



## Postharvest quality of strawberry (*Fragaria × ananassa* Duch.) coated with calcium and nano-chitosan as affected by different storage temperatures

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### ARTICLE INFO

#### Original Article

#### Article history:

Received 30 February 2021

Revised 9 June 2021

Accepted 9 June 2021

Available online 12 August 2021

#### Keywords:

Calcium chloride

Edible coating

Malondialdehyde

Nano-chitosan

Strawberry

DOI: 10.22077/jhpr.2021.4106.1194

P-ISSN: 2588-4883

E-ISSN: 2588-6169

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### ABSTRACT

**Purpose:** The aim of the study was to identify the impacts of storage temperature on strawberries coated with 3% calcium chloride (CaCl<sub>2</sub>) and 0.2% nano-chitosan. **Research method:** Fresh strawberry fruit were immersed in CaCl<sub>2</sub> solution of 3% for 1 min and drained at room temperature before coating with nano chitosan solution of 0.2%. The treated fruit was then stored at 0 °C, 2 °C, 4 °C and 25 °C. Physico-chemical analysis was performed in each three-day interval. **Findings:** Of the four examined temperatures tested, storing the fruit at 0 °C was the most effective in maintaining the overall quality index of strawberries up to 21 days. The treatment also reduced weight loss, preserved ascorbic acid content, antioxidant capacity, and total anthocyanin content, prevented microbial growth and prolonged storage-life of treated strawberries up to 21 days. **Limitations:** the industrial packaging that could affect the actual influences of the studied temperatures was not investigated in this work. **Originality/Value:** storing fresh strawberries coated with CaCl<sub>2</sub> 3%, nano-chitosan 0.2% at 0 °C was the most effective treatment in lengthening the shelf life of the fruit up to 21 days. The combination treatment of coating and storing at 2 °C extends strawberry storage life by 6 days when compared to uncoated fruit.

## INTRODUCTION

Strawberry (*Fragaria × ananassa* Duch.) is a non-climacteric fruit containing a great variety of bioactive compounds including phenolic constituents, anthocyanins, vitamins and minerals (Giampieri et al., 2015). Many of these compounds have biological properties, such as antioxidant and anti-inflammatory activity (Giampieri et al., 2017). However, postharvest handling and storage of fresh strawberries difficult mostly due to their high susceptibility to mechanical injury, water loss, microbial decay, physiological deterioration and high respiration rate (Liu et al., 2018). Reduction of firmness, mechanical injury, and gray mold (*Botrytis cinerea*) induced decay are typical undesirable changes observed during postharvest storage. Therefore, quality attributes decline rapidly after harvesting. The storage life of strawberries may be within a day at room temperature (Mercantila, 1989) or 5-10 days at 0°C (SeaLand, 1991). In recent research, various techniques have been studied to extend the shelf life of fresh strawberries such as cold storage (Han et al., 2004), modified atmosphere packaging (Nielsen & Leufvén, 2008), heat treatments (Civello et al., 1997), bioactive compounds (Liu et al., 2018) and edible coating (Badawy et al., 2017; Sogvar et al., 2016). Nguyen et al. (2020) found that coating strawberries with calcium and nano chitosan effectively maintains the postharvest quality of the fruit. In particular, they reported that coating strawberries with 3 % CaCl<sub>2</sub> combined with 0.2 % nano-chitosan effectively maintaining the postharvest quality of the fruit. However, besides coating technology, storage temperature is one of the critical parameters for monitoring fruit and vegetable storage quality. Adequate low temperature range could suppress both physiological and microbial activities. Several published studies have documented strawberry storage temperatures of 0°C and 2°C (Han et al., 2004; Lu et al., 2018; Yan et al., 2019). Nevertheless, a combination effect of the coating treatment with storage temperatures has not been reported. Moreover, the temperature of the household kitchen refrigerator is usually set at around 4°C while the room temperature is normally 25°C. Therefore, this study aimed to investigate the effects of storage temperature on the quality of strawberries coated with 3% calcium chloride and 0.2% nano chitosan during storage.

## MATERIALS AND METHODS

### Material

Strawberry fruit (*Fragaria x ananassa* Duch.) were harvested when fully ripe from an orchard in Vietnam's Lam Dong province. Sound fruit that is free from mechanical and insect damage fruit was selected for studying and fruit rinsed under tap water. The fruit was drained of water before treatment.

