



Effects of nano-chitosan and chitosan coating on the quality, polyphenol oxidase activity, malondialdehyde content of strawberry (*Fragaria x ananassa* Duch.)

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ABSTRACT

Purpose: The aim of this study was to investigate the effects of nano-chitosan and chitosan coating on physico-chemical properties of strawberries during storage. **Research methods:** Fresh strawberries were coated with different concentrations of chitosan (1%, 1.5%, 2%) or nano-chitosan (0.2%, 0.4%, and 0.8%) and stored in 2°C for 21 days. **Findings:** Coating strawberry with 0.2% and 0.4% nano-chitosan preserved the overall quality index of the fruit up to 21 days. The treatments reduced weight loss, retained firmness, titratable acidity and L-ascorbic acid, significantly retarded malondialdehyde production and inhibited polyphenol oxidase activity of the stored fruit. The 0.2% nano-chitosan treatment reserved total soluble solid and total anthocyanin content better than the 0.4% nano-chitosan. Although 2% chitosan coating showed the positive effects, the overall quality index of the coated fruit was reduced below the acceptable level after 18 days, shorter as compared to the others coated with the lower concentrations of nano-chitosan. **Research limitations:** Nano-chitosan, showing to be the effective coating material in this study, is not popular traded in the industry. **Originality/Value:** The combination of 0.2 % nano-chitosan coating and storing fresh strawberry at 2°C preserved the quality of fruits up to 21 days. The much lower concentrations of nano-chitosan showed higher positive effects as compared to the higher concentrations of chitosan. This would help to reduce the cost of postharvest handlings for the strawberry industry.

INTRODUCTION

Strawberry, a nutritive fruit containing high phytochemicals, vitamins, and minerals, is a highly perishable non-climacteric fruit due to the high rate of respiration, leading to a shorter shelf life after harvesting (Almenar et al., 2007; Hernández-Muñoz et al., 2008).

Chitosan, the N-deacetylated derivative of chitin, mainly found in shrimp skeleton and recognized as safe by the U.S. FDA (2001), is soluble in acid conditions to form the colloid complex, a suitable material for food packaging. Chitosan has potential in controlling plant diseases (Kumar, 2000) including activity against infection caused by bacteria, mold and other pathogens (Goy et al., 2009). Besides, chitosan had shown the antioxidant properties coming from the donation of hydrogen or lone pair of the electron from their structure (Rajalakshmi et al., 2013). Chitosan coatings have been proved to be applicable for prevention the losses of weight, titratable acidity, total soluble solid and bioactive compounds in fruit and vegetables during storage (Gol et al., 2013; Kerch, 2015). The quality, permeability, and storability of chitosan coating have been proved as beneficial effects on fruits such as strawberry (Hernández-Muñoz et al., 2006; Hernández-Muñoz et al., 2008), papaya (Bautista-Baños et al., 2003), and tomato (El Ghaouth et al., 1992).

Recently, nanotechnology has shown the significant results for application in pharmaceutical and food industries, including the food packaging. Nano-based materials are found to be capable of enhancing the barrier properties such as strength, stiffness, or heat resistance of the packaging materials (Silvestre et al., 2011). However, there are a very few researches studying effects of nano-chitosan coating on fresh fruit storage, particularly on strawberry. Nanoparticles of chitosan have shown the more exceptional antifungal ability defending the varieties of microorganism than chitosan particles (Goy et al., 2009). Nano-chitosan based coating has better barrier properties to the internal gas atmosphere and more optimize in the permeability of coating on fruit (Lorevice et al., 2012).

The main purpose of this research was to study the effects of nano-chitosan, and chitosan coating on the postharvest quality of strawberries. The results from this study would be a useful database for further researches involving edible coating in the post-harvesting technology for fruit.

MATERIALS AND METHODS

Materials

Sample preparation

Strawberries (*Fragaria x ananassa* Duch.) were harvested from the orchard in Lamdong province, Vietnam in September 2017 when reached the market maturity stage.

Strawberry was washed under tap water to remove contaminants before being dipped into chitosan (1%, 1.5%, 2%) and nano-chitosan solutions (0.2%, 0.4%, 0.8%) for 1 min. The fruit then drained on stainless steel racks for 30 min at room temperature (Han et al., 2005). The nano-pure water (Barnstead EasyPure II, ThermoScientific, USA) treated fruit were used as control samples. The treated samples were stored at 2°C and tested after each 3-day intervals (Brat et al., 2007).

Edible coating formulations

Chitosan solution was prepared by dissolving chitosan powder (Sigma-Aldrich, St. Louis, MO, USA) in 1% acetic acid solution (Merck Chemicals Ltd., Darmstadt, Germany) (Han et

al., 2005). Meanwhile, the nano-chitosan solution was supplied by Dalat Nuclear Research Institute, Vietnam (NRI) with the particle size was at 250 nm.

Analytical methods

Measurement of the overall quality index

The visual quality scores of samples were evaluated by the trained people following the score and description in Figure 1.

Measurement of weight loss (%)

Strawberry was weighed after being coated, 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 as follow (1):

$$Weight\ loss\ (\%) = \frac{m_1 - m_2}{m_1} \times 100 \tag{1}$$

Where m_1 : the weight of sample before storage (g), m_2 : the weight of the sample after storage intervals (g)

		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.			

