Salicylic Acid and Chitosan Effects on Fruit Quality When Applied to Fresh Strawberry or During Different Periods of Cold Storage

Khalid F. Almutairi,[a,]* Abdulaziz R. Alharbi,[a] Mohamed Ewis Abdelaziz,[b,c] and Walid F. A. Mosa [d]

One of the biggest problems that threaten the production of strawberry in the world is the rapid damage and high rate of deterioration after harvest or during cold storage. Therefore, the current study was conducted to investigate the possibility of decreasing the post-harvest damage percentages and increasing the fruit quality of *Fragaria* x *ananassa* cv. 'Estavana' after harvest immediately or during the cold storage period. The strawberry plants were dipped for 3 to 5 minutes at 25 °C in a solution of 10 L made from distilled water and containing salicylic acid (SA) at 0, 250, 500, and 1000 mg/L or chitosan (CHIT) at 0, 2.5, 5, and 10 mg/L during the period of 0, 3, 6, 9, 12, and 16 days after harvesting. The results showed that the fruit firmness was notably decreased, and the loss and decay percentages were increased by increasing the period of storage, but it could be decreased by using SA or CHIT. Fruit content from soluble solids, total sugars and anthocyanin was significantly increased in the 16 days stored fruits treated with 500 mg/L SA or 50mg/L CHIT. Treating the fresh harvested without or with SA or CHIT increased the fruit content from vitamin C. The highest fruit content from titratable acidity was in the fresh harvested fruits compared with treated fruits with SA or CHIT.

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*Contact information: a: Department of Plant Production, College of Food Science and Agriculture, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia; b: The National Research and Development Center for Sustainable Agriculture (Estidamah), Riyadh, Kingdom of Saudi Arabia; c; Department of Vegetable Crops, Faculty of Agriculture, Cairo University, Giza, 12613, Egypt; d: Plant Production Department (Horticulture-Pomology), Faculty of Agriculture, Saba Basha, Alexandria University, Alexandria 21531, Egypt; *Corresponding author: almutairik@ksu.edu.sa*

INTRODUCTION

Strawberry fruit is a popular food and is rich in many different bioactive compounds and antioxidants, including terpenoids, flavonoids, carotenoids, anthocyanins, and vitamin E (Pang *et al*. 2020; Kim *et al*. 2021). Strawberries are susceptibile to mechanical damage, dehydration, and microbial deterioration, and as a result, present a challenging post harvest protocol (Benavides and Franco 2023). Because strawberries are highly perishable and susceptible to fungal infections, their shelf life is usually fewer than five days at room temperature, and their quality properties deteriorate quickly after harvest (Cordenunsi *et al*. 2005; Feliziani and Romanazzi 2016; Barkaoui *et al*. 2021). It has been investigated whether bioactive edible coatings with CHIT can reduce or eliminate fungal and mechanical fruit damage (Benavides and Franco 2023). Edible coating treatments can provide a protective layer on the product's surface (Deng *et al*. 2017). When applied to fresh fruit, these coatings can prevent microbial attack and help maintain the fruit's desirable composition and marketability. Additionally, coatings can prevent moisture loss, create a modified internal atmosphere, and regulate the concentration of gases such as O_2, CO_2, and fragrance compounds to more desirable levels (Gutiérrez and Álvarez 2017). Edible coatings are a suitable replacement for preserving postharvest crops (No *et al*. 2007). Coatings have been demonstrated to slow down the rates of carbohydrate breakdown, which delays maturity (Yan *et al*. 2019), and to function as moisture and gas barriers, controlling microbial growth and preserving colour and texture (Chaudhary *et al*. 2020).

CHIT, an environmentally safe and non-toxic edible polymer with antifungal properties, stimulates plant defense mechanisms. It is frequently used to preserve postharvest fruits and vegetables as a food additive and a successful replacement for synthetic fungicides (Romanazzi *et al*. 2017). Moreover, CHIT coating can alter the internal atmosphere of the fruit by lowering oxygen levels and/or increasing carbon dioxide levels. This leads to reduced respiration rates and metabolic activity, delays in sugar accumulation and starch degradation, and potential initiation of fermentation processes (Silva *et al*. 2017). Furthermore, CHIT has a number of special advantages, including the capacity to form films, biocompatibility, biodegradability, antibacterial activity, and non-toxicity (Jiang *et al*. 2018a,b).

The coating of fruit with CHIT has been demonstrated to have higher total soluble solids and antioxidant activity while decreasing hardness, decay, and weight loss (Adiletta *et al*. 2019). By minimizing losses in weight, soluble solids, vitamin C, titratable acidity, and firmness, CHIT coating preserves fruit quality during storage (Lin *et al*. 2020). By lowering the postharvest respiration rate of fruits, the coating's use of polysaccharides such as CHIT acts as an effective oxygen and moisture barrier, delaying the deterioration of the product (Jung *et al*. 2020). An edible coating of CHIT can preserve the fruit freshness by minimizing microbial degradation, softening, and oxidative stress (Ghosh *et al*. 2021). Besides, CHIT works as an antimicrobial and antitranspirant agent, elicitor, and stimulator for both plant growth and beneficial microorganisms (Kipkoech *et al*. 2021). Additionally, the use of CHIT in coating mango fruit reduces the weight loss and the fruit content from TSS percentages, as well as preserves the peel firmness (Limon *et al*. 2021). Furthermore, CHIT is a great film-forming agent and biocompatible material for usage in the perishable fruit product sector. Fresh fruit coated with CHIT is thought to be safe for ingestion by humans because of its antibacterial and non-toxic qualities (Zhang *et al*. 2021). CHIT edible coating effectively reduces moisture loss, respiration rates, ethylene production, fruit ripening, softening, and decomposition, while preserving fruit quality and extending the postharvest life of various crops (Kumarihami *et al*. 2022).