### Methods

#### Experimental design

According to Nguyen et al. (2020), fresh strawberries were soaked in calcium chloride 3% and nano-chitosan (NCTS) 0.2% supplied by Dalat Nuclear Research Institute for 1 hour at room temperature for forming the coating layer on the fruit surface before being stored at 0 °C, 2 °C, 4 °C, and 25 °C. Physico-chemical analyses were performed in each three-day interval. Each treatment was done in triplicate.

## Analytical methods

### Determination of the overall quality index

A 1 to 5 visual rating scale (Fig. 1) was used (Nguyen & Nguyen, 2021) to evaluate decay rate and shriveling. The minimum acceptable quality was established at 3. When the rating of at least one of the quality attributes was at 3 or lower, the treatments were terminated.



**Fig. 1.** The visual quality scores and descriptors for strawberry (Nguyen & Nguyen, 2021) with (5.0) Excellent; (4) Very good; (3) Acceptable; (2) Poor; (1) Very poor.

### Determination of weight loss

Fruit was evaluated for its weight loss that was expressed as differences between the initial weight and the weight recorded at each sampling time (Hernández-Muñoz et al., 2008). The initial weight was measured right after coating using a digital balance (TXB-622L, Shimadzu Co, LTD., Japan). The weight loss percentage was calculated by the following formula (1):

$$\text{Weight loss (\%)} = \frac{m_0 - m}{m_0} \times 100 \quad (1)$$

Where

$m_0$ : initial weight of sample before storage (g)

$m_s$ : weight of sample at sampling time (g)

### Determination of firmness

Fruit for each treatment was cut in to halves and each half was measured its firmness in the center zone using a Digital Fruit Hardness Tester (FR-5120, Lutron electronic enterprise Co., LTD., Taiwan) with the cross-head speed was  $2 \text{ mm s}^{-1}$  and the penetration depth was 2 mm (Hernández-Muñoz et al. 2006). The firmness was recorded as the maximum penetration force (N) reached during tissue breakage.

### Determination of titratable acidity (TA) and total soluble solid (TSS)

Homogenize 10 g of strawberry puree using a blender with 100 mL of distilled water and centrifuge the mixture at 4000 rpm for 10 min (UNIVERSAL 320R, Andreas Hettich GmbH & Co. KG, Germany). The supernatant was collected for measuring TA and TSS.

The titratable acidity (TA) of strawberry fruit was measured using the pH titration method (Hernández-Muñoz et al., 2006) using 0.1M NaOH until pH 8.1 was reached. pH values were assessed using a pH meter (HI 9126, Hanna Instruments Inc., Romania). The TA was calculated as the following equation (2):

$$\text{TA (g citric acid/100 g FW)} = \frac{\text{Volume of NaOH (mL)} \times 0.1 \text{ M} \times 0.064}{10 \text{ g of sample}} \times 100 \quad (2)$$

A digital refractometer (RX- 5000, Atago Co., LTD., Japan) was used to measure TSS at 25 °C. The results of TSS were expressed as percentage (%).

### Determination of L-ascorbic acid content (AAC)

Following the method of Kapur et al. (2012) with slight modifications, 5 g of strawberry fruit were homogenized with 50 mL metaphosphoric acid – acetic acid solution before centrifugation at 4000 rpm for 15 min (UNIVERSAL 320R, Andreas Hettich GmbH & Co. KG, Germany). Then, mix 4 mL of the supernatant collected with 0.23 mL of 3% bromine water, 0.13 mL of 10% thiourea and 1 mL of 2,4-dinitrophenyl hydrazine and incubate at 37 °C for 3 h before cooling for 30 min and adding 5 mL of chilled 85%  $\text{H}_2\text{SO}_4$ . The absorbance was taken at 521 nm (GENESYS 10 UV-Vis, Thermo Fisher Scientific, Inc., USA). The results were expressed as milligrams of L-ascorbic acid per 100 g of fruit fresh weight basis (mg L-ascorbic/100g FW).