Fig. 1. The overall rating chart of strawberry showing photographs of strawberry at different stages of visual deterioration correspond with subjective scores and descriptions

Measurement of firmness (N)

The firmness of strawberry was measured following the method of [Hernández-Muñoz et al. \(2006\)](#), using a Digital Fruit Hardness Tester (FR- 5120, Lutron electronic enterprise Co., LTD., Taiwan).

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

The juice of strawberry after slightly squeezing was used for measuring the total soluble solid (TSS), using the digital refractometer (RX- 5000, Atago Co., LTD., Japan) ([Hernández-Muñoz et al., 2008](#)). The results were expressed as a percentage.

According to [AOAC \(1990\)](#), 10 g puree of strawberry was mixed with 50 mL distilled water and filtered. Titratable acidity (TA) of samples was measured using the titrate method with 0.1M NaOH. The endpoint reading during titration was monitored by pH meter (HI 9126, Hanna Instruments Inc., Romania) and the results were expressed as grams of citric acid per 100 g fresh weight of strawberry and calculated as the below formula (2):

$$\text{Titratable Acidity (g 100 g}^{-1}\text{)} = \frac{\text{Volume of NaOH (mL)} \times 0.1 \text{ M} \times 0.064}{10 \text{ g of sample}} \times 100 \quad (2)$$

Measurement of antioxidant capacity by DPPH method (%)

Antioxidant capacity of strawberry was determined using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) (Sigma-Aldrich Pte. Ltd., St. Louis, MO, USA) ([Hangun-Balkir & McKenney, 2012](#)). The absorbance of samples was measured at 517 nm, using a UV-VIS spectrophotometer (GENESYS 10 UV-Vis, Thermo Fisher Scientific, Inc., USA). The antioxidant capacity was expressed as the percentage of DPPH radical scavenging capacity (3):

$$\text{DPPH scavenging} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100 \quad (3)$$

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. \(2012\)](#), 5g of strawberry was homogenized with 50mL of metaphosphoric acid - acetic acid solution. This mixture was centrifuged at $1937 \times g$ in 15 min at 27°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 in 3h. The absorbance of samples was measured at 512 nm after adding 5 mL of chilled 85% H₂SO₄.

Determination of total anthocyanin content (mg 100g⁻¹)

The predominant anthocyanin in strawberry was pelargonidin-3-glucoside. For measuring the absorbance of the total anthocyanin content (4) in strawberry, the pH differential method was applied ([Cordenunsi et al., 2003](#)). Briefly, 5g of the sample was extracted with 0.025 M potassium chloride buffer, pH 1.0 at 50 °C in 3h and then centrifuged at $2142 \times g$ for 20 min at 4 °C. The sample solution was diluted using two buffers: 0.025 M potassium chloride buffer (pH 1.0) and 0.4 M sodium acetate buffer (pH 4.5) ([Yang et al., 2010](#)). Each diluted solution had recorded the absorbance at 496 nm and 700 nm.

$$\text{Total anthocyanin content (} \frac{\text{mg}}{\text{L}} \text{)} = \frac{A \times MW \times DF \times 1000}{\epsilon \times l} \quad (4)$$

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: molecular weight, DF: dilution factor, ϵ : the molar absorptivity of $15600 \text{ M}^{-1} \text{ cm}^{-1}$ (Giusti et al., 1999).

Determination of total phenolic content (mg GAE 100g⁻¹)

Total phenolic content (TPC) in strawberry was determined using Folin-Ciocalteu assay (Boeing et al., 2014). The samples were extracted using acetone:water (7:3 v/v) (Merck Chemicals Ltd., Darmstadt, Germany). The absorbance of sample was recorded at 765 nm. The results were expressed as milligram gallic acid equivalent per 100g fresh weight (mg 100g⁻¹).

Determination of malondialdehyde ($\mu\text{mol g}^{-1}$)

Malondialdehyde (MDA) content was determined using the 2-thiobarbituric acid (Merck Chemicals Ltd., Darmstadt, Germany) (TBA) reaction (Yang et al., 2010). The sample was homogenized with ice-cold 0.1% trichloroacetic acid (Sigma-Aldrich Pte. Ltd., St. Louis, MO, USA) (TCA) in a cooled mortar and pestle. The supernatant collected from centrifugation of the mixture at $2142 \times g$ 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 was recorded at 532 nm and 600 nm. The concentration of lipid peroxides together with oxidatively modified proteins of fruit were quantified in terms of MDA level using an extinction coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$ and expressed as $\mu\text{mol g}^{-1}$ (Yang et al., 2010).

Polyphenol oxidase assay (Unit g⁻¹)

Polyphenol oxidase extraction was based on a method of (Holzwarth et al., 2012). Strawberry purée was stirred continuously with cold acetone (−20 °C) for 5 min. The mixture was filtered using filter paper Whatman No.2 (GE- Healthcare, Chicago, Illinois, USA) before mixing the solution collected 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 was measured at 420 nm. The reaction time was 2 mins, and the activity was expressed in units with one unit = $0.001 * \Delta A_{420} / \text{min} / \text{g}$ fresh weight (Zhang & Xingfeng, 2015).

