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Effects of storage temperature on postharvest physico-chemical attributes of nano-chitosan coated strawberry (*Fragaria × ananassa* Duch.)

Duyen Huu Huong Nguyen[1,2], Ha Vu Hong Nguyen[1,2*]

1, *Department of Food Technology, School of Biotechnology, International University*
2, *Vietnam National University, Ho Chi Minh city*

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***Corresponding author:**
Department of Food Technology, School of Biotechnology, International University; *Vietnam National University, Ho Chi Minh city*.
Email: nvhha@hcmiu.edu.vn

ABSTRACT

Purpose: Recently, there are researches showed positive effects of nano-chitosan in prolonging the postharvest quality and shelf life of strawberry, however, influences of storage temperatures on the nano-chitosan coated fruit have been overlooked. Therefore, in this work, changes of physiological traits of strawberry (*Fragaria × ananassa* Duch.) coated with 0.2% nano-chitosan and stored at different temperatures were studied. **Research Method:** Strawberry was coated with 0.2% nano-chitosan and stored at different temperatures (2°C, 5°C, 10°C and 25°C) for 12 days. The effects of temperatures on the coated fruits were tested by measuring visual quality, weight loss, antioxidant properties, malondialdehyde content, firmness, total soluble solid, polyphenol oxidase activity in three days intervals. **Findings:** After storing 0.2% nano-chitosan coated strawberry at four different temperatures, 2°C showed the most effective one as maintaining the overall quality of strawberry higher than the acceptable/marketable level after 12 days; meanwhile, fruits stored at 25°C were quickly decayed after 3 days. The treatments at low temperatures (2°C, and 5°C) significantly reduced weight loss, maintained firmness, total soluble solid, polyphenol oxidase activity and malondialdehyde content of the stored fruits. **Limitations**: Nano-chitosan has not been widely traded. **Originality/Value:** Coating strawberry with nano-chitosan and storing at 2°C effectively maintained the postharvest quality of strawberry as well. This treatment is quite simple and would be useful for stakeholders in the strawberry supply chain.

INTRODUCTION

Strawberry (*Fragaria* × *ananassa* Duch.) is high perishable non-climacteric fruit. Besides, its attractive color and flavor, strawberry is also containing a great variety of bioactive compounds including antioxidants, anthocyanins, vitamins, and minerals. However, it has short shelf life and high sensitivity to storage conditions (Hernández-Muñoz et al., 2008). In uncontrolled conditions the fruit is easy to be decayed by microorganism such as mold or bacteria.

Temperature is one of the critical parameters monitoring fruit and vegetable storage quality. Storage life of fresh strawberry may be within a day at room temperature (Mercantila, 1989) or 5-10 days at 0°C (SeaLand, 1991) or less than 5 days at 4°C (Han et al., 2004).

Chitosan, the N-deacetylated derivative of chitin, mainly found in shrimp skeleton, have potential in controlling plant diseases (Kumar, 2000) including activities against infection caused by bacteria, mold, and other pathogens (Goy et al., 2009). Recently, it has been found that nano-chitosan provided positive effects in prolonging the quality and shelf life of strawberry as compared to chitosan (Nguyen & Nguyen, 2020). Nano-based materials are found owning the properties of enhancing the barrier such as strength, stiffness or heat resistance (Silvestre et al., 2011). In addition, nanoparticles of chitosan have shown the greater antifungal ability defensing the varieties of microorganism than chitosan particles (Goy et al., 2009). Besides, nano-chitosan based coating has higher barrier properties to the internal gas atmosphere and more optimize in permeability of coating on fruits (Gardesh et al., 2016; Lorevice et al., 2012; Nguyen & Nguyen, 2020; Nguyen et al., 2020).

Nowadays, there are several postharvest approaches studied to prolong the shelf life of strawberry such as cold storage (Martínez et al., 2018), X-ray irradiation (Yoon et al., 2020), ozon treatment (Nayak et al., 2020), atmosphere cold plasma treatment (Rana et al., 2020) and edible coating (Jiang et al., 2020; Nguyen & Nguyen, 2020; Nguyen et al., 2020), however, studying about storage of the coated berry at different temperatures seems to be lacking in literature.

The main purpose of this research was to investigate effects of storage temperatures on the postharvest quality of nano-chitosan coated strawberry. The results from this current work would be a useful database for further researches involving edible coating in the post-harvesting technology for fruits, particularly strawberry.

MATERIALS AND METHODS

Sample preparation

Strawberry (*Fragaria* × *ananassa* Duch.) that reached the ready-to-eat stage was harvested from the orchard in Lamdong province, Vietnam. Damaged fruits were discarded. The chosen fruits must have over 75% of red color as well as the absence of mechanical damaged. The fruits were rinsed in tap water to remove contaminants before treatments.

Edible coating formulation

The 0.2% nano-chitosan solution was supplied by Dalat Nuclear Research Institute (NRI) with the particle size was at 250nm.

Experimental design

The experimental design was followed Nguyen and Nguyen (2020) with some modifications. At first, strawberries were dipped into 0.2% nano-chitosan solutions for 1 min and then drained on stainless steel racks for 30 mins (Han et al., 2005). Then, the coated fruit were

stored at four different temperatures (2, 5, 10, and 25°C) for 12 days. The physico-chemical parameters of coated samples were tested after each 3-day intervals (Brat et al., 2007).

Measurement of the overall quality index

The overall quality index was a critical parameter for evaluating the storage life and acceptable level of strawberry. Visual quality scores of samples evaluated using 1-5 rating scale (Table *1*). The overall appearance of strawberry was based on the description of color, firmness, weight loss, and shriveling rate in rating scale from 1 to 5 where; 1= very poor; 2= poor; 3=acceptable for marketability; 4=good; 5= excellent (Nguyen & Nguyen, 2020). When one of attribute equaled 3 or lower, the treatments were stopped.

Measurement of weight loss (%)

The weight loss of strawberry was measured using a top loading balance (TXB- 622L, Shimadzu Co, LTD., Japan). The weight loss was the differences between the initial weight and the weight recorded after each storage intervals (Hernández-Muñoz et al., 2008).
The percentage of weight loss was calculated (1):

$$\text{Weight loss (\%)} = \frac{m1-m2}{m1} \text{x } 100 \quad (1)$$

Where m_1 weight of sample before storage (g), and m_2 weight of sample after storage intervals (g).

Measurement of firmness (N)

According to Hernández-Muñoz et al. (2008), the firmness of strawberry was measured by texture analysis using a Digital Fruit Hardness Tester (FR- 5120, Lutron electronic enterprise Co., LTD., Taiwan). The maximum penetration force (N) was assessed during tissue breakage as the measurement value of firmness. The depth was 2 mm and the cross-head speed was 2 $mm.s^{-1}$.