### Determination of total anthocyanin content (TAC)

The total anthocyanin content of fruit was measured using the pH-differential method Giusti and Wrolstad (2001). In detail, 2 g of strawberries was homogenized with 0.025M potassium chloride buffer at pH 1.0 and heated at 50 °C for 3 h. Following a 20-minute centrifugation at 4000 rpm (UNIVERSAL 320R, Andreas Hettich GmbH & Co. KG, Germany), the supernatant was diluted with 0.025 M potassium chloride buffer, pH 1.0, and 0.4 M sodium acetate buffer, pH 4.5 with the appropriate dilution factor and allowed to stand for 15 minutes to equilibrate. The absorbance for each dilution was taken at 496 nm and 700 nm. TAC was expressed as pelargonidin-3-glucoside (pg-3-glu) equivalents and calculated by the equation (3):

$$\text{Total anthocyanin content (mg/L)} = \frac{A \times MW \times DF \times 10^3}{\epsilon \times l} \quad (3)$$

Where

A (absorbance of diluted sample) =  $(A_{496} - A_{700})_{\text{pH } 1.0} - (A_{496} - A_{700})_{\text{pH } 4.5}$

MW (molecular weight) = 433.39 g/mol, for pg-3-glu

$\epsilon$  (molar absorptivity) = 15600, for pg-3-glu (Giusti et al., 1999)

DF (dilution factor)

#### Determination of total phenolic content (TPC)

The total phenolic content of strawberries was determined using the Folin–Ciocalteu assay (Nguyen et al., 2020). Strawberry fruit (1 g) was homogenized with 10 mL of acetone: water (7:3, v/v) and centrifuged at 4000 rpm for 10 min at 4°C. Then, mix 1 mL of the supernatant with 5 mL of 10% Folin–Ciocalteu reagent and 4 mL of 7.5% sodium carbonate. The mixture was stood at room temperature for 60 min before measuring the absorbance at 765 nm (GENESYS 10 UV-Vis, Thermo Fisher Scientific, Inc., USA). The results were expressed as milligrams of gallic acid per 100 g of fruit fresh weight basis (mg GAE/100g FW).

#### Determination of antioxidant capacity (AC)

The antioxidants capacity of strawberries was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging assay Hangun-Balkir and McKenney (2012). Briefly, 2 g of fresh sample was homogenized with 80% ethanol and centrifuged at 4000 rpm for 15 min at 4 °C to collect the supernatant. Then, add 2 mL of 0.1 mM DPPH reagent to 2 mL of the supernatant (equivalent 80% ethanol volume as control) and keeps the mixture at room temperature for 30 min in the dark before measuring the absorbance at 517 nm using a UV-spectrometer. Antioxidant capacity was expressed as the percentage of DPPH radical scavenging capacity (4):

$$\% \text{DPPH scavenging} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100 \quad (4)$$

Where

$A_{\text{control}}$ : The absorbance of control

$A_{\text{sample}}$ : The absorbance of sample

#### Determination of malondialdehyde content (MDA)

According to (Liu et al., 2018), 1g of fresh strawberries was homogenized in 10 mL of ice-cold 0.1% trichloroacetic acid (TCA) before centrifugation at 4000 rpm for 10 min (UNIVERSAL 320R, Andreas Hettich GmbH & Co. KG, Germany). Then, mix 1 mL of the supernatant with 4 mL of 0.25% thiobarbituric acid (TBA) with 10% TCA. After incubation at 95°C for 15 min, the mixture was quickly cooled in an ice bath and centrifuged at 4000 rpm for 10 min. The absorbance of the mixture was taken at 532 and 600 nm. The malondialdehyde content was calculated and expressed as micromoles of MDA per gram of fruit fresh weight basis ( $\mu\text{mol/g FW}$ ) (5):



$$\text{MDA content } (\mu\text{mol/g}) = \frac{(A_{532} - A_{600}) \times \text{DF}}{\varepsilon \times l} \quad (5)$$

Where

$\varepsilon$  (molar extinction coefficient):  $155 \text{ mM}^{-1} \cdot \text{cm}^{-1}$ , for MDA

DF: dilution factor

### Statistical analysis

This study was designed as a one-way factor experiment. Data measured were statistically analyzed by one-way analysis of variance (ANOVA) using Minitab software (version 17) and presented as mean  $\pm$  standard deviation (SD) with  $p < 0.05$  as a significant level.