Statistical analysis

The statistical results were analyzed using the Minitab statistical software (Version 17.0, Minitab Pty Ltd., Australia) with Fisher's one-way ANOVA and expressed as means \pm S.D with $p < 0.05$ as significant differences at confidence intervals of 95%.

RESULTS AND DISCUSSION

The overall quality index

During storage at 2 °C, all samples showed the loss of visual appearances (Fig. 2). However, the storage-life of coated strawberries was much longer than that of the control. This could be due to barrier properties of chitosan protecting the fruit from physical, chemical, and biological deterioration (Kester & Fennema, 1986). Overall, the higher overall appearance of coated strawberry correlated with the lower level of dehydration and darkening during storage (Hernández-Muñoz et al., 2008). Increasing nano-chitosan concentrations seemed to decrease the overall quality index of fruit (Fig. 2a). In contrast, the increase of chitosan concentrations showed positive effects on prolonging the shelf life of strawberries (Fig. 2b). Moreover, with higher concentrations of chitosan at 1.5% and 2%, the quality of strawberries was reduced to

an acceptable level after 18 days. Meanwhile, the lower nano-chitosan concentrations (0.2% and 0.4%) maintained the quality of the fruit until 21 days.

Weight loss

The weight loss of uncoated berry was 8.84% at 12th day. This data was significant ($p < 0.05$) higher than those of coated samples (Fig. 3). Among coated samples, after 15 days of storage, the lowest weight loss was observed from the 2% chitosan treatment, while the highest value was recorded in the 1% chitosan one (2.65% vs 7.57%) (Fig. 3b). There were insignificant differences ($p > 0.05$) found in 0.2%, 0.4% NCS and 1.5%, 2% CS samples after 18th day. In this study, chitosan and nano-chitosan coating layers worked as the physical barriers to prevent dehydration and shriveling and therefore reduced weight loss of fruit.

Firmness

During nine days of storage, there were increases in the firmness of coated strawberries and control sample. At 12th day, the firmness of the uncoated strawberry was rapidly decreased to 1.73N, ranked as the lowest value (Fig. 4). Generally, chitosan coating slowed down the respiration rate of fresh fruit and vegetables (Hernández-Muñoz et al., 2006), consequently delayed the fruit ripening (Velickova et al., 2013). The coating also controlled the loss of moisture from the fruit and therefore retained the texture of fruit (Han et al., 2004). After 21 days of storage, the coating strawberry with 0.2% NCS was the most effective treatment in preventing the loss of fruit firmness.

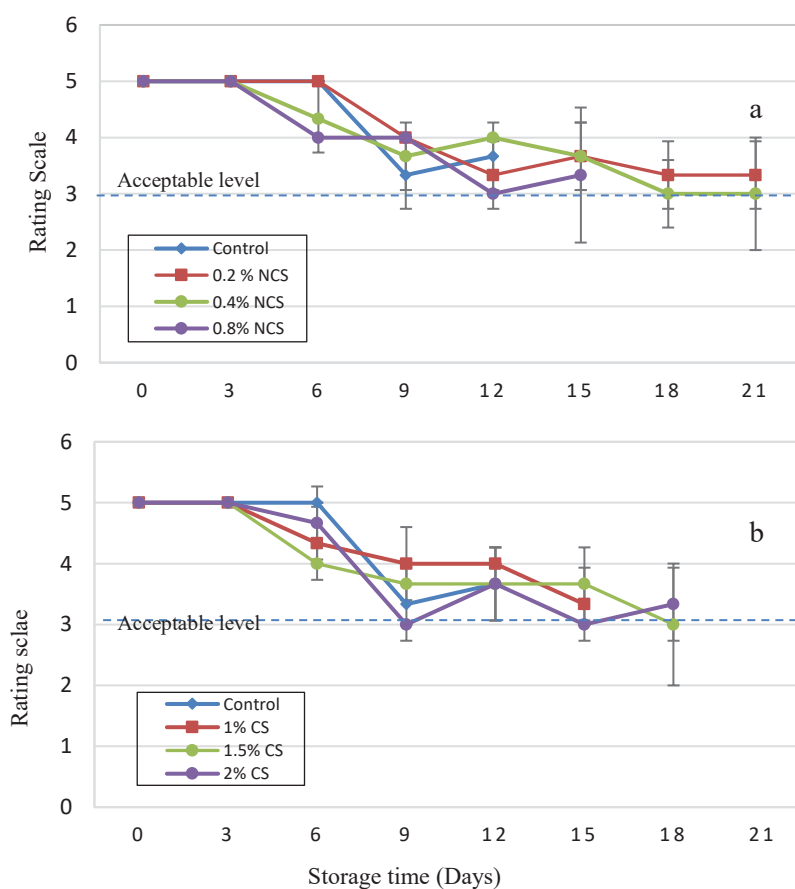


Fig. 2. Effects of coating on the overall quality index of strawberry coated with (a) nano-chitosan; (b) chitosan and stored at 2 °C