Determination of total soluble solid (%) and titratable acidity (%)

The total soluble solid content of strawberry was measured using the digital refractometer (RX- 5000, Atago Co., LTD., Japan) (Hernández-Muñoz et al., 2008). The results of TSS were expressed as a percentage.

According to AOAC (1990), sample was prepared by mixing 5g puree of strawberry with 50 mL distilled water. Titratable acidity (TA) of samples was assessed using the titrate method with 0.1M NaOH. The endpoint reading (pH 8.1) during titration was monitored by pH meter (HI 9126, Hanna Instruments Inc., Romania). The results of TA test were expressed as grams of citric acid per 100 g fresh strawberry and calculated using the follow formula (2):

$$\text{\% Titratable Acidity (TA)} = \frac{\text{Volume of NaOH (ml)} \times 0.1 \text{ M} \times 0.064}{\text{10 g of sample}} \times 100 \quad (2)$$

Measurement of antioxidant capacity by DPPH method (%)

Antioxidant capacity (Ac) of strawberry was determined following the methods of Hangun-Balkir and McKenney (2012) using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) (Sigma-Aldrich Pte. Ltd., St. Louis, MO, USA). The absorbance of control sample and sample were measured at 517 nm, using (GENESYS 10 UV-Vis, Thermo Fisher Scientific, Inc., USA). The results of Ac were expressed as the percentage of DPPH radical scavenging capacity using the following formula (3):

Table 1. Visual quality scores and descriptors for strawberry (Nguyen & Nguyen, 2020)

	Scores and description		
	Decay percentages	Shriveling	Photographs at different stages of visual
5 Excellent	0%	Calyx is stiff and green. No sign of shriveling. Field-fresh.	
4 Good	0%	Calyx is green but slightly less stiff than at harvest. Minor signs of shriveling.	
3 Acceptable	0%	Calyx may appear dry and wilted.	
2 Poor	1- 5%	Fruit is started to dry and calyx is obviously shriveled.	
1 Very poor	over 10%	Calyx may appear very dry and yellowish or brownish-green.	

$$\% \text{ DPPH scavening} = \frac{(A_{control} - A_{sample})}{A_{control}} \text{ x } 100 \quad (3)$$

Where $A_{control}$ the absorbance of control, A_{sample} the absorbance of sample.

Determination of L-Ascorbic acid (mg.100g^{-1})

According to Kapur et al. (2012), sample was prepared by homogenizing with metaphosphoric acid - acetic acid solution. This mixture was centrifuged at 4000 x g in 20 min at 25°C using centrifugation (UNIVERSAL 320R, Andreas Hettich GmbH & Co. KG, Germany). Then, 4 ml supernatant was mixed with 0.23 mL of 3% bromine water, 0.13 mL of 10% thiourea (Sigma-Aldrich Pte. Ltd., St. Louis, MO, USA), 1 ml of 2,4-dinitrophenyl hydrazine (Sigma-Aldrich Pte. Ltd., St. Louis, MO, USA) and incubated 37°C for 3hrs. The absorbance of samples was measured at 512 nm, using UV-VIS spectrophotometer (GENESYS 10 UV-Vis, Thermo Fisher Scientific, Inc., USA) after adding 5mL of chilled 85% H_2SO_4.

Determination of total anthocyanin content (mg.100g^{-1})

Pelargonidin-3-glucoside was the most predominant anthocyanin presented in strawberry. The total anthocyanin content (TAC) of strawberry was measured as the pH differential method using a UV-VIS spectrophotometer (GENESYS 10 UV-Vis, Thermo Fisher Scientific, Inc., USA) (Cordenunsi et al., 2003). Briefly, 5g of sample was extracted with 0.025M potassium chloride buffer, pH 1.0 at 50°C in 3h and then the mixture was centrifuged at 5000 rpm for 20 min at 4°C using centrifugation (UNIVERSAL 320R, Andreas Hettich

GmbH & Co. KG, Germany). The sample solution was diluted follow the dilution factor, using two buffers: 0.025M potassium chloride buffer (pH 1.0) and 0.4M sodium acetate buffer (pH 4.5) (Yang et al., 2010). Each diluted solution had recorded the absorbance at 496 nm and 700 nm.

Total anthocyanin content was calculated following the equation (4) (Giusti & Wrolstad, 2001):

$$\text{Total anthocyanin content } \left(\frac{\text{mg}}{\text{L}}\right) = \frac{\text{A}\times\text{MW}\times\text{DF}\times 1000}{\varepsilon\times 1} \quad (4)$$

Where A the absorbance of diluted sample, A= $(A_{496} - A_{700})_{\text{pH 1.0}}$ - $(A_{496} - A_{700})_{\text{pH 4.5}}$, MW the molecular weight ,DF the dilution factor, and ε the molar absorptivity of 15600 (Giusti et al., 1999).

Determination of total phenolic content (mg.100g^{-1})

Total phenolic content (TPC) in strawberry was determined using Folin- Ciocalteu assay (ISO:14502, 2005). The samples were extracted by using 70% acetone (Merck Chemicals Ltd., Darmstadt, Germany). The absorbance of sample was recorded at 765nm, using the UV-VIS spectrophotometer (GENESYS 10 UV-Vis, Thermo Fisher Scientific, Inc., USA). In addition, gallic acid used as a standard substance. The results were expressed as mg Gallic acid equivalent per 100g fresh weight (mg.100g^{-1}).

Determination of Malondialdehyde (µmol.g^{-1})

According to Yang et al. (2010), malondialdehyde (MDA) content of fruit was determined basing on the 2- thiobarbituric acid (Merck Chemicals Ltd., Darmstadt, Germany) (TBA) reaction. Briefly, the sample was homogenized with of ice-cold 0.1% trichloroacetic acid (Sigma-Aldrich Pte. Ltd., St. Louis, MO, USA) (TCA). The supernatant collected from centrifugation of the mixture at 5000 x g for 10 min at 4°C was thoroughly mixed with 10% TCA containing 0.25% TBA and incubated at 95°C in 3 min. The absorbance of the sample was recorded at 532 nm and 600 nm, using UV-VIS spectrophotometer (GENESYS 10 UV-Vis, Thermo Fisher Scientific, Inc., USA). The concentration of lipid peroxides together with oxidatively modified proteins of fruit were thus quantified in terms of MDA level using an extinction coefficient of 155 $mM^{-1}\cdot cm^{-1}$ and expressed as µmol.g^{-1}.