## RESULTS AND DISCUSSION

### The overall quality index

Fig. 2 illustrates the influences of four different storage temperatures on the overall quality of calcium treated strawberry fruit. It is reported that temperature is one of the most important factors in postharvest handling and storage of fresh fruit due to its useful effects on delaying biological reaction rate as well as microbial growth (Ayala-Zavala et al., 2004). As shown in Fig. 2, lowering temperature prolonged the storage-life of strawberries. To be more specific,  $0^{\circ}\text{C}$  was the most effective storage temperature for calcium treated fruit, which maintained the overall quality of strawberries up to the 21<sup>st</sup> day. Meanwhile, fruit stored at  $25^{\circ}\text{C}$  became spoiled at 3<sup>rd</sup> day while  $2^{\circ}\text{C}$  and  $4^{\circ}\text{C}$  maintained an acceptable quality up to 15 and 18 days, respectively. Nguyen and Nguyen (2021) found that strawberries stored at lower temperatures had the highest overall quality index when compared to strawberries stored at higher temperatures during the same storage periods. Ayala-Zavala et al. (2004) recorded that  $0^{\circ}\text{C}$  effectively suppressed fungal decay of strawberries. This could be the main reason that helps to maintain the high scores of the overall quality index for strawberries during storage. In comparison to results reported by Nguyen and Nguyen (2021) whose recorded an extension of the quality overall index of strawberry stored at  $2^{\circ}\text{C}$  up to 12 days, coating strawberry with 3%  $\text{CaCl}_2$  + 0.2% NCTS and stored at the same temperature in this work effectively prolonged the quality up to 18 days.

### Weight loss

At all studied temperatures, weight loss of the berry fruit increased during storage with the greatest loss was observed at  $25^{\circ}\text{C}$ . Indeed, after 3 days of storage, the weight loss of fruit stored at  $25^{\circ}\text{C}$  significantly ( $p < 0.05$ ) increased up to 12.25%. Interestingly, no remarkable differences ( $p > 0.05$ ) were obtained between three other temperatures at any point of time during the storage period. Fruit weight loss is mainly related to fruit respiration and transpiration rates, and the reaction rate at which water is lost depends on the storage temperature. Proper cold storage might contribute to preventing weight loss of the berry fruit (Hernández-Muñoz et al., 2008). In conclusion,  $0^{\circ}\text{C}$ ,  $2^{\circ}\text{C}$ , and  $4^{\circ}\text{C}$  were recommended for delaying the weight loss of fresh strawberries treated with  $\text{CaCl}_2$  and NCTS.

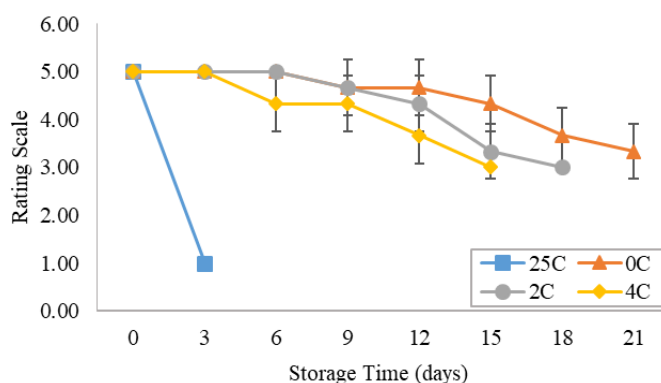


Fig. 2. The overall quality index of strawberry stored at 0°C (▲), 2°C (●), 4°C (◆) and 25°C (■)

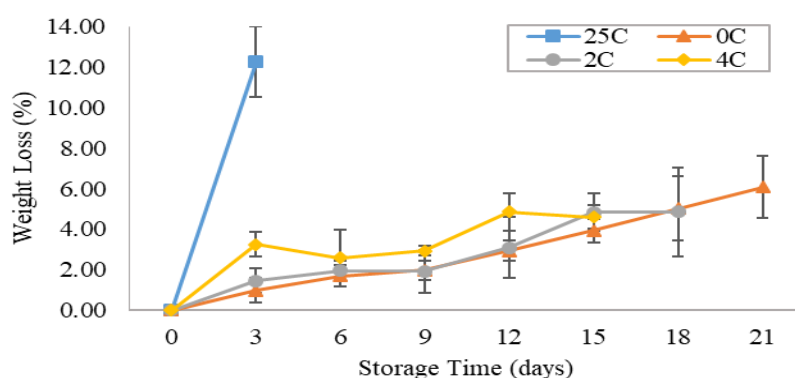


Fig. 3. The weight loss of strawberry stored at 0°C (▲), 2°C (●), 4°C (◆) and 25°C (■)