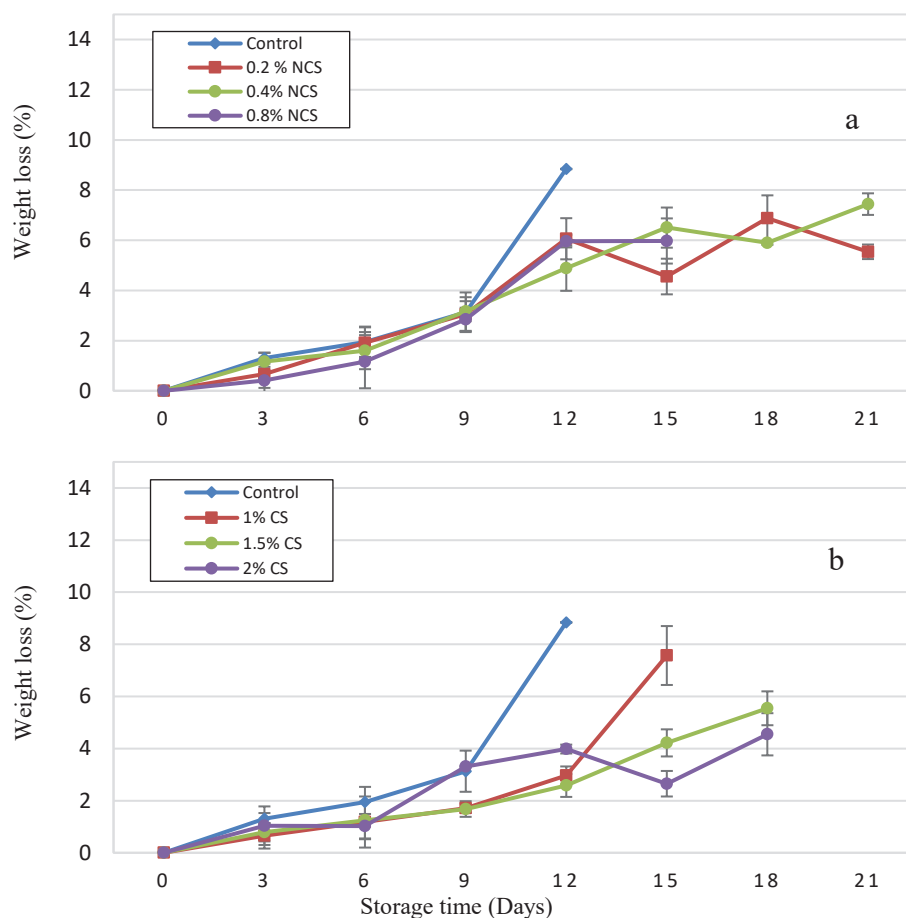


Fig. 3. Effects of coating on the weight loss percentage of strawberry coated with (a) nano-chitosan; (b) chitosan and stored at 2 °C

Total soluble solids, titratable acidity and L-Ascorbic acid

There were decreases in total soluble solids of all samples during storage (Table 1a). This reduction could be explained by the hydrolysis of sucrose during the respiration of fruit (Yang et al., 2010). The similar phenomenon was recorded in coated apple (Baldwin et al., 2011) and coated strawberry with chitosan (Vargas et al., 2006).

By comparison with the initial value, no significant differences in titratable acidity values of both coated and uncoated strawberries were observed ($p < 0.05$) (Table 1b). These results agreed with findings of Hernández-Muñoz et al. (2008), who had proved coating strawberry did not change titratable acidity after storage at 10 °C.

The concentrations of L-ascorbic acid (L-AA) in the control sample and strawberries treated with chitosan and nano-chitosan were shown in Table 1c. At 12th day, there was a decrease of L-AA in uncoated strawberry; meanwhile, it remained in 0.2% NCS and 0.8% NCS coated strawberry ($p < 0.05$). This loss of vitamin C might occur due to the high respiration rate of strawberry (Gol et al., 2013). Also, coating treatments had been reported to prevent the degradation of L-AA in fruit (Gol et al., 2013). It could be expected from the current study that nano-chitosan and chitosan coating would delay the loss of vitamin C in strawberries.

Table 1. Effects of chitosan and nano-chitosan concentrations on total soluble solid, titratable acidity and L-ascorbic acid of strawberry stored at 2 °C

