litchi fruit during storage. *Food Research International*, *44*, 1905–1911.

Ennab, H., El-Shemy, M., and Alam-Eldein, S. (2020). Salicylic acid and putrescine to reduce post-harvest storage problems and maintain quality of Murcott mandarin fruit. *Agronomy*, *10*(1), 115–122.

Gill, S. S., and Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiologhy and Biochemistry*, *48*, 909–930.

Gohari, G., Molaei, S., Kheiry, A., Ghafouri, M., Razavi, F., Lorenzo, J., and Juárez-Maldonado, A. (2021). Exogenous application of proline and L-cysteine alleviates internal browning and maintains eating quality of cold stored flat 'Maleki' peach fruits. *Horticulturae*, *27*, 11–23.

Hanif, A., Saeed, A., Jaskani, M. J., and Rashid, A. (2020). Papaya treatment with putrescine maintained the overall quality and promoted the antioxidative enzyme activities of the stored fruit. *Scientia Horticulturae*, *267*, 1078–198.

Harker, F. R., Elgar, H. J., Watkins, C. B., Jackson, P. J., and Hallett, I. C. (2000). Physical and mechanical changes in strawberry fruit after high carbon dioxide treatments. *Postharvest Biology and Technology*, *19*, 139–146.

He, X., and Hwang, H. M. (2016). Nanotechnology in food science: Functionality, applicability, and safety assessment. *Journal of Food and Drug Analysis*, *24*, 671–681.

Hernandez-Lopez, G., Ventura-Aguilar, R. I., Correa-Pacheco, Z. N., Bautista-Banos, S., and Barrera-Necha, L. L. (2020). Nanostructured chitosan edible coating loaded with a-pinene for the preservation of the postharvest quality of *Capsicum annum* L. and *Alternaria alternate* control. *International Journal of Biological Macromolecules*, *165*, 1881–1888.

Hernández-Muñoza, P., Almenara, E., José, J., and Gavara, R. (2006). Effect of calcium dips and chitosan coatings on postharvest life of strawberries (*Fragaria ananassa*). *Postharvest. Biology and Technology*, *39*, 247–253.

Hosseini, M. S., Zahedi, M., Abadía, J., and Karimi, M. (2018). Effects of postharvest treatments with chitosan and putrescine to maintain quality and extend shelf-life of two banana cultivars. *Food Science and Nutrition*, *13*, 45–57.

Hu, H., Li, X., Dong, C., and Chen, W. (2011). Effects of wax treatment on quality and postharvest physiology of pineapple fruit in cold storage. *African Journal of Biotechnology*, *10*, 7592–7603.

Jesus Filho, M. D., Scolforo, C. Z., Saraiva, S. H., Gomes Pinheiro, C. H. J., Silva, P. I., and Della Lucia, S. M. (2018). Physicochemical, microbiological and sensory acceptance alterations of strawberries caused by gamma radiation and storage time. *Scientia Horticulturae*, *238*, 187–194.

Jhalegar, M. J., Sharma, R. R., Pal, R. K., and Rana, V. (2012). Effect of postharvest treatments with polyamines on physiological and biochemical attributes of kiwifruit (*Actinidia deliciosa*) cv. Allison. *Fruits*, *67*, 13–22.

Keutgen, A., and Pawelzik, E. (2007). Modifications of strawberry fruit antioxidant pools and fruit quality under NaCl stress. *Journal of Agricultural and Food Chemistry*, *55*, 4066−4072.

Li, H., Wang, Y., Liu, F., Yang, Y., Wu, Z., Cai, H., Zhang, Q., and Wang, Y. (2015). Effects of chitosan on control of postharvest blue mold decay of apple fruit and the possible mechanisms involved. *Scientia Horticulturae*, *186*, 77–83.

Liang, Y. C., Shen, Q. R., and Ma, T. S. (2003). Effects of silicon on salinity tolerance of two barley cultivars. *Journal of Plant Nutrition*, *19*, 173–183.

Liu, C. H., Zhang, H., Sheng, K., Liu, W., and Zheng, L. (2018). Effects of melatonin treatment on the postharvest quality of strawberry fruit. *Postharvest Biology and Technology*, *139*, 47–55.

Liu, J., Tian, S., Menga, X., and Xu, Y. (2007). Effects of chitosan on control of postharvest diseases and physiological responses of tomato fruit. *Postharvest Biology and Technology*, *44*, 300–306.

Ma, Q., Zhang, T., Zhang, P., and Wang, Z. Y. (2016). Melatonin attenuates postharvest physiological deterioration of cassava storage roots. *Journal of Pineal Research*, *60*, 424–434.

Mahmoudi, R., Razavi, F., Rabiei, V., Gohari, G. H., and Palou, L. (2022) Application of glycine betaine coated chitosan nanoparticles alleviate chilling injury and maintain quality of plum (*Prunus domestica* L.) fruit. *International Journal of Biological Macromolecules*, *207*, 965–977.

Martin-Tanguy, J. (2001). Metabolism and function of polyamines in plants: Recent development (new approaches). *Plant Growth Regulation*, *34*, 135–148.