Polyphenol oxidase assay (U.g^{-1})

Polyphenol oxidase (PPO) extraction was based on a described method of Holzwarth et al. (2012) described with some modifications. Samples were prepared by stirring strawberries continuously with cold acetone (−20°C) for 5 min. The mixture was filtered using filter paper Whatman No.2 (GE- Healthcare, Chicago, Illinois, USA). Then, the sample was mixed with 40 mmol.l^{-1} catechol (Sigma-Aldrich Pte. Ltd., St. Louis, MO, USA) and 0.1 mol.l^{-1} phosphate buffer (pH 6.5) (Merck Chemicals Ltd., Darmstadt, Germany). The absorbance of PPO was measured at 420nm, using UV-VIS spectrophotometer (GENESYS 10 UV-Vis, Thermo Fisher Scientific, Inc., USA). The reaction time for PPO was 2 mins, and the activity was expressed in units with one unit = 0.001*ΔA_{420}/min/g fresh weight (FW) (Zhang & Xingfeng, 2015).

Statistical analysis

All statistical analyses were performed using the Minitab statistical software (Version 18.0, Minitab Pty Ltd., Australia). The statistics data were analyzed by comparing the means of different levels of single factors using one-way ANOVA. The statistical results were expressed as means ± S.D with $p < 0.05$ as significant differences.

RESULTS AND DISCUSSION

The overall quality index

An overall rating table (Table 1) was built to illustrate different deteriorative stages of strawberry with corresponding scores and descriptions of the overall quality index based on the fruit visual appearance and freshness (Nunes & Cecilia, 2015).

The overall quality index of strawberry had mentioned as one of critical features to evaluate the quality, especially the assessment of customer (Hardenburg et al., 1986). According to the statistics in Fig. 1A, the higher storage temperature caused the higher loss of the overall quality. Indeed, the overall quality index of strawberry at 25°C decreased below the acceptable level rapidly after 2nd day. Meanwhile, at 5°C and 2°C, the quality of berries maintained over 9 days and 12 days, respectively. It had been proven in a research of Nguyen and Nguyen (2020), nano-chitosan coating extended the storage life of strawberry until 21 days and enhanced the physico-chemical quality as weight loss, firmness or titratable acidity. Li and Kader (1989) reported that temperature had direct effects on respiration rate and biological reactions. The higher temperature, the higher respiration rate. This leads to increasing water loss, reducing turgidity and consequently causing shriveling and faster depletion of nutrients (Ayala-Zavala et al., 2004). Besides, lower storage temperatures positively affected the fruit quality and decreased the fungal decay incidence (Ayala-Zavala et al., 2004). It could be suggested from the present study that 2°C should be considered as the suitable temperature for maintaining the overall quality of nano-chitosan coated strawberry.

Weight loss (%)

All fruits had gradually increased weight losses throughout the storage time (Fig. 1B). The weight loss was roughly 4% when fruits stored at 25°C after 3 days. This value was significantly ($p<0.05$) higher than those of the samples stored at the lower temperatures (Fig. 1B). The weight loss of sample at 2°C at 12nd day was approximately 2.5% showing the positive impact in lower the reduction while those at 10°C had lost the same value after 6 days of storage (Fig. 1B). However, strawberry stored at 2°C and 5°C showed the insignificant difference ($p>0.05$) for 9 days. As surrounding temperature strongly impact the rate of respiration, the weight loss would much depend on the storage temperature (Hernández-Muñoz et al., 2008). It is clearly that a higher temperature caused a higher percentage of weight loss (Nunes et al., 1998). Furthermore, prolonging storage time caused the increment of weight loss. The gradually increase of this value by time was showed in samples stored at all temperatures (Fig. 1B**)**. Nano-chitosan coating was proven to prevent the dehydration on the surface of strawberry by working as physical barriers (Nguyen & Nguyen, 2020), therefore, consequently reducing weight loss. In this present study, cold storage temperature from 2°C to 5°C is recommended for preventing weight loss of 0.2% nano-chitosan coated strawberry.

Firmness (N)

It could be seen from Fig. 1C, reduction of firmness in fruit occurred faster when fruit were stored at higher temperatures. Indeed, the firmness of strawberry stored at 25°C reduced remarkably, from 3.0 N to 0.73 N after 3 days, meanwhile at 2°C and 5°C, the firmness was insignificantly changed after 9 dyads and 12 days, respectively. It was reported that strawberry was softened during storage due to the activities of pectin methylesterase hydrolyzing the middle lamella of the cell walls (Perkins-Veazie, 2010). According to Hernández-Muñoz et al. (2008), cold storage help to suppress or delay the enzyme activities, therefore effectively preventing fruit softening. However, there were slight increases of firmness found in fruit stored at 2°C and 5°C for 9 days and 6 days, respectively. This could

be related to increasing pectin viscosity (gelling behavior) in structure of cell wall during storage (Shin et al., 2007). The solubilization of pectin under the activities of β-galactosidase and pectin methylesterase during fruit ripening changed the bonding between some polymers was directly related to fruit softening and fruit firmness (Brummell, 2006; Brummell & Harpster, 2001; Goulao et al., 2012).