Salicylic acid (SA) is a relatively basic phenolic molecule that can be utilized as a safe and natural substitute chemical to regulate horticulture crops' postharvest quality, and it is a naturally occurring substance that has the potential to significantly delay the fruit ripening (Mo *et al*. 2008). The application of SA effectively controls ethylene production, the respiration process, and enhances ascorbic acid content in fruits (Huang *et al*. 2008), lowering the post-harvest damages in horticultural crops (Asghari and Aghdam 2010), inducing the disease resistance (Shafiee *et al*. 2010), the preservation of post-harvest quality (Champa *et al*. 2015), and an extension of horticultural crop storage life. Additionally, it is frequently used to manage the firmness of crops to prolong their postharvest life (Kazemi *et al*. 2011). The preharvest treatment with SA significantly mitigates the harmful effects of heat and high light stress on photosystem, and it induces the photosynthesis process (Zhao *et al*. 2011). Furthermore, Khan *et al*. (2012) documented that SA is essential for the organization of several plant physiological systems, including proline metabolism, antioxidant defense system, nitrogen absorption, photosynthesis, plant-water connections, and resistance to abiotic stressors. Besides, it was documented by many authors that SA is included in the improving resistances to various biotic and abiotic stresses, including heat, heavy metals toxicity, pathogens, and oxidative decomposition (Agnihotri *et al*. 2018; Ali *et al*. 2023), and cell wall enforcement (Jia *et al*. 2021). Treating peach cv. 'Flordaking' with SA at concentrations of 1, 2, and 3 mM improved fruit quality attributes such as flesh firmness, titratable acidity, and ascorbic acid content during storage. Conversely, these treatments reduced fruit weight loss, soluble solids content, membrane seepage, chilling injury, ethylene production, color deterioration, disease incidence, decomposition, and delayed the ethylene climacteric peak after six weeks of low-temperature storage (Ali *et al*. 2021). Besides, SA plays vital roles in raising the plant stress tolerance by activation the plant defense mechanisms (Alam *et al*. 2022), improvement of photosynthesis (George *et al*. 2022), the enhancing the plant tolerance to drought (Balfagón *et al*. 2022), cold (Raza *et al*. 2023), heat (Lafuente, and Romero 2022), and diseases (Khan *et al*. 2023). Besides, SA regulates the protein pattern, enhances the antioxidative system, growth and photosynthesis process, and Superoxide dismutase (SOD), Catalase (CAT), and Peroxidase (POD) activities under cold stress (Hernanz *et al*. 2023; Raza *et al*. 2023; Singh 2023).

Therefore, the current study was conducted to increase the shelf life and maintain the strawberry fruits during storage and reduce their decay and fungal attack by the application of CHIT and SA.

EXPERIMENTAL

The current work was performed to study the effect of the dipping of strawberry fruits (*Fragaria* x *ananassa* cv. 'Estavana') for 3 to 5 min at 25 °C with SA (LHCHEM company, Jinan City, Shandong Province, China) at 0, 250, 500, and 1000 mg/L and CHIT (Realfine Chemicals (Shanghai) Co., Ltd., Wuxi, Jiangsu, China) at 0, 2.5, 5, and 10 mg/L during the period of 0, 3, 6, 9, 12 and 16 days after harvesting. The volume of the used solution was 10 L from distilled water. Split-Plot design was used to perform this experiment where the time was the main factor, and SA or CHIT was the sub main factor in eight replicates were used as blocks. During the storage periods, several parameters were measured.

Physical Fruit Characteristics

A total of 40 fruits for each treatment (10 fruits as a replicate) were chosen to perform the study during the experiment times and the fruit quality was measured as follows: Fruit firmness (lb/inch^2) was assessed by using a Magness and Taylor pressure tester (mod. FT 02 (0-2 lb, Alfonsine, Italy). The number of strawberry fruits that had rotted and showed signs of pitted peel or pathogen incidence relative to the overall quantity of strawberries was used to calculate the percentage of fruit decay, which was then represented as a percentage following each storage period (Huang *et al*. 2023). By comparing the difference between the fruits' original and final weights using an electronic balance, the percentage of fruit weight loss was calculated (PA4102 OHAUS Corporation, USA) and expressed in percentage (%).

Fruit Chemical Characteristics

Total soluble solids (TSS) were assessed by triplicate with a digital refractometer (Atago N1; Atago Co. Ltd., Tokyo, Japan) at 20 °C and expressed as %.