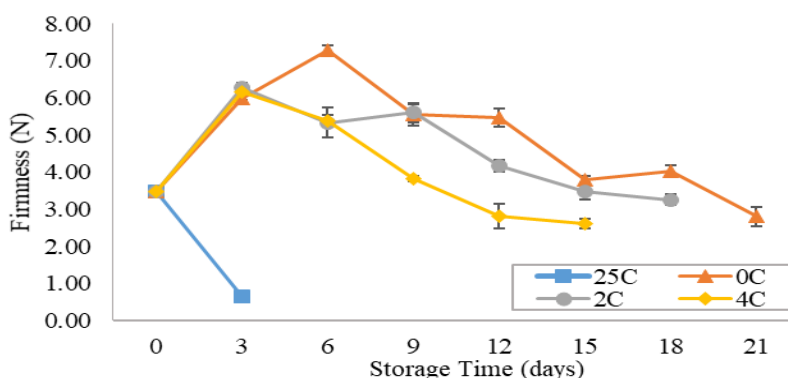


Fig. 4. The firmness of strawberry stored at 0°C (▲), 2°C (●), 4°C (◆) and 25°C (■)

### Firmness

The fruit firmness of strawberries was significantly affected by storage temperature (Fig. 4). Firmness is a crucial parameter that influences the consumer acceptability of fresh produce. It is obvious to see that at 0°C, 2°C, 4°C, there were significant increases in fruit firmness after 6 days of storage. This could be due to water loss from fruit cells during storage, resulting in a higher solute concentration and proportion of fibrous substances, and thus an increase in the force required to break up the fruit flesh (Nguyen et al., 2020).

As illustrated in Fig. 4, strawberries stored at 4°C and 25°C showed significant decreases ( $p < 0.05$ ) in firmness at the end of storage when compared to the initial value and the highest

reduction belonged to the 25°C samples. Strawberries were softened remarkably during storage, inducing short postharvest life and susceptibility to fungal contamination (Sogvar et al., 2016). Furthermore, fruit softening or loss of fruit firmness may be caused by pectin solubilization, which resulted in the degradation of the middle lamella of cortical parenchyma cells (Sogvar et al., 2016). Meanwhile, no significant differences ( $p < 0.05$ ) were observed between the initial and final values of this index for fruit stored at 2°C. Furthermore, 0°C maintained the firmness value for up to 18 days. The results indicated that 0°C and 2°C was the most appropriate temperature for maintaining the firmness of strawberries coated with 3% CaCl<sub>2</sub>+ 0.2 % NCTS.

### Titratable acidity (TA)

From the results presented in Fig. 5, storage temperatures had a considerable effect on the TA content of strawberries. The titratable acidity of the fruit remained unchanged after the 21<sup>st</sup>, 18<sup>th</sup> and 15<sup>th</sup> days of storage at 0°C, 2°C and 4°C, respectively, in comparison to the initial value. Meanwhile, storing strawberries at 25°C significantly ( $p < 0.05$ ) reduced the TA level to 31% after 3 days. There is a fact that during storage, fruit has to use organic acids as substrates for its respiration process, and the respiration rate depends on storage temperatures (Hernández-Muñoz et al., 2008). Therefore, different temperatures induced changes in TA values. It is suggested from this work that the cold storage conditions at 0°C, 2°C, and 4°C were recommended for maintaining TA in strawberries.

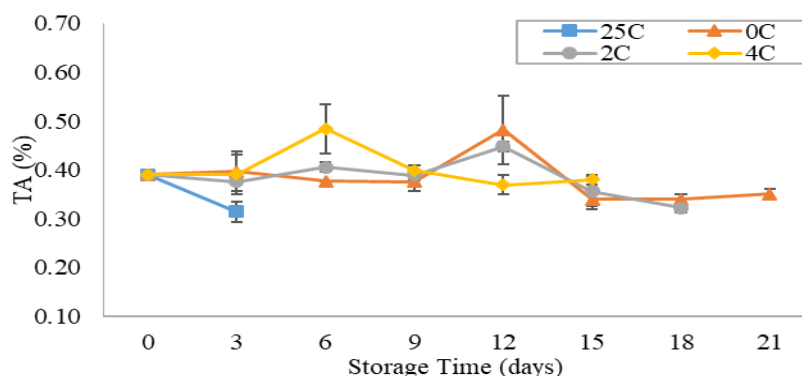


Fig. 5. The titratable acidity of strawberry stored at 0°C (▲), 2°C (●), 4°C (◆) and 25°C (■)