	Initial	3d	6d	9d	12d	15d	18d	21d
(a) Total soluble solids (%)								
Control	7.4 ± 0.4 ^{aA}	7.1 ± 0.5 ^{cdA}	5.7 ± 0.3 ^{abcB}	5.8 ± 0.8 ^{aB}	5.2 ± 0.5 ^{aB}	ES	ES	ES
0.2% NCS	7.4 ± 0.4 ^{aA}	7.6 ± 0.3 ^{bcA}	6.1 ± 0.3 ^{abB}	5.7 ± 0.5 ^{aBC}	5.3 ± 0.2 ^{aC}	5.9 ± 0.5 ^{abBC}	5.6 ± 0.2 ^{aBC}	5.3 ± 0.3 ^{aC}
0.4% NCS	7.4 ± 0.4 ^{aB}	9.7 ± 0.5 ^{aA}	5.8 ± 0.3 ^{abcC}	5.6 ± 0.7 ^{aC}	5.3 ± 0.1 ^{aC}	5.3 ± 0.3 ^{bc}	5.3 ± 0.9 ^{aC}	4.4 ± 0.4 ^{bd}
0.8% NCS	7.4 ± 0.4 ^{aA}	7.1 ± 0.5 ^{cdAB}	6.2 ± 0.7 ^{aBC}	5.1 ± 0.6 ^{aD}	5.3 ± 0.3 ^{aD}	5.9 ± 0.7 ^{abCD}	ES	ES
1% CS	7.4 ± 0.4 ^{aA}	6.1 ± 0.4 ^{dB}	5.4 ± 0.1 ^{eBC}	5.0 ± 0.6 ^{aC}	5.3 ± 0.2 ^{aC}	5.5 ± 0.5 ^{abBC}	ES	ES
1.5% CS	7.4 ± 0.4 ^{aB}	9.1 ± 1.7 ^{aA}	5.4 ± 0.2 ^{bcC}	5.3 ± 0.2 ^{aC}	5.0 ± 0.7 ^{aC}	5.3 ± 0.3 ^{bc}	6.1 ± 0.3 ^{aC}	ES
2% CS	7.4 ± 0.4 ^{aB}	8.7 ± 0.9 ^{abA}	5.7 ± 0.2 ^{abcCD}	5.4 ± 0.3 ^{aD}	5.3 ± 0.2 ^{aD}	6.2 ± 0.1 ^{aC}	5.4 ± 0.1 ^{dD}	ES
(b) Titratable acidity (%)								
Control	0.20 ± 0.00 ^{abc}	0.33 ± 0.06 ^{abA}	0.30 ± 0.00 ^{abAB}	0.17 ± 0.06 ^{aC}	0.20 ± 0.10 ^{bBC}	ES	ES	ES
0.2% NCS	0.20 ± 0.00 ^{aC}	0.33 ± 0.06 ^{abA}	0.30 ± 0.00 ^{abAB}	0.20 ± 0.00 ^{aC}	0.20 ± 0.00 ^{bC}	0.27 ± 0.06 ^{abcB}	0.20 ± 0.00 ^{abc}	0.20 ± 0.00 ^{aC}
0.4% NCS	0.20 ± 0.00 ^{abc}	0.27 ± 0.06 ^{bAB}	0.30 ± 0.00 ^{abA}	0.17 ± 0.06 ^{aC}	0.20 ± 0.00 ^{bBC}	0.27 ± 0.06 ^{abcAB}	0.30 ± 0.10 ^{aA}	0.27 ± 0.06 ^{aAB}
0.8% NCS	0.20 ± 0.00 ^{ab}	0.33 ± 0.06 ^{abA}	0.33 ± 0.06 ^{aA}	0.17 ± 0.06 ^{ab}	0.20 ± 0.00 ^{bB}	0.23 ± 0.06 ^{bcB}	ES	ES
1% CS	0.20 ± 0.00 ^{abc}	0.30 ± 0.00 ^{abA}	0.30 ± 0.00 ^{abA}	0.17 ± 0.06 ^{aC}	0.23 ± 0.06 ^{abB}	0.20 ± 0.00 ^{bcB}	ES	ES
1.5% CS	0.20 ± 0.00 ^{aC}	0.33 ± 0.06 ^{abA}	0.27 ± 0.06 ^{bB}	0.20 ± 0.00 ^{aC}	0.30 ± 0.00 ^{aAB}	0.30 ± 0.00 ^{abAB}	0.27 ± 0.06 ^{abB}	ES
2% CS	0.20 ± 0.00 ^{de}	0.37 ± 0.06 ^{aA}	0.30 ± 0.00 ^{abABC}	0.23 ± 0.06 ^{cdE}	0.27 ± 0.06 ^{abBCD}	0.33 ± 0.06 ^{aAB}	0.17 ± 0.06 ^{bE}	ES
(c) L- Ascorbic acid (mg 100g⁻¹)								
Control	53.1 ± 0.7 ^{aA}	39.7 ± 1.5 ^{bB}	41.8 ± 0.2 ^{cB}	44.4 ± 5.1 ^{abB}	42.1 ± 2.8 ^{cB}	ES	ES	ES
0.2% NCS	53.1 ± 0.7 ^{aAB}	50.2 ± 0.1 ^{aB}	43.0 ± 5.8 ^{cd}	40.3 ± 4.1 ^{bcD}	51.4 ± 1.4 ^{abAB}	47.6 ± 0.8 ^{abBC}	44.3 ± 1.4 ^{aCD}	56.5 ± 5.34 ^{aA}
0.4% NCS	53.1 ± 0.7 ^{aA}	51.2 ± 3.9 ^{aAB}	49.2 ± 1.1 ^{aAB}	41.1 ± 3.0 ^{abcd}	46.4 ± 3.0 ^{cdeBC}	39.8 ± 4.7 ^d	38.0 ± 4.1 ^{aD}	48.8 ± 4.34 ^{aAB}
0.8% NCS	53.1 ± 0.7 ^{aAB}	48.0 ± 6.2 ^{abc}	40.1 ± 3.0 ^d	45.5 ± 0.3 ^{aCD}	54.6 ± 2.1 ^{aA}	43.8 ± 1.9 ^{bcCD}	ES	ES
1% CS	53.1 ± 0.7 ^{aA}	31.9 ± 1.5 ^{cC}	52.9 ± 1.9 ^{aA}	35.5 ± 2.3 ^{cC}	44.5 ± 3.6 ^{deB}	33.6 ± 1.1 ^{dC}	ES	ES
1.5% CS	53.1 ± 0.7 ^{aA}	48.3 ± 6.5 ^{aABC}	44.0 ± 1.8 ^{bcC}	44.7 ± 0.1 ^{abC}	46.6 ± 2.8 ^{cdBC}	50.6 ± 1.2 ^{aAB}	38.2 ± 2.6 ^{aD}	ES
2% CS	53.1 ± 0.7 ^{aAB}	54.1 ± 1.8 ^{aA}	48.4 ± 2.4 ^{abc}	41.36 ± 1.63 ^{abd}	49.0 ± 0.5 ^{bcBC}	50.7 ± 1.2 ^{aABC}	42.3 ± 5.4 ^{aD}	ES