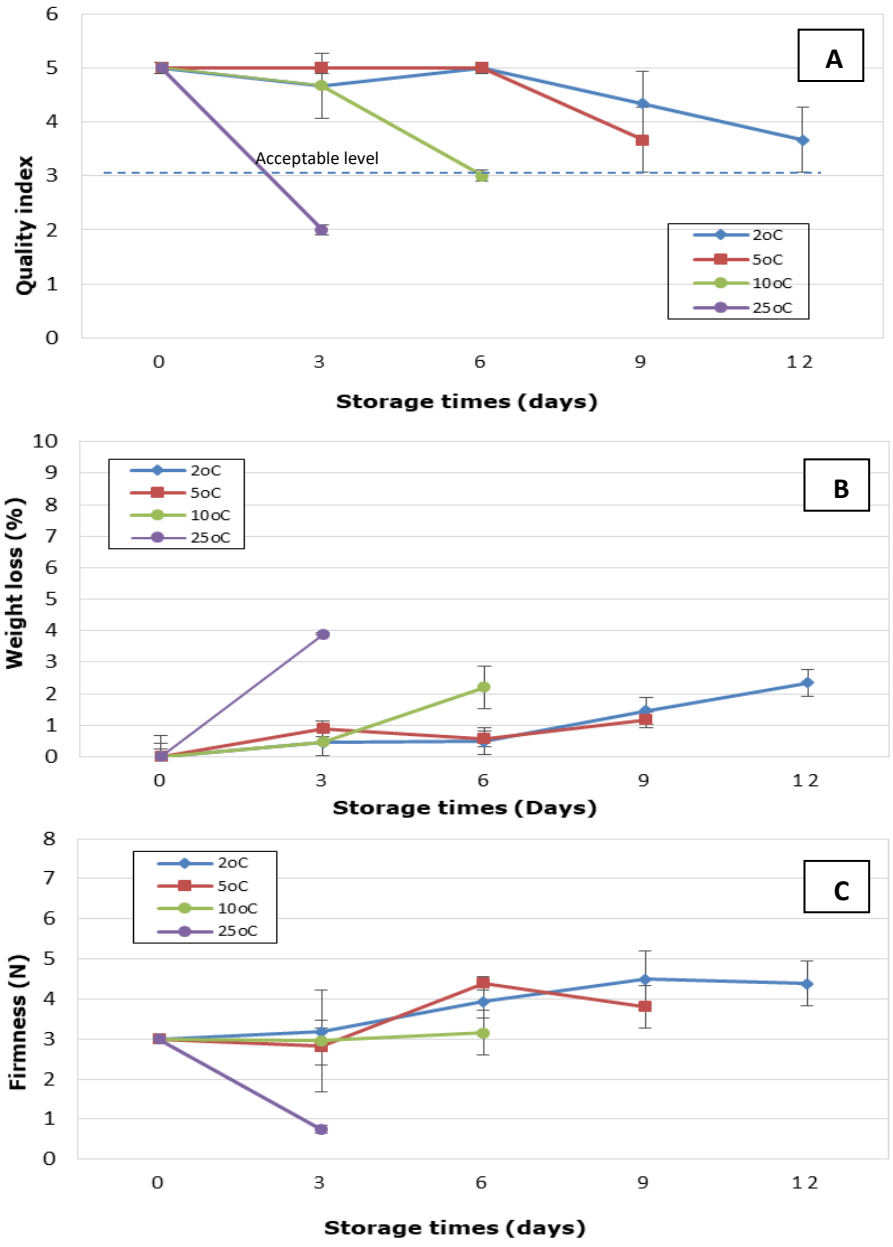

Fig. 1. Effects of storage temperature combined with 0.2% nano-chitosan on overall quality index (A), weight loss (B) and firmness (C) of strawberry stored at 2°C (-♦-), 5°C (-■-), 10°C (-•-) and 25°C (-•-). The error bars represent standard deviations of triplicate assays with the confidence interval of 95 %.

Total soluble solids (TSS), titratable acidity (TA) and L-Ascorbic acid

Total soluble solids (TSS) of strawberry stored at different temperatures were shown in Table 2. TSS value of strawberry stored at 2°C, 5°C, and 10°C were remained at the initial value ($p < 0.05$) after 12 days, 9 days and 6 days, respectively (Table 2). However, at 25°C, total soluble solid content rapidly decreased to 4.2 % at the 3rd day when fruits start to be rotten. In general, the temperature was an important factor affecting TSS of fruit during storage. Ayala-Zavala et al. (2004) found that the carbohydrate of strawberry was conserved when stored fruit at cold temperature. The decrease of TSS value could be explained by sucrose hydrolysis and the utilization of the reducing sugars during fruit respiration (Yang et al., 2010). The higher temperature and respiration rate, the higher loss of TSS used for metabolic activity (Ayala-Zavala et al., 2004; Cordenunsi et al., 2003). According to Nguyen and Nguyen (2020), strawberries treated with nano-chitosan did not showed significant differences in TA and TSS compared to the untreated one.

As obtained for titratable acidity (TA) from Table 2, at different temperatures, expect 25°C, the storage duration significantly affected to the amounts of TA in fruit. They increased from the initial value and then decreased before deteriorated. Storing strawberry at 2°C again showed to be the most effective temperature in maintaining the TA of the fruits. Indeed, after 9 days, the value remained twice as higher as the initial amounts. It has been reported that changing of titratable acidity during storage was related to the attainment of maturity and ripening of strawberry (Maftoonazad et al., 2008; Yang et al., 2010) or mango (Islam et al., 2013).

At all temperatures, the highest amounts of L-AA in strawberry were observed at the first 3 days (Table 2). However, the highest L-AA levels could not be achieved by lowering the temperature. Indeed, although having a short shelf life for only 3 days, the fruits stored at 10°C and 25^0C contained the highest amounts of L-AA as compared to those preserved at 2°C and 5°C at the same storage interval. Storing the fruits at 5°C showed the efficiency in preventing the loss of L-AA after 9 days (Table 2). Meanwhile, lowering stored temperature to 2°C significantly reduced ($p<0.05$) the L-AA value after 9 days compared to the 5°C. The changes of L-AA might due to the synthesis from D-galacturonic acid released from cell wall pectin (Nguyen et al., 2020). Pectin is a crucial cell wall component of building blocks whose release D-galacturonic acid upon enzymatic hydrolysis process during fruit ripening (Liu et al., 2018). Therefore, significant higher contents of L-AA in strawberries stored at 10°C and 25°C (Table 2**)** could be relevant to significant lower firmness of fruit stored at those temperatures as compared to those stored at 2°C and 5°C (Fig. 1C). This observation supported for a reported study of Sahari et al. (2004). The maintenance of ascorbic acid content in fruit and vegetables directly related to temperature management of fruit after harvest, cultivars and storage durations (Kalt et al., 1999; Lee & Kader, 2000).

Total phenolic content (TPC), total anthocyanin content (TAC) and malondialdehyde (MDA)

During storage at different temperatures, TPC of the fruit reduced gradually (Table 3), but no significant changes ($p>0.05$) were observed between temperatures at the same storage days, except 10°C at 3rd day. However, there were significant decreases ($p<0.05$) of TPC were detected the same temperatures at different storage duration. Although 2°C was the most effective temperature in maintaining the overall quality of strawberry, there was almost 60% of TPC reduction observed after 12 days. The decrease of TPC occurred as the normal ripening processes of non-damaged fruit changing the cell membrane permeability in phenolic metabolism. Furthermore, the decrease of TPC in strawberry likely reflected the rising of PPO activity (Petriccione et al., 2015). Low storage temperature damaged the cell wall of strawberry and released PPO inside (Valenzuela et al., 2017). According to Nunes et al.

(2005), the more PPO contained, the more enzymatic browning reaction occurred leading the oxidation of phenolic compounds. TPC of strawberry stored at 25°C was the highest value in comparison with other temperatures at the 3rd day (Table 3). Similarly, this phenomenon was recorded as strawberry stored at 20°C in a study of Shin et al. (2007). It could be concluded that the total phenolic contents in strawberry were significantly affected by the storage temperature and storage time. Nunes et al. (2005) stated that the decrease in TPC might be partly a consequence of the degradation of anthocyanin content. The same observation was recorded in this study (Table 3).