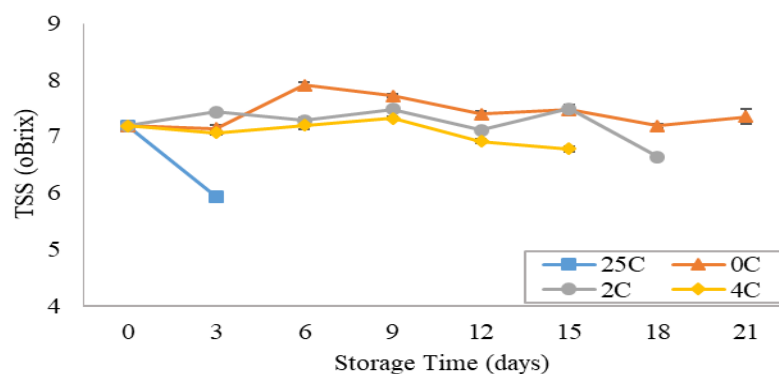


Fig. 6. The total soluble solids of strawberry stored at 0°C (▲), 2°C (●), 4°C (◆) and 25°C (■)



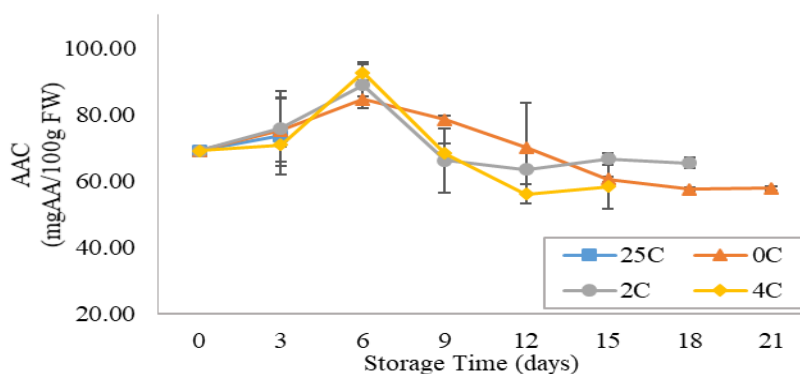


Fig. 7. The L-ascorbic acid of strawberry stored at 0°C (▲), 2°C (●), 4°C (◆) and 25°C (■)

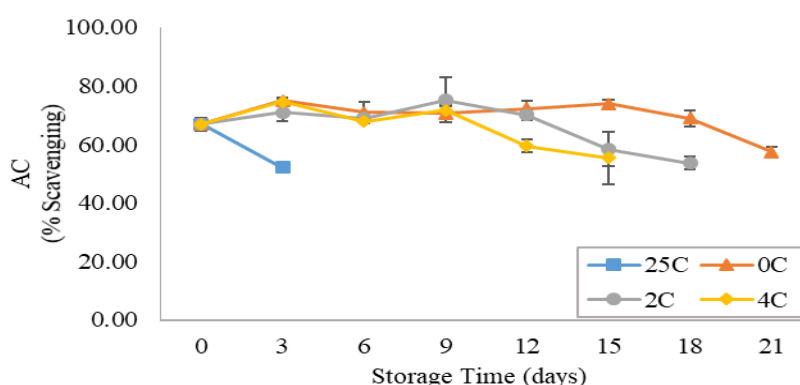


Fig. 8. The antioxidant capacity of strawberry stored at 0°C (▲), 2°C (●), 4°C (◆) and 25°C (■)

### Total soluble solids (TSS)

The effect of different storage temperatures on total soluble solids (TSS) level of strawberry fruit is shown in Fig.6. Among four temperatures studied, 0°C was the most effective one in conserving the TSS value of strawberries after 21 days ( $p>0.05$ ). Meanwhile, there was a significant decrease ( $p<0.05$ ) in TSS of strawberries stored at 25°C after 3 days. Storing strawberries at 2°C and 4°C also reduced TSS content at the end of storage life ( $p<0.05$ ) but to a lesser extent. These results indicated that temperature had a considerable effect on the TSS of strawberries. It was stated that temperature had significant impacts on rates of biological reactions including respiration, which consumes sugar as substrate (Ayala-Zavala et al., 2004). Thus, the higher temperature might lead to the faster depletion of carbohydrates used for respiratory metabolism. The findings are in agreement with those of Ayala-Zavala et al. (2004), who found that cold storage at 0°C maintained the TSS level of strawberries after 13 days.