Total Titratable Acidity (TA)

The TA was determined according to an AOAC method (AOAC 2005) by triplicate using an automatic titration device (877 Titrino plus, Metrohm ion analyses CH9101, Herisau, Switzerland) with 0.1 N NaOH up to pH 8.1, using 1 mL diluted juice in 25 mL distilled H_2O, and the results were expressed as g malic acid per 100 g fw (Celikel *et al*. 2008). Malondialdehyde (MDA) content was assayed using the TBA method, where 2.0 g of frozen juice sac was extracted with 5 mL of 10% (m/v) TCA solution and centrifuged (10000 × g at 4 °C for 20 min). Afterwards, 2.0 mL of the supernatant was mixed by adding the same volume of 0.67% TBA (dissolved in 50 mM NaOH) solution, followed by a boiling water bath for 20 min, and then quickly cooled in an ice bath. Finally, the absorbance of the supernatant was recorded at three specific wavelengths (450 nm, 532 nm, and 600 nm) using a UV-Vis spectrophotometer (model: TU-1950, Persee General Instrument Co., Ltd., Beijing, China), with the results were reported as millimole per gram fruit weight (Bakpa and Zhang 2022).

Fruit Total Sugars

The quantity was determined by using phenol sulfuric acid, and fruit reducing sugars content was determined calorimetrically (Nielsen 2010). The content of Vitamin C mg/100 mL juice was determined by the titration of 2,6-dichlorophenol-indo-phenol (Huang *et al*. 2023).

Antioxidant Enzyme Activity Assays

A 10 g fruit sample was chosen and homogenized using a Kinematica tissue processor (Kinematica AG Werkstrasse 7 c-d Switzerland) in 25 mL of ice-cold extraction buffer and 0.5 g of polyvinyl-polypyrrolidone (PVPP) (Crl-6010, Kriens-LU, Switzerland). For the catalase (CAT) and superoxide dismutase (SOD) estimation, the extraction buffer used was 50 mM sodium phosphate with a pH of 7.8. The homogenized mixture was centrifuged at 27,000 g for 50 min at 4 °C, and the resulting supernatants were used to measure CAT and SOD activity (Wang *et al*. 2005).

The reaction combination for CAT analysis consisted of 2.8 mL H_2O_2 (40 mM in 50 mM sodium phosphate buffer, pH 7.0) and 0.2 mL enzyme extract. The decrease of H_2O_2 (substrate) was determined by measuring the decrease in absorbance at 240 nm during a 120-s period using a spectrophotometer (Model UH4150AD UV-Vis-NIR Spectrophotometer Hitachi, Ltd., Tokyo, Japan). The activity was expressed as unit g^{-1} FW, where one unit of catalase equals one molecule of H_2O_2 per mass of fruit per minute at 30 °C. For the SOD activity assay, a volume of approximately 3 mL in the SOD activity experiment comprised 65 mM sodium phosphate cradle (pH 7.8). Finally, 13 mM methionine, 75 mM nitro-blue tetrazolium (NBT), 10 mM EDTA, and 2 mM riboflavin were mixed, along with 0.1 mL of the enzyme extract. After illumination of the combinations for 10 minutes with light (60 Mol m^{-2} s^{-1}), the absorbance at 560 nm was evaluated using a spectrophotometer. The reaction solution took 3 min to settle. The reaction was measured in units of g^{-1} FW, where one unit represented the amount of chemical that caused a 50% reduction in the SOD-inhibitable NBT per mass per hour.

Total Phenols Content

Phenol content was measured by the reduction of phosphotungstic-phosphomolybdic acid to blue pigments in an alkaline solution, following the Folin method as described by Salem *et al*. (2018). A 100 µL aliquot of the diluted sample (1/100) in ethanol was mixed with 400 µL of 1/10 diluted Folin-Ciocalteu reagent. After 5 minutes, 500 µL of a 10% (w/v) sodium carbonate solution was added. Following a 1-hour incubation at room temperature, the absorbance was measured at 765 nm in triplicate. The total polyphenol content was expressed as mg gallic acid equivalents per g dry weight of fruit (mg GAE/g DW).

Total Anthocyanin Content (TAC)

One mL of each fruit extract sample was separately added to 980 µL of KCl buffer (pH 1.0) and NaOAc buffer (pH 4.5). After a 15-min incubation at room temperature, the absorbance was measured at 510 nm and 700 nm using a spectrophotometer, with 50% ethanol as a blank. The total anthocyanin content (TAC) was calculated using Eq. 1, and the results were expressed as mg of cyanidin-3-glucoside equivalents per 100 g of dry weight.

$$\text{TAC} = (A*\text{MW}*\text{DF}*1000/\mathcal{E}*L) \quad (1)$$

where, A: Absorbance = [(A 510 nm to A 700 nm)] pH 1.0 – [(A 510 nm to A 700 nm)] pH 4.5; MW: molecular weight (449.2 g mol^{-1}); DF: dilution factor; L: is the cell path length (1 cm); $\mathcal{E}$: molar absorptivity coefficient of cyanidin- 3-glucoside (26900 L mol^{-1} cm^{-1}) (Jakobek *et al*. 2007).

Statistical Analysis

The obtained results were statistical analysis using Split-Plot Design by using CoHort Software (Pacific Grove, CA, USA), and the Least Significant Difference (LSD) at 0.05% was used to compare the means of treatments (Mishra *et al*. 2019).

RESULTS AND DISCUSSION

The data in Table 1 show that the fruit firmness was significantly increased in the fresh harvested fruits, especially when they were treated with 1000 SA. On the contrary, the fruit firmness was decreased by increasing the period of the storage. For 9 days stored fruits, the the statistical analysis indicated that there are no significant differences between the treatments. Additionally, the treating the stored fruits for 3 or 12 days with CHIT show that there are no significant differences between the different concentrations.