The anthocyanin was the major component in strawberry that contributed to the red color of fruit (Timberlake & Bridle, 1982). There was the significant decrease ($p<0.05$) in the anthocyanin concentration of strawberry stored at all storage temperature (Table 3). The decrease of anthocyanin concentration was also recorded when 'Oso Grande' and 'Mazi' cultivar stored at 6°C (Cordenunsi et al., 2003) or strawberry handled at 7.5°C during 12 days (Ayala-Zavala et al., 2005). It has been reported that TAC changes during fruit storage depend on the initial amount relating to cultivar (Cordenunsi et al., 2003) and the oxidation of anthocyanin (Ivanova et al., 2012). At the end of storage time, fruit stored at 2°C showed the significant effects ($p<0.05$) in delaying the degradation of TAC compared to 5°C at 9th day. It is obviously that temperature and storage time significantly affected TAC of strawberry.

The level of lipid peroxides in the progress of fruit ripening was measured through MDA, a secondary end product of oxidative lipid degradation (Hodges et al., 1999). For the preservation of fruit and vegetables, ROS accumulation was able to cause oxidative damage to membranes, lipids, proteins and nucleic acids, forming toxic products such as MDA (Yang et al., 2010). Following the data in Table 3, the significant increases of MDA ($p<0.05$) were observed in fruit stored at 2°C and 5°C. The higher temperature might promote faster the production of MDA. Srivalli et al. (2003) reported that heat stress led to a loss of cellular homeostasis accompanied by the formation of ROS. The high-temperature stress also interfered with the degradation of tissues and changed the intensity of oxidative processes (increased the ROS level) (Savicka & Škute, 2010).

Table 2. Effects of storage temperature combined with 0.2% nano-chitosan on total soluble solid (a), titratable acidity (b), and L-ascorbic acid (c) of strawberry.

	Initial	Day 3	Day 6	Day 9	Day 12
Total soluble solid (°Brix)					
2°C	6.3 ± 0.5^{aA}	7.3 ± 0.7^{aBC}	6.6 ± 0.2^{aAB}	8.2 ± 0.5^{aC}	7.2 ± 0.9^{B}
5°C	6.3 ± 0.5^{aA}	7.2 ± 0.3^{aB}	6.2 ± 0.1^{abA}	6.8 ± 0.4^{bAB}	ES
10°C	6.3 ± 0.5^{aA}	6.7 ± 0.6^{aA}	5.5 ± 0.6^{bB}	ES	ES
25°C	6.3 ± 0.5^{aA}	4.2 ± 0.1^{bB}	ES	ES	ES
Titratable acidity (%)					
2°C	0.2 ± 0.0^{aA}	0.3 ± 0.1^{bB}	0.4 ± 0.0^{aC}	0.4 ± 0.0^{aC}	0.1 ± 0.0^{D}
5°C	0.2 ± 0.0^{aA}	0.4 ± 0.0^{bC}	0.4 ± 0.1^{aC}	0.3 ± 0.0^{aB}	ES
10°C	0.2 ± 0.0^{aA}	0.6 ± 0.1^{aB}	0.3 ± 0.1^{aA}	ES	ES
25°C	0.2 ± 0.0^{aA}	0.1 ± 0.0^{cB}	ES	ES	ES
L-Ascorbic acid ($mg.100g^{-1}$)					
2°C	34.8 ± 0.8^{aA}	40.4 ± 1.4^{bB}	19.9 ± 2.5^{aD}	26.2 ± 2.1^{bC}	23.7 ± 1.3^{C}
5°C	34.8 ± 0.8^{aA}	41.2 ± 0.7^{bB}	23.3 ± 4.7^{aC}	37.9 ± 0.8^{aAB}	ES
10°C	34.8 ± 0.8^{aA}	50.7 ± 1.3^{aB}	26.0 ± 3.3^{aC}	ES	ES
25°C	34.8 ± 0.8^{aA}	53.1 ± 6.8^{aB}	ES	ES	ES

Data are expressed as mean ± S.D, ES: end of shelf life. Values with different letters (a-e) within column, (A-E) with row are significantly different ($p<0.05$).

Table 3. Effects of storage temperature combined with 0.2% nano-chitosan on total phenolic content (a), total anthocyanin content (b), malondialdehyde (c), antioxidant capacity (d), and polyphenol oxidase activity (e) of strawberry.

	Initial	Day 3	Day 6	Day 9	Day 12
Total phenolic content (mg.100g^{-1})					
2°C	252.1 ± 0.3^{aA}	184.8 ± 23.8^{bAB}	175.9 ± 7.7^{aB}	174.4 ± 52.1^{aBC}	101.8 ± 27.8^{C}
5°C	252.1 ± 0.3^{aA}	201.4 ± 11.8^{abAB}	165.6 ± 34.3^{aB}	141.4 ± 33.6^{aB}	ES
10°C	252.1 ± 0.3^{aA}	147.3 ± 18.1^{cB}	168.3 ± 56.5^{aAB}	ES	ES
25°C	252.1 ± 0.3^{aA}	236.3 ± 21.0^{aA}	ES	ES	ES
Total anthocyanin content (mg.100g^{-1})					
2°C	16.7 ± 2.2^{aA}	6.9 ± 2.2^{bC}	9.1 ± 1.2^{aBC}	11.2 ± 0.8^{aB}	6.5 ± 0.8^{C}
5°C	16.7 ± 2.2^{aA}	9.6 ± 0.2^{abB}	9.6 ± 2.5^{aB}	8.4± 1.2^{bB}	ES
10°C	16.7 ± 2.2^{aA}	12.5 ± 2.5^{aAB}	10.1 ± 3.9^{aB}	ES	ES
25°C	16.7 ± 2.2^{aA}	12.8 ± 1.6^{aB}	ES	ES	ES
Malondialdehyde (µmol.g^{-1})					
2°C	34.0 ± 3.0^{aA}	58.3 ± 6.4^{cC}	61.0 ± 6.0^{aC}	63.1 ± 6.1^{bC}	82.8 ± 18.2^{B}
5°C	34.0 ± 3.0^{aA}	46.3 ± 5.2^{dC}	60.2 ± 9.1^{aD}	79.02 ± 4.9^{aB}	ES
10°C	34.0 ± 3.0^{aA}	67.1 ± 8.2^{bB}	72.4 ± 16.0^{aB}	ES	ES
25°C	34.0 ± 3.0^{aA}	72.1 ± 8.0^{aB}	ES	ES	ES
Antioxidant capacity (%)					
2°C	73.4 ± 0.1^{aA}	79.8 ± 3.1^{aB}	76.2 ± 4.9^{aAB}	52.0 ± 1.3^{aC}	49.1 ± 2.1^{C}
5°C	73.4 ± 0.1^{aA}	70.7 ± 5.5^{bA}	72.0 ± 3.8^{aA}	49.0 ± 2.2^{aB}	ES
10°C	73.4 ± 0.1^{aA}	73.5 ± 1.9^{abA}	69.1 ± 0.6^{aB}	ES	ES
25°C	73.4 ± 0.1^{aA}	68.6 ± 6.3^{bA}	ES	ES	ES
Polyphenol oxidase activity (U.g^{-1})					
2°C	12.6 ± 0.0^{aA}	8.3 ± 0.0^{aC}	9.4 ± 0.4^{aB}	9.8 ± 0.1^{aB}	6.3 ± 0.3^{D}
5°C	12.6 ± 0.0^{aA}	8.6 ± 0.9^{aB}	7.8 ± 0.1^{bB}	2.6 ± 0.1^{bC}	ES
10°C	12.6 ± 0.0^{aA}	3.8 ± 0.5^{bB}	3.9 ± 0.1^{cB}	ES	ES
25°C	12.6 ± 0.0^{aA}	1.3 ± 0.0^{cB}	ES	ES	ES