Martinez-Romero, D., Serrano, M., Carbonell, A., Burgos, L., Riquelme, F., and Valero, D. (2002). Effects of postharvest putrescine treatment on extending shelf life and reducing mechanical damage in apricot. *Journal of Food Science*, *67*, 1706–1712.

Meena, M., Pilania, S. H., Pa, A., Mandhania, S. H., Bhushan, B., Kumar, S., Gohari, G., and Sahara, V. (2020). Cu-chitosan nano-net improves keeping quality of tomato by modulating physio-biochemical responses. *Scientific Reports*, *21914*, 1–10.

Meng, X. H., Li, B. Q., Liu, J., and Tian, S. P. (2008). Physiological responses and quality attributes of table grape fruit to chitosan preharvest spray and postharvest coating during storage. *Food Chemistry*, *106*, 501–508.

Mirdehghan, S. H., and Rahemi, M. (2007). Seasonal changes of mineral nutrients and phenolics in pomegranate (*Punica granatum* L.) fruit. *Scientia Horticulturae*, *111*, 120–127.

Nasr, F., Pateiro, M., Rabiei, V., Razavi, F., Formaneck, S., Gohari, G. H., and Lorenzo, J. M. (2021). Chitosan-phenylalanine nanoparticles (Cs-Phe Nps) extend the postharvest life of persimmon (*Diospyros kaki*) fruits under chilling stress. *Coatings*, *11*, 819.

Nguyen, L. P. L., Zsom, T., Sao Dam, M., Baranyai, L., and Hitka, G. (2020). Comparison of 1-MCP treatment on four melon cultivars using different temperatures. *Journal of Applied Botany and Food Quality*, *93*, 122–129.

Palma, F., Carvajal, F., Ramos, J. M., Jamilena, M., and Garrido, D. (2015). Effect of putrescine application on maintenance of zucchini fruit quality during cold storage: Contribution of GABA shunt and other related nitrogen metabolites. *Postharvest Biology and Technology*, *99*, 131–140.

Petriccione, M., Mastrobuoni, F., Pasquariello, M. S., Zampella, L., Nobis, E., Capriolo, G., and Scortichini, M. (2015). Effect of chitosan coating on the postharvest. Quality and antioxidant enzyme system response of strawberry fruit during cold storage. *Foods*, *4*(4), 501–523.

Piero, A. R., Puglisi, I., Rapisarda, P., and Petrone, G. (2005). Anthocyanins accumulation and related gene expression in red orange fruit induced by low temperature. *Journal of Agricultural and Food Chemistry*, *53*, 9083–9088.

Pineli, L. O., Moretti, C. L., Dos Santos, M. S., Campos, A. B., Brasileiro, A. V., Cordova, A. C., and Chiarello, M. D. (2011). Antioxidants and other chemical and physical characteristics of two strawberry cultivars at different ripeness stages. *Journal of Food Composition and Analysis*, *24*, 11–16.

Razzaq, K., Khan, A. S., Malik, A. U., Shahid, M., and Ullah, S. (2014). Role of putrescine in regulating fruit softening and antioxidative enzyme systems in 'Samar Bahist Chaunsa' mango. *Postharvest Biology and Technology*, *96*, 23–32.

Resende, N. S., Gonçalves, G. A. S., Reis, K. C., Tonoli, G. H. D., and Boas, E. (2018). Chitosan/cellulose nanofibril nanocomposite and its effect on quality of coated strawberries. *Journal of Food Quality*, *2016,* 233–254.

Ribeiro, C., Vicente, A. A., Teixeira, J. A., and Miranda, C. (2007). Optimization of edible coating composition to retard strawberry fruit senescence. *Postharvest Biology and Technology*, *44*, 63–70.

Saini, R. S., Sharma, K. D., Kaushik, R. A., and Dhankhar, O. P. (2006). Laboratory manual analytical techniques in horticulture. *Agrobios (India). IST Edition*, *2*, 12–26.

Serrano, M., Martinez-Romero, D., Guillen, F., and Valero, D. (2003). Effects of exogenous putrescine on improving shelf life of four plum cultivars. *Postharvest Biology and Technology, 30*(3), 259–271.

Song, H., Yuan, W., Jin, G., Wang, W., Wang, X., Yang, L., and Zhang, Y. (2016). Effects of chitosan/nano-silica on postharvest quality and antioxidant capacity of loquat fruit during cold storage. *Postharvest Biology and Technology, 119*, 41–48.

Sudhakar, C., Lakshmi, A., And Giridarakumar, S. (2001). Changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (*Morus alba* L.) under NaCl salinity. *Plant Science*, *141*, 613–619.

Sun, D., Liang, G., Xie, J., Lei, X., and Mo, Y. (2010). Improved preservation effects of litchi fruit by combining chitosan coating with ascorbic acid treatment during postharvest storage. *African Journal of Biotechnology*, *9*, 3272–3279.