The results in Table 2 show that fruit decay percentages in the fresh fruits were zero. However, the decay percentages were significantly increased by increasing the time of storage, where the period of 16 or 12 days registered the highest percentages. The usage of SA or CHIT decreased the decay percentages in the stored fruits. Moreover, the percentage of decay was also minimized by raising the used concentration from SA or CHIT.

Table 1. Effect of Storage Time and Postharvest Treatment of SA and CHIT on Fruit Firmness

Fruit Firmness (lb/inch^2)							
Treatment	Concentration (mg/L)	Time of the storage (days)					
		0	3	6	9	12	16
SA	0	2.47^{ab}	2.27^{a-e}	2.00^{a-e}	1.77^{a-e}	1.60^{de}	1.67^{b-e}
	250	2.40^{a-d}	2.32^{a-e}	2.00^{a-e}	1.75^{a-e}	1.72^{a-e}	1.80^{a-e}
	500	2.35^{a-e}	1.75^{a-e}	1.85^{a-e}	2.37^{a-e}	1.77^{a-e}	1.82^{a-e}
	1000	2.52^{a}	1.92^{a-e}	2.20^{a-e}	2.45^{a-c}	1.77^{a-e}	1.77^{a-e}
CHIT	0	2.32^{a-e}	2.35^{a-e}	2.07^{a-e}	1.57^{de}	2.25^{a-e}	1.55^{e}
	2.5	2.32^{a-e}	2.10^{a-e}	1.75^{a-e}	1.75^{a-e}	2.22^{a-e}	1.62^{c-e}
	5	2.37^{a-e}	2.20^{a-e}	2.00^{a-e}	2.37^{a-e}	2.52^{a}	1.60^{de}
	10	2.37^{a-e}	2.37^{a-e}	2.07^{a-e}	2.20^{a-e}	2.05^{a-e}	1.92^{a-e}
$LSD_{0.05}$		0.41					

The treatments that have the same letters mean that there are no significant differences between them.

Table 2. Effect of Storage Time and Postharvest Treatment of SA and CHIT on the Fruit Decay Percentage

Decay %							
Treatment	Concentration (mg/L)	Time of the storage (days)					
		0	3	6	9	12	16
SA	0	0 x	4.57^{t}	8.57^{o}	14.34^{k}	25.15^{c}	33.83^{a}
	250	0 x	3.75^{u}	5.99^{r}	11.78^{m}	20.65^{f}	27.37^{b}
	500	0 x	3.19^{uv}	5.93^{r}	10.02^{n}	17.57^{h}	23.29^{d}
	1000	0 x	2.76^{vw}	5.12st	8.65^{o}	13.35 l	20.10^{g}
CHIT	0	0 x	4.58^{t}	8.65^{o}	14.36^{k}	25.20^{c}	34.13^{a}
	2.5	0 x	3.23^{uv}	6.97^{q}	10.12^{n}	17.75^{h}	23.52^{d}
	5	0 x	2.90^{vw}	5.39^{rs}	9.11^{o}	15.98i	21.17^{e}
	10	0 x	2.43^{w}	4.50^{t}	7.61^{p}	15.17j	17.69^{h}
$LSD_{0.05}$		0.50					

The treatments that have the same letters mean that there are no significant differences between them.

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Table 3. Effect of Storage Time and Postharvest Treatment of SA and CHIT on the Fruit Loss Percentage

Loss %							
Treatment	Concentration (mg/L)	Time of the storage (days)					
		0	3	6	9	12	16
SA	0	0v	1.15^{rs}	1.52^{op}	2.08^{n}	6.93^{ef}	8.91^{a}
	250	0v	1.26^{qr}	1.71^{o}	2.31^{m}	6.73f	7.69^{d}
	500	0v	1.167^{rs}	1.58^{op}	2.16^{n}	6.18^{g}	8.19^{b}
	1000	0v	1.13^{rs}	1.53^{op}	2.10^{n}	5.92^{h}	7.84^{cd}
CHIT	0	0v	1.13^{rs}	1.53^{op}	2.09^{n}	7.03^{e}	7.90^{c}
	2.5	0v	0.94^{stu}	1.27^{qr}	1.74^{o}	5.09j	6.74^{f}
	5	0v	0.86^{tu}	1.17^{rs}	1.60^{op}	4.57^{k}	6.05^{gh}
	10	0v	0.77^{u}	1.04^{rst}	1.42^{pq}	4.16^{l}	5.52^{i}
$LSD_{0.05}$		0.17					

The treatments that have the same letters mean that there are no significant differences between them.

Table 3 shows that the loss percentages were significantly enhanced by the storage of the strawberry fruits. The highest percentage was observed in 16 days of stored fruits. The percentage of fruit loss was increased by increasing the period of the storage and using SA or CHIT played a good role in decreasing the fruit loss percentage. The efficacy of SA or CHIT was increased in parallel to increasing the used concentration.

As shown in Table 4, the storage of strawberry fruits increased the fruit content from TSS % where the period of 16 days registered the highest percentage from TSS in particularly when 500 mg/L SA or 10 mg/L CHIT. Increasing the used concentration from SA or CHIT was more effective in improving the fruit content from soluble solids during the storage period. In the 16 days stored fruits, the differences between the effect of 250 and 1000 mg/L SA or between 2.5 and 5 mg/L CHIT were not significant.