### L-ascorbic acid content (AAC)

Ascorbic acid (AA) is considered as an important nutritional component of strawberry fruit; thus, it is crucial to maintain AA content during storage at proper temperature. The variation in the L-ascorbic acid content (AAC) as a function of temperatures and storage time for strawberries is presented in Fig. 7. The increasing trend of AAC was observed in the first 6 days of storage for all temperatures before the degradation occurred. The delaying of AAC loss could be achieved by applying 0°C and 2°C which maintained AAC up to 18 and 21 days, respectively. It is claimed that ascorbic acid is synthesized during the storage period due to

activities of enzyme groups involving synthesis, oxidation and recycling (Gomez & Lajolo, 2008) and the synthesis process was affected by lowering the temperature (Cordenunsi et al., 2005). In this study, the obtained results supported the findings of Cordenunsi et al. (2005) and Nguyen and Nguyen (2021), who revealed the positive effect of low temperature on the retention of AAC during storage of fresh produce.

#### **Antioxidant capacity (AC)**

The antioxidant capacity (AC) of strawberries treated with 3 %  $\text{CaCl}_2$  + 0.2 % NCTS was affected by storage temperatures (Fig. 8). The initial AC value was 66.97 % and it declined ( $p < 0.05$ ) after 12 days of storage at low temperatures. The AC of fruit stored at 25°C decreased remarkably to 52.19% after 3 days ( $p < 0.05$ ). The AC of those stored at 0°C, 2°C and 4°C, on the other hand, was preserved until day 18, 15, and 12, respectively. The trend of AC seems similar to that of TPC (Fig. 9). When TPC remained, AC would also be maintained (Nguyen et al., 2020). It was stated that low temperatures can retard respiration, slow down deterioration reactions caused by enzymes and, as a result, preserve more antioxidants (Cordenunsi et al., 2003; Cordenunsi et al., 2005; Yoruk & Marshall, 2003). That could explain why the lower storage temperature, the more effective the antioxidant protection.

#### **Total phenolic content (TPC)**

It can be seen that low temperatures effectively preserved TPC of strawberries coated with  $\text{CaCl}_2$  and NCTS within 12 days (Fig. 9). The TPC of fruit stored at 25 °C was significantly reduced by about 11.9% after 3 days only. Although 0°C was the most effective temperature in preserving the overall quality index of strawberries there was approximately 13.6% and 20.8% of TPC reduction on the 15<sup>th</sup> day and the 18<sup>th</sup> day, respectively, in comparison with the initial value (Fig. 9). This phenomenon supported a previous study of Ayala-Zavala et al. (2004) which recorded that storing strawberries at 0°C was not effective in enhancing the TPC of the fruit as compared to 5 °C and 10 °C. Nguyen and Nguyen (2021) reported that decreasing TPC could be due to the ripening process, releasing increasing polyphenol oxidase enzymes (PPO) that degrade mono- and diphenolic compounds in the fruit. In addition, it was stated that low storage temperature could damage cell walls of the fruit, releasing more PPO in the damaged areas, therefore stimulating phenolic degradation (Valenzuela et al., 2017; Yoruk & Marshall, 2003). Furthermore, the decline in TPC could be due to cell structure degradation during fruit senescence stage (Gol et al., 2013). Besides, the increasing of TPC of strawberries stored at low temperatures from the initial day to the 6<sup>th</sup> day could be due to phenolic production via phenylpropanoid metabolism (Singh et al., 2010).

#### **Total anthocyanin content (TAC)**

Anthocyanins are pigments responsible for the development of red color in strawberry fruit (Gol et al., 2013). As presented in Figure 10, total anthocyanin content (TAC) was affected by storage temperature. TAC increased remarkably ( $p < 0.05$ ) from 23.16 to 37.66 mg/100gFW within 3 days at 25°C (Fig. 10). At the same time, those of fruit stored at 0°C, 2°C, and 4°C remained unchanged after 12 days of storage ( $p > 0.05$ ). Several studies have revealed that higher storage temperatures might accelerate the ripening and anthocyanins accumulation process (Ayala-Zavala et al., 2004; Cordenunsi et al., 2003; Cordenunsi et al., 2005; Kalt et al., 1999). In addition, no significant differences ( $p > 0.05$ ) were observed between samples stored at 0 °C, 2 °C, and 4 °C on the 15<sup>th</sup> and 18<sup>th</sup> days. Therefore, suitable temperatures for preserving TAC of 3%  $\text{CaCl}_2$ +NTCS strawberry fruit were 0°C, 2°C, and 4°C.