Data are expressed as mean ± S.D, ES: end of storage life. Values with different letters (a-e) within column, (A-E) with row are significantly different ($p<0.05$).

Antioxidant capacity

Several previous studies have shown that strawberry was a good source of natural antioxidants (Wang et al., 1996). The antioxidant capacity of strawberry stored at 2°C, 5°C, 10°C and 25°C was shown in Table 3. The antioxidant capacity of strawberry stored at 2°C, 5°C declined after the 6th day and remained until 12th day. Cordenunsi et al. (2005) explained that the decrease in antioxidant activity was correlated with a higher content of dehydroascorbic acid and a decrease in ascorbic acid (Table 2). However, the antioxidant capacity of fruit stored at different temperatures was the insignificant difference ($p>0.05$). It showed that temperature did not affect the antioxidant capacity of strawberry. This supported the reported study of Shin et al. (2008).

Polyphenol oxidase

The enzymatic browning reaction, oxidized these phenolic compounds, was one of the most important cause of color deterioration in fruit. The enzyme related to this was polyphenol oxidase (PPO) (Yang et al., 2010). In Table 3, the value of PPO content in strawberry at 5°C and 2°C increased to the maximum of 8.6 U g^{-1} at the 3rd day and 9.4 U g^{-1} at the 9th day, respectively. This increase of PPO activity directly related to the decrease of TPC and TAC (Table 3). Petriccione et al. (2015) had observed the same phenomenon that the decrease of TPC in strawberry likely reflected the rising of PPO activity. Low storage temperature might be damaged strawberry cell and released more PPO enzyme in the initial stage of storage (Valenzuela et al., 2017). However, PPO was inactivated when storage in low temperature in long time and it started to rapidly decline PPO activity for lengthening the shelf life (Yang et al., 2010). According to the overall quality index of strawberry (Fig. 1a), 2°C showed the

most effective one to maintain the overall quality of strawberries and inhibit the PPO activity during storage.

CONCLUSIONS

In the present study, 0.2% nano-chitosan coated strawberries were stored at four different temperatures for investigating effects of temperature on their physico-chemical quality. As discussed above, lowering storage temperature was the effective way to prolong strawberry's storage life, prevent the decrement of firmness and the weight loss. Besides, the MDA content delayed, PPO content was inactivated and the loss of TSS in strawberry was also reduced when storing at 2°C. The TPC synthesis was favored at 5°C but an additional decrease in temperature did not improve the final amount of this compound. However, the low temperature brought the negative effects during storage in TAC and L-AA content of strawberry causing the reduction of anthocyanin content and vitamin C. The antioxidant capacity of strawberry was not affected by temperature. Therefore, strawberry stored at 2°C had the greater effect on physico-chemical properties of fruit than others storage temperatures.

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Conflict of interest
The authors have no conflict of interest to report.

REFERENCES

AOAC. (1990). Titrable Acidity 942.15. AOAC Official Methods of Analysis, 15th ed.

Ayala-Zavala, J. F., Wang, S. Y., Wang, C. Y., & González-Aguilar, G. A. (2004). Effect of storage temperatures on antioxidant capacity and aroma compounds in strawberry fruit. *LWT-Food Science and Technology*, *37*(7), 687-695. https://doi.org/10.1016/j.lwt.2004.03.002

Ayala-Zavala, J. F., Wang, S. Y., Wang, C. Y., & González-Aguilar, G. A. (2005). Methyl jasmonate in conjunction with ethanol treatment increases antioxidant capacity, volatile compounds and postharvest life of strawberry fruit. *European Food Research and Technology*, *221*(6), 731-738. https://doi.org/10.1007/s00217-005-0069-z

Brat, P., Mennen, L., George, S., Scalbert, A., Bellamy, A., Amiot-Carlin, M., & Chaffaut, L. (2007). Determination of the polyphenol content of fruits and vegetables. Establishment of a database and estimation of the polyphenol intake in the French diet. *Acta Horticulturae*, *744*, 61-70. https://doi.org/10.17660/actahortic.2007.744.5

Brummell, D. A. (2006). Cell wall disassembly in ripening fruit. *Functional Plant Biology*, *33*(2), 103-119. https://doi.org/10.1071/fp05234

Brummell, D. A., & Harpster, M. H. (2001). Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. *Plant Molecular Biology*, *47*(1-2), 311-339.