Table 4. Effect of Storage Time and Postharvest Treatment of SA and CHIT on TSS %

TSS%							
Treatment	Concentration (mg/L)	Time of the storage (days)					
		0	3	6	9	12	16
SA	0	8.07^{r}	8.40^{o-r}	8.55^{n-r}	9.35^{jk}	10.45^{e-h}	11.57^{bc}
	250	8.15^{qr}	8.82^{k-q}	9.00^{j-o}	9.87^{hi}	10.75^{d-g}	11.72^{ab}
	500	8.12^{r}	8.65^{m-r}	8.72^{l-r}	9.55^{ij}	10.72^{d-g}	12.12^{a}
	1000	8.22^{qr}	8.67^{m-r}	9.32^{j-l}	10.15^{gh}	10.77^{d-g}	11.50^{bc}
CHIT	0	8.10^{r}	8.07^{r}	8.20^{qr}	8.92^{k-p}	10.05^{h}	10.95^{de}
	2.5	8.07^{r}	8.45^{o-r}	8.65^{m-r}	9.27^{j-m}	10.42^{e-h}	11.47^{bc}
	5	8.27^{p-r}	8.30^{p-r}	8.42^{o-r}	9.12^{j-n}	10.30^{f-h}	11.15^{cd}
	10	8.20^{qr}	8.75^{k-r}	8.90^{k-p}	10.20^{h}	10.82^{d-f}	12.12^{a}
LSD$_{0.05}$		0.38					

The treatments that have the same letters mean that there are no significant differences between them.

Table 5 shows that when the strawberry fruits were stored, anthocyanin was increased in parallel to increasing the period of the storage, where the highest concentration of anthocyanin was in the 16 days stored fruits. The SA and CHIT helped to improve the fruit coloring by increasing the content of anthocyanin, where the 250, 500, and 1000 SA or 2.5, 5, and 10 mg/L CHIT in 16 days stored fruits gave the highest concentrations.

Table 5. Effect of Storage Time and Postharvest Treatment of SA and CHIT on Anthocyanin Content

Anthocyanin (mg/100g)							
Treatment	Concentration (mg/L)	Time of the storage (days)					
		0	3	6	9	12	16
SA	0	30.72^{p}	35.32^{m-o}	37.675^{l-n}	42.17^{h-j}	48.82^{de}	51.95^{a-c}
	250	31.37^{p}	35.67^{m-o}	39.52^{kl}	42.92^{h-j}	49.82^{cd}	51.90^{a-c}
	500	31.75^{p}	34.77^{no}	38.10^{lm}	43.12^{hi}	52.10^{a-c}	52.25^{a-c}
	1000	31.45^{p}	36.20^{m-o}	41.80^{h-k}	46.72^{ef}	47.65^{d-f}	52.77^{ab}
CHIT	0	30.62^{p}	34.50^{o}	36.67^{m-o}	40.40^{jk}	45.75^{fg}	50.07^{b-d}
	2.5	31.70^{p}	35.47^{m-o}	37.40^{l-o}	40.60^{i-k}	46.05^{fg}	51.45^{a-c}
	5	31.55^{p}	34.87^{no}	36.95^{m-o}	40.72^{i-k}	46.50^{e-g}	50.15^{b-d}
	10	31.15^{p}	36.67^{m-o}	44.17^{gh}	47.17^{ef}	48.70^{de}	53.62^{a}
LSD$_{0.05}$		1.80					

Treatments that take the same letters mean that there are no significant differences between them.

The results in Table 6 show that the storage of the strawberry fruits gradually increased the fruit content from the total sugars percentages and the highest percentages were noticed when the fruits were treated with SA or CHIT. The differences between the effects of the different concentrations of SA or CHIT were not significant. The fruit content from total sugars was significantly low in fresh harvested fruits and when the fruits were stored for 3, 6 and 9 days after harvested.

Table 6. Effect of Storage Time and Postharvest Treatment of SA and CHIT on Total Sugars Content %

Total sugars %							
Treatment	Concentration (mg/L)	Time of the storage (days)					
		0	3	6	9	12	16
SA	0	6.62^{l-n}	6.77^{j-n}	7.22^{i-l}	7.6^{f-i}	8.40^{a-d}	8.71^{ab}
	250	6.55^{l-n}	6.82^{j-n}	6.82^{j-n}	7.45^{g-j}	8.35^{b-d}	8.99^{a}
	500	6.52^{l-n}	6.57^{l-n}	6.70^{k-n}	7.37^{h-k}	8.13^{b-f}	9.00^{a}
	1000	6.27^{n}	6.42^{mn}	6.50^{l-n}	7.227^{i-l}	7.93^{c-h}	8.70^{ab}
CHIT	0	6.50^{l-n}	6.80^{j-n}	6.87^{j-n}	7.65^{f-i}	8.30^{b-e}	8.53^{a-c}
	2.5	6.55^{l-n}	6.52^{l-n}	6.87^{j-n}	7.20^{i-l}	8.01^{c-g}	8.77^{ab}
	5	6.45^{mn}	6.55^{l-n}	6.50^{l-n}	7.147^{i-m}	7.80^{d-i}	9.01^{a}
	10	6.45^{mn}	6.27^{n}	6.30^{n}	6.85^{j-n}	7.72^{e-i}	8.44^{a-c}
$LSD_{0.05}$		0.40					

The treatments that take the same letters mean that there are no significant differences between them.