Terry, L. A., and Joyce, D. C. (2004). Elicitors of induced disease resistance in postharvest horticultural crops: A brief review. *Postharvest Biology and Technology*, *32*, 1–13.

Valero, D., Martinez-Romero, D., and Serrano, M. (2002). The role of polyamines in the improvement of the shelf life of fruit. *Trends in Food Science and Technology*, *13*, 228–234.

Van, T. B., Duyen, H. H., and Ha, V. H. (2020). Combination effects of calcium chloride and nano-chitosan on the postharvest quality of strawberry (*Fragaria ananassa* Duch.). *Postharvest Biology and Technology*, *162*, 103–111.

Vieira, J. M., Flores-López, M. L., De Rodríguez, D. J., Sousa, M. C., Vicente, A. A., Martins, J. T. (2016). Effect of chitosan–*aloe vera* coating on postharvest quality of blueberry (*Vaccinium corymbosum*) fruit. *Postharvest Biology and Technology*, *11*, 88–97.

Vivek, K., and Subbarao, K. V. (2016). Effect of edible chitosan coating on combined ultrasound and NaOCl treated kiwi fruits during refrigerated storage. *International Food Research Journal*, *25*(1), 101–108.

Walters, D. R. (2003). Polyamines and plant disease. *Phytochemistry*, *64*, 97–107.

Wang, S. Y., and Gao, H. Y. (2013). Effect of chitosan-based edible coating on antioxidants, antioxidant enzyme system, and postharvest fruit quality of strawberries (*Fragaria aranassa* Duch.). *Food Science and Technology*, *52*, 71–79.

Wannabussapawich, B., and Seraypheap, K. (2018). Effects of putrescine treatment on the quality attributes and antioxidant activities of "Nam Dok Mai No. 4" mango fruit during storage. *Scientia Horticulturae*, *233*, 22–28.

Wszelaki, A. L., and Mitcham, E. J. (2003). Effect of combinations of hot water dips, biological control and controlled atmospheres for control of gray mold on harvested strawberries. *Postharvest Biology and Technology, 27*, 255–264.

Xiao, L., Gu, C., Zhu, D., Huang, Y., Luo, Y., and Zhou, Q. (2021). Development and characterization of an edible chitosan/zein-cinnamaldehyde nano-cellulose composite film and its effects on mango quality during storage. *Food Science and Technology, 140*, 58–65.

Xing, Y., Lin, H., Cao, D., Xu, Q., Han, W., Wang, R., Che, Z., and Li, X. (2015). Effect of chitosan coating with cinnamon oil on the quality and physiological attributes of China jujube fruits. *Hindawi Publish. Corporation Research International*, *54*, 10–15.

Xing, Y., Li, X., Xu, Q., Yun, J., Lu, Y., and Tang, Y. (2011). Effects of chitosan coating enriched with cinnamon oil on qualitative properties of sweet pepper (*Capsicum annuum* L.). *Food Chemistry*, *124*, 1443–1450.

Xing, Y., Li, W., Wang, Q., Li, X., Xu, Q., and Guo, X. (2019). Antimicrobial nanoparticles incorporated in edible coatings and films for the preservation of fruits and vegetables. *Molecules*, *24*(9), 1695.

Xing, Y., Yang, H., Guo, X., Bia, X., Liu, X., Xua, Q., Wang, Q., Lia, W., Lia, X., Shui, Y., Chen, C., Zheng, Y. (2020). Effect of chitosan/Nano-TiO2 composite coatings on the postharvest quality and physicochemical characteristics of mango fruits. *Scientia Horticulturae*, *263*, 109–119.

Xing, Y., Yang, S., Xu, Q., Xu, L., Zhu, D., Li, X., Shui, Y., Liu, X., and Bi, X. (2021). Effect of chitosan/Nano-TiO2 composite coating on the postharvest quality of blueberry fruit. *Coatings*, *21*, 18–27.

Yang, Q., Wang, F., and Rao, J. (2016). Effect of putrescine treatment on chilling injury, fatty acid composition and antioxidant system in Kiwifruit. *Social Psychiatry*, *45*, 11–2535.

Yu, T., Wang, L., Yin, Y., Wang, Y., and Zheng, X. (2008). Effect of chitin on the antagonistic activity of *Cryptococcus laurentii* against *Penicillium expansum* in pear fruit. *International Journal of Food Microbiology*, *122*, 44–48.

Yu, Y., Zhang, S., Ren, Y., Li, H., Zhang, X., and Di, J. (2012). Jujube preservation using chitosan film with nano-silicon dioxide. *Journal of Food Engineering*, *113*, 408–414.

Zhang, Y., Zhang, M., and Yang, H. (2015). Postharvest chitosan-g-salicylic acid application alleviates chilling injury and preserves cucumber fruit quality during cold storage. *Food Chemistry*, *174*, 558–563.

Zokaei Khosroshahi, M. R., Esna-Ashari, M., and Ershadi, A. (2007). Effect of exogenous putrescine on postharvest life of strawberry fruit. *Scientia Horticulturae*, *114*, 27–32.

Received: May 27, 2023; accepted: February 27, 2024