Cordenunsi, Genovese, M. I., do Nascimento, J. R. O., Hassimotto, N. M. A., dos Santos, R. J., & Lajolo, F. M. (2005). Effects of temperature on the chemical composition and antioxidant activity of three strawberry cultivars. *Food Chemistry*, *91*(1), 113-121. https://doi.org/10.1016/j.foodchem.2004.05.054

Cordenunsi, Nascimento, J. d., & Lajolo, F. (2003). Physico-chemical changes related to quality of five strawberry fruit cultivars during cool-storage. *Food Chemistry*, *83*(2), 167-173. https://doi.org/10.1016/S0308-8146(03)00059-1

Gardesh, A. S. K., Badii, F., Hashemi, M., Ardakani, A. Y., Maftoonazad, N., & Gorji, A. M. (2016). Effect of nanochitosan based coating on climacteric behavior and postharvest shelf-life extension of apple cv. Golab Kohanz. *LWT - Food Science and Technology*, *70*, 33-40.

https://doi.org/10.1016/j.lwt.2016.02.002
Giusti, M. M., Rodriguez-Saona, L., & Wrolstad, R. (1999). Spectral characteristics, molar absorptivity and color of pelargonidin derivatives. *Journal of Agricultural and Food Chemistry, 47*(11), 4631-4637. https://doi.org/10.1021/jf981271k
Giusti, M. M., & Wrolstad, R. E. (2001). Characterization and measurement of anthocyanins by UV-visible spectroscopy. *Current Protocols in Food Analytical Chemistry, 00*, F1.2.1-F1.2.13. https://doi.org/10.1002/0471142913.faf0102s00
Goulao, L., Fernandes, J., Lopes, P., & Amâncio, S. (2012). *Tackling the cell wall of the grape berry*: Bentham Science Publishers.
Goy, R. C., Britto, D. D., & Assis, O. B. (2009). A review of the antimicrobial activity of chitosan. *Polímeros, 19*(3), 241-247. https://doi.org/10.1590/S0104-14282009000300013
Han, Lederer, C., McDaniel, M., & Zhao, Y. (2005). Sensory evaluation of fresh strawberries (*Fragaria ananassa*) coated with chitosan-based edible coatings. *Journal of Food Science, 70*(3), 172-178. https://doi.org/10.1111/j.1365-2621.2005.tb07153.x
Han, Zhao, Y., Leonard, S., & Traber, M. (2004). Edible coatings to improve storability and enhance nutritional value of fresh and frozen strawberries (*Fragaria× ananassa*) and raspberries (*Rubus ideaus*). *Postharvest Biology and Technology, 33*(1), 67-78. https://doi.org/10.1016/j.postharvbio.2004.01.008
Hangun-Balkir, Y., & McKenney, M. L. (2012). Determination of antioxidant activities of berries and resveratrol. *Green Chemistry Letters and Reviews, 5*(2), 147-153. https://doi.org/10.1080/17518253.2011.603756
Hardenburg, R. E., Watada, A. E., & Wang, C. Y. (1986). *The commercial storage of fruits, vegetables, and florist and nursery stocks.* Agriculture Handbook, USDA. pp.66-130.
Hernández-Muñoz, P., Almenar, E., Del Valle, V., Velez, D., & Gavara, R. (2008). Effect of chitosan coating combined with postharvest calcium treatment on strawberry (*Fragaria× ananassa*) quality during refrigerated storage. *Food Chemistry, 110*(2), 428-435. https://doi.org/10.1016/j.foodchem.2008.02.020
Hodges, D. M., DeLong, J. M., Forney, C. F., & Prange, R. K. (1999). Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta, 207*(4), 604-611. https://doi.org/10.1007/s004250050524
Holzwarth, M., Korhummel, S., Carle, R., & Kammerer, D. R. (2012). Evaluation of the effects of different freezing and thawing methods on color, polyphenol and ascorbic acid retention in strawberries (*Fragaria× ananassa* Duch.). *Food Research International, 48*(1), 241-248. https://doi.org/10.1016/j.foodres.2012.04.004
Islam, M., Khan, M., Sarkar, M., Absar, N., & Sarkar, S. (2013). Changes in acidity, TSS, and sugar content at different storage periods of the postharvest mango (*Mangifera indica* L.) influenced by bavistin DF. *International Journal of Food Science, 2013*, 1-8. https://doi.org/10.1155/2013/939385
ISO:14502. (2005). Content of total polyphenols in tea - Colorimetric method using Folin-Ciocalteu reagent.
Ivanova, V., Vojnoski, B., & Stefova, M. (2012). Effect of winemaking treatment and wine aging on phenolic content in Vranec wines. *Journal of Food Science and Technology, 49*(2), 161-172. https://doi.org/10.1007/s13197-011-0279-2
Jiang, Y., Yu, L., Hu, Y., Zhu, Z., Zhuang, C., Zhao, Y., & Zhong, Y. (2020). The preservation performance of chitosan coating with different molecular weight on strawberry using electrostatic spraying technique. *International Journal of Biological Macromolecules, 151*, 278-285. https://doi.org/10.1016/j.ijbiomac.2020.02.169
Kalt, W., Forney, C. F., Martin, A., & Prior, R. L. (1999). Antioxidant capacity, vitamin C, phenolics, and anthocyanins after fresh storage of small fruits. *Journal of Agricultural and Food Chemistry, 47*(11), 4638-4644. https://doi.org/10.1021/jf990266t
Kapur, A., Hasković, A., Čopra-Janićijević, A., Klepo, L., Topčagić, A., Tahirović, I., & Sofić, E. (2012). Spectrophotometric analysis of total ascorbic acid content in various fruits and vegetables. *Bull Chem Technol Bosnia Herzegovina, 38*(4), 39-42.

Kumar, M. N. R. (2000). A review of chitin and chitosan applications. *Reactive and Functional Polymers*, *46*(1), 1-27. https://doi.org/10.1016/S1381-5148(00)00038-9

Lee, S. K., & Kader, A. A. (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biology and Technology*, *20*(3), 207-220. https://doi.org/10.1016/s0925-5214(00)00133-2

Li, C., & Kader, A. A. (1989). Residual effects of controlled atmospheres on postharvest physiology and quality. *Journal of the American Society for Horticultural Science*, *114*, 629-634.

Liu, C., Zheng, H., Sheng, K., Liu, W., & Zheng, L. (2018). Effects of melatonin treatment on the postharvest quality of strawberry fruit. *Postharvest Biology and Technology*, *139*, 47-55. https://doi.org/10.1016/j.postharvbio.2018.01.016

Lorevice, M. V., Moura, M. R. D., Aouada, F. A., & Mattoso, L. H. (2012). Development of novel guava puree films containing chitosan nanoparticles. *Journal of Nanoscience and Nanotechnology*, *12*(3), 2711-2717. https://doi.org/10.1166/jnn.2012.5716

Maftoonazad, N., Ramaswamy, H. S., & Marcotte, M. (2008). Shelf-life extension of peaches through sodium alginate and methyl cellulose edible coatings. *International Journal of Food Science and Technology*, *43*(6), 951-957. https://doi.org/10.1111/j.1365-2621.2006.01444.x

Martínez, K., Ortiz, M., Albis, A., Gilma Gutiérrez Castañeda, C., Valencia, M. E., & Grande Tovar, C. D. (2018). The effect of edible chitosan coatings incorporated with Thymus capitatus essential oil on the shelf-life of strawberry (*Fragaria* × *ananassa*) during cold storage. *Biomolecules*, *8*(4), 155. https://doi.org/10.3390/biom8040155

Mercantila, F. (1989). *Guide to food transport: fruit and vegetables*: Mercantila Publishers.