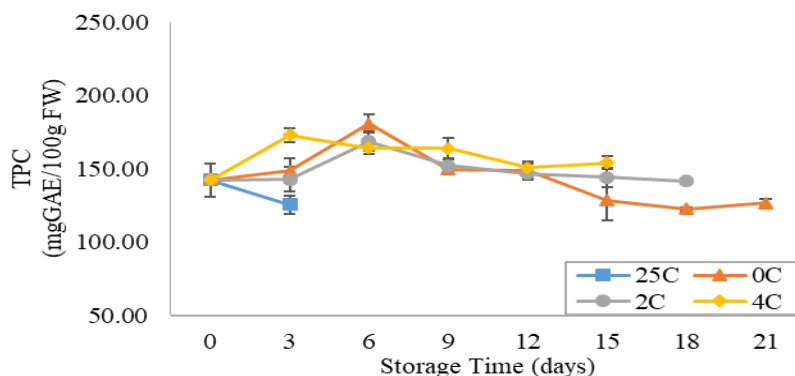


Fig. 9. The total phenolic content of strawberry stored at 0°C (▲), 2°C (●), 4°C (◆) and 25°C (■)

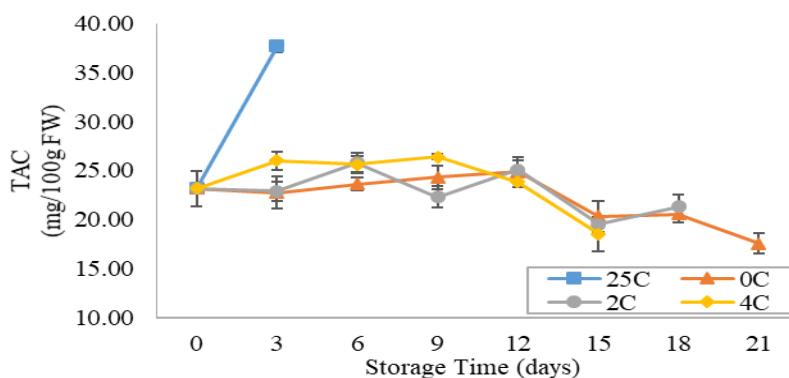


Fig. 10. The total anthocyanin content of strawberry stored at 0°C (▲), 2°C (●), 4°C (◆) and 25°C (■)

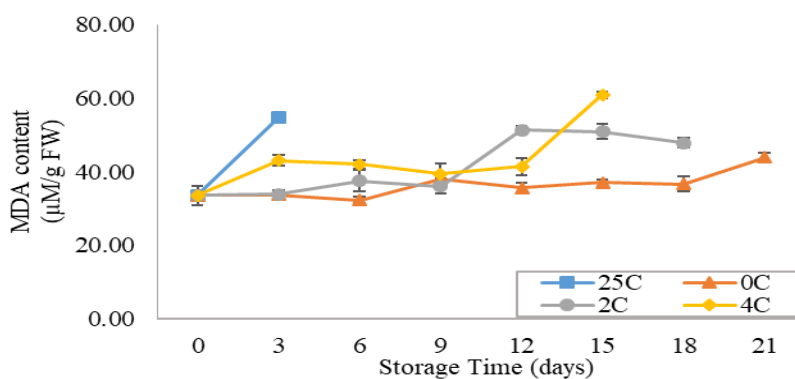


Fig. 11. The malondialdehyde content of strawberry stored at 0°C (▲), 2°C (●), 4°C (◆) and 25°C (■)

### Malondialdehyde content (MDA) at different storage temperatures

At each storage temperature, there was a significant increase ( $p < 0.05$ ) in MDA content between the initial day and the last day of storage (Fig. 11). However, among the temperatures tested, 0°C proved to be the most effective in delaying MDA production. Indeed, the fruit stored at 0°C had the lowest MDA level compared with those at the other three temperatures. It was reported that the higher temperature promoted faster the accumulation of MDA (Nguyen & Nguyen, 2021). In addition, the degradation of cell membrane and changes

in the intensity of oxidative processes were influenced by high temperature storage (Savicka & Škute, 2010).

## CONCLUSION

It was suggested from the current work that 0°C should be considered as the appropriate temperature to prolong the shelf-life of calcium and NCTS treated strawberries. Besides, cold storage at 0°C and 2°C is recommended for reducing weight loss, delaying fruit softening, maintaining chemical characteristics including AAC, AC, and TAC. At the same time, fruit stored at 25 °C quickly deteriorated after 3 days.

## Conflict of interest

The authors have no conflict of interest to report.

## Acknowledgement

The authors would like to thank the infrastructure support of International University-Vietnam National University Ho Chi Minh city, Vietnam.

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