The data in Table 7 show that treating fresh fruits with 500 mg/L SA significantly increased their Vitamin C (VC) content. Additionally, treating fruits with 500 or 1000 mg/L SA and also by 2.5, 5, or 10 mg/L CHIT also greatly improved the VC. The results showed that the storage of strawberry fruits for 3, 6, 12, and 16 days solely or by treating them with 250, 500, and 1000 SA or by 2.5, 5, and 10 mg/L decreased VC. Additionally, there is an inverse relationship between the vitamin C content in the fruit and the storage duration, with the lowest values observed after 12 or 16 days of storage. This trend was noted whether the fruit was untreated or treated with 250, 500, and 1000 mg/L SA or 2.5, 5, and 10 mg/L CHIT.

Table 7. Effect of Storage Time and Postharvest Treatment of SA and CHIT on Vitamin C Levels

Vitamin C (mg/100 mL)							
Treatment	Concentration (mg/L)	Time of the storage (days)					
		0	3	6	9	12	16
SA	0	58.20^{b}	51.42^{f}	45.02^{k}	42.00^{n}	35.32^{s}	36.47^{qr}
	250	57.90^{b}	50.65^{fg}	46.57^{i}	42.60^{mn}	36.32^{qr}	36.32^{qr}
	500	59.27^{a}	52.37^{e}	48.20^{h}	44.20^{l}	37.87^{p}	36.70^{qr}
	1000	58.15^{b}	53.00^{e}	48.90^{h}	44.90^{kl}	38.32^{p}	36.90^{q}
CHIT	0	57.97^{b}	51.40^{f}	46.62^{i}	42.85^{m}	35.80^{rs}	36.47^{qr}
	2.5	58.27^{b}	52.60^{e}	48.45^{h}	44.12^{l}	37.9^{p}	36.62^{qr}
	5	58.27^{b}	54.47^{d}	50.12^{g}	45.72^{j}	39.37^{o}	37.57^{p}
	10	58.00^{b}	55.22^{c}	50.70^{fg}	46.25^{ij}	39.65^{o}	37.97^{p}
$LSD_{0.05}$		0.67					

The treatments that take the same letters mean that there are no significant differences between them.

The results in Table 8 show that the fresh harvested fruits were characterized by a high content of titratable acidity. Fresh fruit treated with 250, 500, and 1000 SA or with 2.5, 5, and 10 mg/L contained high quantities of titratable acidity. Additionally, the 3 and 6 days stored fruit contained higher titratable acidity than fruits stored for 9, 12, and 16 days, only or after treatment with 250, 500, and 1000 mg/L SA and 2.5, 5, and 10 mg/L CHIT. Additionally, the results showed that as the period of storage increased, the percentage of titratable acidity decreased.

Table 8. Effect of Storage Time and Postharvest Treatment of SA and CHIT on the Fruit Titratable Acidity

Titratable acidity %							
Treatment	Concentration (mg/L)	Time of the storage (days)					
		0	3	6	9	12	16
SA	0	0.87^{a}	0.83^{e}	0.82^{ef}	0.79^{ij}	0.77^{l-n}	0.71^{q}
	250	0.85^{cd}	0.83^{e}	0.81^{e-g}	0.78^{jk}	0.76^{m-o}	0.70^{qr}
	500	0.86^{a-c}	0.82^{ef}	0.81^{f-h}	0.78^{j-l}	0.76^{n-p}	0.70^{qr}
	1000	0.87^{ab}	0.81^{e-g}	0.80^{gh}	0.77^{k-n}	0.75^{op}	0.69^{r}
CHIT	0	0.87^{a}	0.83^{e}	0.81^{e-g}	0.78^{jk}	0.77^{m-o}	0.70^{qr}
	2.5	0.86^{b-d}	0.82^{ef}	0.81^{f-h}	0.78^{j-l}	0.76^{n-p}	0.70^{qr}
	5	0.85^{d}	0.82^{e-g}	0.80^{gh}	0.77^{l-n}	0.75^{op}	0.70^{r}
	10	0.85^{d}	0.81^{f-h}	0.80^{hi}	0.77^{k-m}	0.75^{p}	0.69^{r}
$LSD_{0.05}$		0.01					

The treatments that take the same letters mean that there are no significant differences between them.

The results in Table 9 show that the fruit content from phenol was significantly increased in the 3 days of stored fruits. Additionally, fruits stored for 3 days after treatment with 250, 500, and 1000 SA or with 2.5, 5, and 10 mg/L CHIT also contained a high quantity of phenols. Moreover, the 6 or 9 days of stored fruits solely or after treatment with SA or CHIT are characterized by a high phenol content. By increasing the storage period, the fruit content from phenols was decreased even with using SA or CHIT.