Nayak, S. L., Sethi, S., Sharma, R., Sharma, R., Singh, S., & Singh, D. (2020). Aqueous ozone controls decay and maintains quality attributes of strawberry (*Fragaria* × *ananassa* Duch.). *Journal of Food Science and Technology*, *57*(1), 319-326. https://doi.org/10.1007/s13197-019-04063-3

Nguyen, D. H., & Nguyen, H. V. (2020). Effects of nano-chitosan and chitosan coating on the postharvest quality, polyphenol oxidase activity and malondialdehyde content of strawberry (*Fragaria* × *ananassa* Duch.). *Journal of Horticulture and Postharvest Research*, *3*(1), 11-24. https://doi.org/10.22077/JHPR.2019.2698.1082

Nguyen, V. T., Nguyen, D. H., & Nguyen, H. V. (2020). Combination effects of calcium chloride and nano-chitosan on the postharvest quality of strawberry (*Fragaria* × *ananassa* Duch.). *Postharvest Biology and Technology*, *162*, 111103. https://doi.org/10.1016/j.postharvbio.2019.111103

Nunes, M. C. N., Brecht, J. K., Morais, A. M. M. B., & Sargent, S. A. (1998). Controlling temperature and water loss to maintain ascorbic acid levels in strawberries during postharvest handling. *Journal of Food Science*, *63*(6), 1033-1036. https://doi.org/10.1111/j.1365-2621.1998.tb15848.x

Nunes, M. C. N., Brecht, J. K., Morais, A. M. M. B., & Sargent, S. A. (2005). Possible influences of water loss and polyphenol oxidase activity on anthocyanin content and discoloration in fresh ripe strawberry (cv. Oso Grande) during storage at 1 C. *Journal of Food Science*, *70*(1), 79-84. https://doi.org/10.1111/j.1365-2621.2005.tb09069.x

Nunes, N., & Cecilia, M. (2015). Correlations between subjective quality and physicochemical attributes of fresh fruits and vegetables. *Postharvest Biology and Technology*, *107*, 43-54. https://doi.org/10.1016/j.postharvbio.2015.05.001

Perkins-Veazie, P. (2010). Growth and ripening of strawberry fruit. *Horticultural Reviews*, *17*, 267-297. https://doi.org/10.1002/9780470650585.ch8

Petriccione, M., Mastrobuoni, F., Pasquariello, M. S., Zampella, L., Nobis, E., Capriolo, G., & 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. https://doi.org/10.3390/foods4040501

Rana, S., Mehta, D., Bansal, V., Shivhare, U., & Yadav, S. K. (2020). Atmospheric cold plasma (ACP) treatment improved in-package shelf-life of strawberry fruit. *Journal of Food Science and Technology*, *57*(1), 102-112. https://doi.org/10.1007/s13197-019-04035-7

Sahari, M. A., Boostani, F. M., & Hamidi, E. Z. (2004). Effect of low temperature on the ascorbic acid content and quality characteristics of frozen strawberry. *Food Chemistry*, *86*(3), 357-363. https://doi.org/10.1016/j.foodchem.2003.09.008

Savicka, M., & Škute, N. (2010). Effects of high temperature on malondialdehyde content, superoxide production and growth changes in wheat seedlings (*Triticum aestivum* L.). *Ekologija*, *56*(1), 26-33. https://doi.org/10.2478/v10055-010-0004-x

SeaLand, M. (1991). *Shipping guide to perishables*. SeaLand Services Inc., Iselim, New Jersey, USA.

Shin, Y., Liu, R. H., Nock, J. F., Holliday, D., & Watkins, C. B. (2007). Temperature and relative humidity effects on quality, total ascorbic acid, phenolics and flavonoid concentrations, and antioxidant activity of strawberry. *Postharvest Biology and Technology*, *45*(3), 349-357. https://doi.org/10.1016/j.postharvbio.2007.03.007

Shin, Y., Ryu, J. A., Liu, R. H., Nock, J. F., & Watkins, C. B. (2008). Harvest maturity, storage temperature and relative humidity affect fruit quality, antioxidant contents and activity, and inhibition of cell proliferation of strawberry fruit. *Postharvest Biology and Technology*, *49*(2), 201-209. https://doi.org/10.1016/j.postharvbio.2008.02.008

Silvestre, C., Duraccio, D., & Cimmino, S. (2011). Food packaging based on polymer nanomaterials. *Progress in Polymer Science*, *36*(12), 1766-1782. https://doi.org/10.1016/j.progpolymsci.2011.02.003

Srivalli, B., Chinnusamy, V., & Khanna-Chopra, R. (2003). Antioxidant defense in response to abiotic stresses in plants. *Journal of Plant Biology-New Delhi*, *30*(2), 121-140.

Timberlake, C., & Bridle, P. (1982). *Distribution of anthocyanins in food plants*: Academic Press, New York.

Valenzuela, J. L., Manzano, S., Palma, F., Carvajal, F., Garrido, D., & Jamilena, M. (2017). Oxidative stress associated with chilling injury in immature fruit: Postharvest technological and biotechnological solutions. *International Journal of Molecular Sciences*, *18*(7), 1467. https://doi.org/10.3390/ijms18071467

Wang, H., Cao, G., & Prior, R. L. (1996). Total antioxidant capacity of fruits. *Journal of Agricultural and Food Chemistry*, *44*(3), 701-705. https://doi.org/10.1021/jf950579y

Yang, F., Li, H., Li, F., Xin, Z., Zhao, L., Zheng, Y., & Hu, Q. (2010). Effect of nano-packing on preservation quality of fresh strawberry (*Fragaria ananassa* Duch. cv Fengxiang) during Storage at 4° C. *Journal of Food Science*, *75*(3), 236-240. https://doi.org/10.1111/j.1750-3841.2010.01520.x

Yoon, Y. S., Ameer, K., Song, B. S., Kim, J. K., Park, H. Y., Lee, K. C., Eun, J. B., & Park, J. H. (2020). Effects of X-ray irradiation on the postharvest quality characteristics of 'Maehyang' strawberry (*Fragaria× ananassa*). *Food Chemistry*, *325*, 126817. https://doi.org/10.1016/j.foodchem.2020.126817

Zhang, X., & Xingfeng, S. (2015). Characterisation of polyphenol oxidase and peroxidase and the role in browning of loquat fruit. *Czech Journal of Food Science*, *33*(2), 109-117. https://doi.org/10.17221/384/2014-CJFS