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

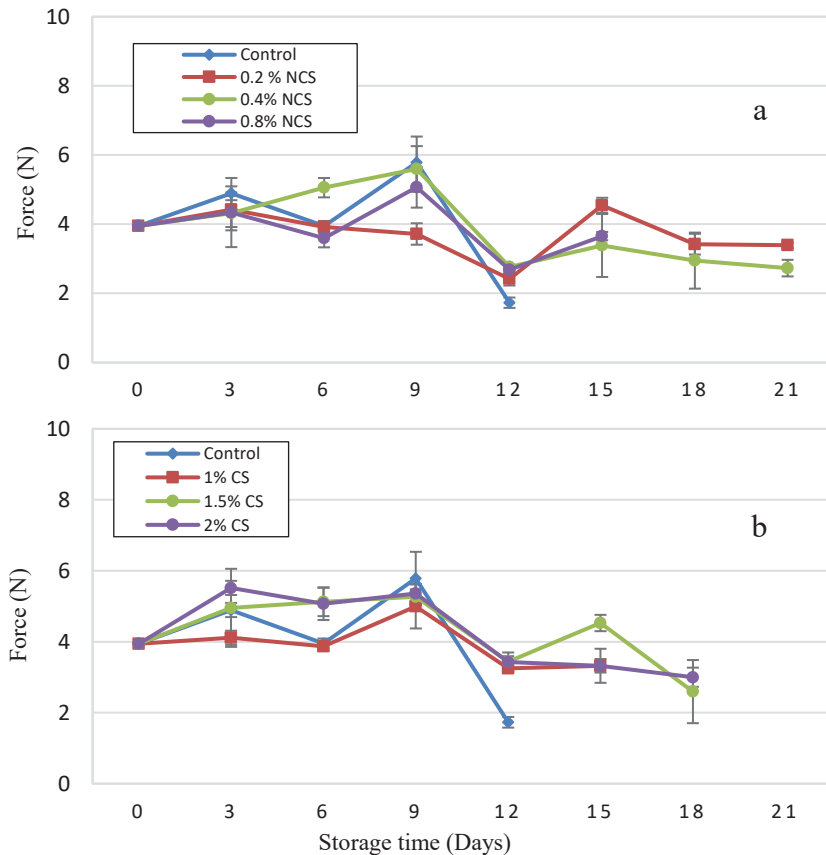


Fig. 4. Effects of coating on the firmness of strawberry coated with (a) nano-chitosan; (b) chitosan and stored at 2 °C

Table 2. Effects of chitosan and nano-chitosan concentration on total phenolic, total anthocyanin, malondialdehyde content, antioxidant capacity and polyphenol oxidase activity of strawberry stored at 2 °C