Table 9. Effect of Storage Time and Postharvest Treatment of SA and CHIT on Phenols

Phenols (mg/g dry weight)							
Treatment	Concentration (mg/L)	Time of the storage (days)					
		0	3	6	9	12	16
SA	0	106.00^{op}	165.50^{a}	153.00^{b}	141.25^{e}	121.50^{l}	108.00^{op}
	250	106.75^{op}	156.00^{b}	145.00^{cd}	138.00^{f}	125.00^{k}	112.50^{n}
	500	105.75^{op}	153.00^{b}	141.75^{de}	137.00^{fg}	127.00^{jk}	115.75^{m}
	1000	104.75^{p}	148.00^{c}	136.00^{fg}	131.00^{hi}	130.75^{hi}	116.00^{m}
CHIT	0	105.5^{op}	164.25^{a}	147.75^{c}	142.50^{de}	121.50^{l}	108.75^{o}
	2.5	108.00^{op}	153.75^{b}	142.50^{de}	135.75^{fg}	127.00^{jk}	115.25^{m}
	5	106.00^{op}	147.50^{c}	136.50^{fg}	131.00^{hi}	132.00^{h}	116.25^{m}
	10	105.25^{op}	142.50^{de}	133.75^{gh}	128.50^{ij}	134.25^{gh}	116.50^{m}
$LSD_{0.05}$		2.44					

The treatments that take the same letters mean that there are no significant differences between them.

The data in Table 10 show that a high significant concentration of MDA was in fruits stored for 16 days. Moreover, fruit stored for 16 days and treated with 250, 500, and

1000 mg/L SA and also with 2.5, 5, and 10 mg/L CHIT were characterized by a high content of MDA. Its content was also significantly increased in the 12 days solely or after the treatment with 250, 500, and 1000 SA or with 2.5, 5, and 10 mg/L CHIT. In the fresh harvest fruits, MDA concentration was less than that after the storage, whereas when the storage time increased, the concentration of MDA in the fruit increased.

Table 10. Effect of Storage Time and Postharvest Treatment of SA and CHIT on MDA Enzyme

MDA ((µmol/g fruit weight)							
Treatment	Concentration (mg/L)	Time of the storage (days)					
		0	3	6	9	12	16
SA	0	40.40^{z}	51.90^{n}	55.60^{j}	61.5^{f}	65.70^{b}	68.70^{a}
	250	40.57^{yz}	46.72^{s}	52.45^{m}	56.67^{i}	62.47^{e}	65.62^{b}
	500	40.60^{yz}	44.55^{u}	50.55^{p}	55.42^{j}	60.62^{g}	64.27^{c}
	1000	41.00^{y}	43.95^{v}	48.85^{q}	53.40^{l}	63.57^{d}	63.57^{d}
CHIT	0	40.70^{yz}	51.50^{o}	55.50^{j}	61.55^{f}	65.60^{b}	68.52^{a}
	2.5	40.32^{z}	44.92^{t}	50.50^{p}	55.60^{j}	61.57^{f}	65.40^{b}
	5	40.62^{yz}	43.47^{w}	48.87^{q}	54.57^{k}	60.45^{g}	64.45^{c}
	10	40.67^{yz}	42.72^{x}	47.55^{r}	53.72^{l}	58.57^{h}	63.57^{d}
$LSD_{0.05}$		0.34					

The treatments that take the same letters mean that there are no significant differences between them.

At 6 days, stored fruits were characterized by the high significant content of catalase enzyme (CAT), followed by 3 or 9 days stored fruits solely or after the usage of SA or CHIT (Table 11). The lowest concentrations of CAT enzyme were noticed in the 16 days stored fruits or even after the usage of SA or CHIT. The amount of CAT enzyme was significantly different in the fresh fruits compared to fruits treated with SA or CHIT.

Table 11. Effect of Storage Time and Postharvest Treatment of SA and CHIT on CAT Enzyme

CAT (U/Fruit weight)							
Treatment	Concentration (mg/L)	Time of the storage (days)					
		0	3	6	9	12	16
SA	0	48.60i	55.50^{b}	58.55^{a}	54.67^{bc}	40.45^{k}	30.62^{r}
	250	48.57^{i}	53.67^{cd}	55.60^{b}	52.70^{d-f}	41.65^{k}	33.65^{op}
	500	48.52^{i}	52.70^{d-f}	54.62^{bc}	50.5^{gh}	38.60^{l}	32.70^{pq}
	1000	48.50^{i}	51.42^{fg}	53.72^{cd}	52.02^{e-g}	36.62^{m}	31.42^{qr}
CHIT	0	48.62^{i}	55.65^{b}	58.67^{a}	54.62^{bc}	40.55^{k}	30.57^{r}
	2.5	48.77^{i}	52.65^{d-f}	55.45^{b}	51.65^{fg}	43.60^{j}	35.50^{n}
	5	48.57^{i}	50.62^{gh}	53.47^{c-e}	50.57^{gh}	41.55^{k}	34.60^{no}
	10	48.52^{i}	49.67^{hi}	51.70^{fg}	48.67^{i}	38.55^{l}	31.62^{qr}
$LSD_{0.05}$		1.11					

The treatments that take the same letters mean that there are no significant differences between them.

Treating the 3, 6 and 9 days stored fruits with 250, 500, and 1000 mg/L SA or with 2.5, 5, and 10 mg/L CHIT increased the superoxide dismutase (SOD) enzyme (Table 12). The superior concentration of SOD was noticed in the 6 days stored fruits after treatment of 1000 mg/L SA and in the 3, 6 and 9 days stored fruits treated with 10 mg/L CHIT. It was noticed also that SOD decreased gradually by increasing the storage period where its concentration in the 12 and 16 stored fruits was significantly decreased particularly when the fruits were not treated with SA or CHIT.

Table 12. Effect of Storage Time and Postharvest Treatment of SA and CHIT on SOD Enzyme

SOD (U/Fruit weight)							
Treatment	Concentration (mg/L)	Time of the storage (days)					
		0	3	6	9	12	16
SA	0	10.77^{ef}	9.65^{g}	8.55^{i}	7.65j	7.60^{j}	6.90^{k}
	250	10.42^{f}	11.57^{d}	12.67^{b}	11.65^{d}	10.42^{f}	8.60i
	500	10.55^{ef}	12.62^{b}	12.87^{b}	12.02^{cd}	10.95^{ef}	9.12^{h}
	1000	10.52^{ef}	12.75^{b}	13.65^{a}	12.72^{b}	11.55^{d}	9.62^{g}
CHIT	0	10.70^{ef}	9.57^{g}	8.65i	7.65j	7.35j	7.40^{j}
	2.5	10.50^{ef}	11.57^{d}	12.50^{b}	12.35^{bc}	10.67^{ef}	9.57^{g}
	5	10.45^{f}	12.62^{b}	12.72^{b}	12.77^{b}	11.02^{e}	10.50^{ef}
	10	10.57^{ef}	13.47^{a}	13.42^{a}	13.42^{a}	11.60^{d}	10.80^{ef}
LSD$_{0.05}$		0.34					

The treatments that take the same letters mean that there are no significant differences between them.

DISCUSSION

The results showed that the post-implementation of CHIT on strawberry increased their fruit shelf life, and decreased the percentage of decay and fungal diseases. These results were in agreement with Wang and Gao (2013); they reported that untreated strawberry fruits with CHIT may have low anthocyanin concentrations due to pigment degradation and accelerated fruit senescence. Because of its propensity to produce semi-permeable membranes, the CHIT-coated film on the fruit surface can block epidermal stomata and lenticels (Hosseinnejad and Jafari 2016), which decreases water and nutrient loss from the fruit and prevents respiration (Xu *et al*. 2018). Treating kiwifruit with CHIT after harvesting increased the fruit firmness as a result of the creation of a semipermeable layer on the fruit surface, which functions as a protective barrier to minimise the respiration rate, hence reducing metabolic activity and textural changes (Drevinskas *et al*. 2017; Zheng *et al*. 2017). Besides, CHIT may create an altered environment surrounding the fruit surface, which inhibits the breakdown of pectin and postpones the fruit's loss of firmness (He *et al*. 2018). CHIT-based coatings have been effectively applied to a range of fresh fruits and vegetables. They can function as barriers, slow down senescence and maturation, lessen dehydration, and postpone microbiological and fungal decomposition (Pagno *et al*. 2018; Kabanov and Novinyuk 2020). CHIT is a good edible coating material due to its outstanding film-forming properties, high mechanical strength, and antibacterial qualities (Jiao *et al*. 2019). CHIT treatment inhibited fungal and bacterial growth, implying that pathogens grew more slowly in these settings (Youssef and Hashim 2020). Wantat *et al*. (2022) described a CHIT-montmorillonite nanocomposites coating that might lower ethylene generation and respiration rates, hence preserving banana storage quality.

The results of this experiment showed that the usage of SA on strawberry increased their fruit shelf life and decreased decay percentages and fungal diseases. These results are in the same line with those documented by Barman *et al*. (2016). They reported that SA plays a significant function in different physiological processes such as fruit ripening, minimizing fruit damage by increasing ethylene production, and preserving the fruit firmness and color. Additionally, SA has been reported to enhance disease resistance (Zhang et *al*. 2010), reduce chilling (Luo *et al*. 2011), increase the storability of horticultural crops (Valero *et al*. 2011), and have antisenescence properties, delaying the postharvest ripening process (Gimenez *et al*. 2017). These effects lead to improving the fruit content from TSS percentage (Ahmad *et al*. 2013). According to García-Pastor *et al*. (2020), the application of SA resulted in a more intense red hue by raising the concentration of anthocyanins, hence boosting pomegranate profits on the global market. SA can reduce the metabolic activity and transpiration of fruit, thereby inhibiting weight loss (Amiri *et al*. 2021), which is a crucial quality parameter for commercial fruit, as it directly impacts visual quality and freshness (Koyuncu *et al*. 2019; Madhav *et al*. 2021). Batool *et al*. (2022) reported that postharvest treatments with SA significantly preserved total soluble solids, titratable acidity, color profile, ascorbic acid content, and total phenolic content in apricot varieties. These treatments also enhanced antioxidant activity and texture, maintained the visual color of apricots compared to the control, and reduced chilling injury index, weight loss, and decay percentage. The use of SA has been shown to improve storage quality by lowering respiration rates and ethylene production, preventing changes in fruit color and softening, preserving sugars and organic acids, reducing chilling injury, and boosting both pathogen resistance and the antioxidant system (Chen *et al*. 2023).

CONCLUSIONS

1. The percentages of fruit loss and decay were significantly increased in parallel to increasing the period of the storage while, the fruit firmness was high in the fresh harvested fruits particularly, the fruits treated with 1000 mg/L salicylic acid (SA).
2. Treating strawberry fruits with salicylic acid (SA) or chitosan (CHIT) increased the total soluble solids (TSS), total sugars, vitamin C, and anthocyanin content. It was noticed that after 3 days storage, the phenol content was increased especially when they were not treated with SA or CHIT.
3. The CAT and SOD enzyme contents were decreased during the storage period, and the treatment of SA and CHIT increased their content, while malondialdehyde (MDA) was increased by increasing the time of the storage while its concentration was decreased after treatment of SA or CHIT.

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