	Initial	3d	6d	9d	12d	15d	18d	21d
(a) Total phenolic content (mg 100g⁻¹)								
Control	159 ± 14.9 ^{aE}	217.5 ± 8.6 ^{aD}	348.0 ± 0.8 ^{aB}	266.7 ± 1.43 ^{bC}	383.6 ± 34.3 ^{aA}	ES	ES	ES
0.2% NCS	159 ± 14.9 ^{aB}	160.1 ± 9.6 ^{eB}	252.1 ± 29.5 ^{bca}	253.8 ± 11.9 ^{bca}	275.2 ± 5.8 ^{eA}	173.0 ± 11.7 ^{eB}	131.1 ± 14.2 ^{cC}	131.1 ± 14.2 ^{bc}
0.4% NCS	159 ± 14.9 ^{aCD}	173.6 ± 7.2 ^{dCD}	253.6 ± 9.0 ^{bcb}	337.4 ± 2.3 ^{aA}	312.2 ± 58.1 ^{bca}	157.5 ± 13.2 ^{dCD}	144.2 ± 14.7 ^{eD}	193.4 ± 9.1 ^{aC}
0.8% NCS	159 ± 14.9 ^{aC}	203.0 ± 1.6 ^{bcb}	235.0 ± 23.9 ^{caB}	240.1 ± 6.9 ^{edAB}	272.8 ± 43.3 ^{ca}	142.6 ± 0.0 ^{cC}	ES	ES
1% CS	159 ± 14.9 ^{aC}	207.5 ± 1.9 ^{abB}	206.7 ± 1.2 ^{dB}	203.1 ± 2.6 ^{eB}	264.0 ± 29.2 ^{ca}	217.0 ± 0.0 ^{bb}	ES	ES
1.5% CS	159 ± 14.9 ^{aE}	215.9 ± 1.5 ^{aC}	264.7 ± 6.6 ^{bb}	264.8 ± 27.8 ^{bb}	378.8 ± 8.7 ^{aA}	220.4 ± 1.1 ^{bc}	189.5 ± 3.0 ^{bD}	ES
2% CS	159 ± 14.9 ^{aE}	192.5 ± 6.1 ^{eD}	269.1 ± 1.1 ^{bb}	224.7 ± 5.5 ^{dc}	336.7 ± 7.4 ^{abA}	239.3 ± 0.0 ^{ac}	228.5 ± 17.4 ^{ac}	ES
(b) Total anthocyanin content (mg 100g⁻¹)								
Control	15.4 ± 1.0 ^{aAB}	15.6 ± 5.5 ^{aAB}	11.8 ± 0.4 ^{eB}	17.3 ± 1.6 ^{bca}	12.4 ± 1.4 ^{bb}	ES	ES	ES
0.2% NCS	15.4 ± 1.0 ^{aB}	12.6 ± 2.9 ^{abc}	19.2 ± 1.0 ^{aA}	15.2 ± 0.8 ^{cdB}	12.0 ± 0.2 ^{bcd}	11.2 ± 0.7 ^{acd}	13.3 ± 0.8 ^{abC}	10.1 ± 1.3 ^{ad}
0.4% NCS	15.4 ± 1.0 ^{aB}	12.8 ± 4.4 ^{abB}	14.9 ± 1.7 ^{bb}	20.4 ± 1.9 ^{aA}	13.7 ± 0.7 ^{bb}	9.2 ± 1.1 ^{bcC}	8.4 ± 0.7 ^{cC}	7.3 ± 0.1 ^{bc}
0.8% NCS	15.4 ± 1.0 ^{aBC}	14.0 ± 3.5 ^{abCD}	13.7 ± 1.4 ^{bcCD}	19.6 ± 0.8 ^{aA}	17.1 ± 1.0 ^{aAB}	10.9 ± 1.1 ^{abd}	ES	ES
1% CS	15.4 ± 1.0 ^{aAB}	15.3 ± 1.1 ^{aAB}	14.0 ± 3.6 ^{bcAB}	15.7 ± 1.8 ^{cdA}	12.4 ± 0.4 ^{bb}	8.0 ± 0.7 ^{cC}	ES	ES
1.5% CS	15.4 ± 1.0 ^{aA}	8.8 ± 1.3 ^{bd}	11.7 ± 0.2 ^{cC}	14.5 ± 0.8 ^{dAB}	12.6 ± 2.9 ^{bbc}	8.7 ± 0.7 ^{cd}	7.2 ± 0.6 ^{cd}	ES
2% CS	15.4 ± 1.0 ^{aB}	13.8 ± 0.9 ^{abB}	14.6 ± 0.3 ^{bcB}	18.3 ± 0.3 ^{abA}	9.3 ± 0.4 ^{cC}	10.9 ± 1.5 ^{abC}	10.9 ± 1.9 ^{bc}	ES
(c) Malondialdehyde (μmol g⁻¹)								
Control	39.7 ± 3.8 ^{aD}	45.6 ± 4.6 ^{abcCD}	50.6 ± 6.1 ^{eBC}	56.2 ± 0.0 ^{bb}	77.8 ± 0.6 ^{aA}	ES	ES	ES
0.2% NCS	39.7 ± 3.8 ^{aE}	49.6 ± 1.8 ^{abd}	63.9 ± 1.9 ^{aABC}	68.3 ± 0.5 ^{aA}	65.3 ± 3.2 ^{baB}	58.3 ± 6.6 ^{aBC}	60.9 ± 2.3 ^{abC}	57.7 ± 1.3 ^{aC}
0.4% NCS	39.7 ± 3.8 ^{aD}	40.3 ± 2.2 ^{cd}	53.5 ± 2.6 ^{bcB}	57.9 ± 6.0 ^{baB}	54.8 ± 2.0 ^{eB}	45.4 ± 2.8 ^{bcCD}	62.9 ± 3.9 ^{aA}	50.8 ± 2.7 ^{bcB}
0.8% NCS	39.7 ± 3.8 ^{aB}	52.6 ± 4.5 ^{aA}	57.8 ± 0.8 ^{ba}	52.4 ± 4.0 ^{bca}	50.6 ± 7.5 ^{caB}	54.0 ± 9.0 ^{aba}	ES	ES
1% CS	39.7 ± 3.8 ^{aB}	42.7 ± 6.6 ^{bcaB}	41.7 ± 2.7 ^{deAB}	48.4 ± 2.4 ^{cdAB}	40.8 ± 3.0 ^{dAB}	49.4 ± 1.8 ^{bca}	ES	ES
1.5% CS	39.7 ± 3.8 ^{aB}	50.4 ± 6.6 ^{aba}	36.8 ± 0.9 ^{eB}	44.9 ± 5.8 ^{cdAB}	37.9 ± 1.5 ^{dB}	49.9 ± 2.1 ^{bca}	51.6 ± 1.1 ^{ba}	ES
2% CS	39.7 ± 3.8 ^{aC}	50.1 ± 2.1 ^{abAB}	44.8 ± 2.4 ^{dBC}	42.2 ± 6.1 ^{dBC}	43.2 ± 2.3 ^{dBC}	43.7 ± 2.0 ^{eBC}	56.2 ± 6.7 ^{aba}	ES
(d) Antioxidant capacity (%)								
Control	83.2 ± 2.4 ^{aA}	82.8 ± 1.9 ^{abA}	83.2 ± 2.0 ^{abA}	83.2 ± 2.0 ^{aA}	69.3 ± 0.0 ^{eB}	ES	ES	ES
0.2% NCS	83.2 ± 2.4 ^{aA}	83.7 ± 1.8 ^{abA}	81.5 ± 2.4 ^{abAB}	77.5 ± 1.9 ^{eBC}	76.1 ± 4.2 ^{bc}	75.8 ± 3.3 ^{bc}	78.1 ± 2.5 ^{abBC}	75.9 ± 3.5 ^{aC}
0.4% NCS	83.2 ± 2.4 ^{aABC}	85.6 ± 1.7 ^{abA}	84.4 ± 3.2 ^{aAB}	80.4 ± 1.1 ^{bbC}	79.2 ± 1.0 ^{abCD}	79.0 ± 3.4 ^{abCD}	79.6 ± 3.0 ^{abBCD}	75.2 ± 5.1 ^{ad}
0.8% NCS	83.2 ± 2.4 ^{aAB}	86.0 ± 2.1 ^{abA}	84.4 ± 3.2 ^{aAB}	81.9 ± 0.4 ^{abAB}	82.8 ± 7.1 ^{aAB}	79.5 ± 1.0 ^{abB}	ES	ES
1% CS	83.2 ± 2.4 ^{aAB}	85.5 ± 2.5 ^{abA}	76.9 ± 7.4 ^{bb}	80.6 ± 1.6 ^{baB}	80.7 ± 1.1 ^{abAB}	82.2 ± 2.9 ^{aAB}	ES	ES
1.5% CS	83.2 ± 2.4 ^{aAB}	88.4 ± 2.6 ^{aA}	83.6 ± 3.9 ^{abAB}	80.2 ± 0.0 ^{bbC}	78.7 ± 4.2 ^{abBC}	77.3 ± 6.2 ^{abBC}	75.8 ± 3.1 ^{bc}	ES
2% CS	83.2 ± 2.4 ^{aA}	80.3 ± 8.6 ^{ba}	80.2 ± 2.0 ^{abA}	80.3 ± 0.9 ^{ba}	80.2 ± 1.1 ^{ba}	80.7 ± 0.6 ^{abA}	80.2 ± 1.3 ^{aA}	ES
(e) PPO activity (U g⁻¹)								
Control	2.4 ± 0.1 ^{aE}	11.5 ± 1.6 ^{abd}	50.6 ± 1.0 ^{aA}	38.0 ± 0.0 ^{ab}	15.9 ± 2.0 ^{abc}	ES	ES	ES
0.2% NCS	2.4 ± 0.1 ^{aF}	13.0 ± 1.6 ^{ab}	16.7 ± 2.1 ^{dcA}	13.5 ± 0.0 ^{cb}	7.5 ± 0.0 ^{abd}	5.0 ± 0.7 ^{dE}	4.5 ± 0.8 ^{aE}	10.8 ± 0.9 ^{aC}
0.4% NCS	2.4 ± 0.1 ^{aF}	10.7 ± 1.2 ^{bbc}	14.1 ± 3.1 ^{eB}	33.8 ± 2.4 ^{aA}	6.5 ± 0.6 ^{abDE}	8.2 ± 0.0 ^{bcd}	5.5 ± 1.2 ^{aDEF}	3.9 ± 1.3 ^{bEF}
0.8% NCS	2.4 ± 0.1 ^{aB}	4.0 ± 0.3 ^{deb}	35.9 ± 1.0 ^{ba}	32.5 ± 8.6 ^{aA}	4.4 ± 0.9 ^{bb}	6.1 ± 0.0 ^{cb}	ES	ES
1% CS	2.4 ± 0.1 ^{aC}	2.9 ± 1.6 ^{dc}	17.4 ± 1.7 ^{dB}	23.55 ± 0.9 ^{ba}	3.1 ± 0.6 ^{bc}	2.9 ± 0.0 ^{cc}	ES	ES
1.5% CS	2.4 ± 0.1 ^{aD}	6.5 ± 0.0 ^{cC}	23.2 ± 0.3 ^{eB}	25.9 ± 0.6 ^{ba}	6.5 ± 3.4 ^{abc}	5.3 ± 0.4 ^{dC}	4.8 ± 0.2 ^{cC}	ES
2% CS	2.4 ± 0.1 ^{aE}	4.2 ± 0.0 ^{dd}	15.5 ± 1.7 ^{fc}	20.2 ± 0.4 ^{ba}	10.1 ± 0.0 ^{aA}	10.9 ± 0.0 ^{ab}	2.7 ± 0.6 ^{bE}	ES

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

Total phenolic content, total anthocyanin content and malondialdehyde content

The phenolic concentration of uncoated samples increased until reaching the peak at 12th day; meanwhile, those of coated samples fluctuated during storage (Table 2a). This could be due to

the breakdown of the cell structure of the fruit senescence (Macheix & Fleuriet, 1990) and the formation of phenolic complexes during non-enzymatic reactions (Nunes et al., 2005). In this study, the fluctuation of total anthocyanin during storage period was recorded and explained below might also be correlated with the changes of TPC in strawberry.

The anthocyanin concentrations (TAC) of all fruit samples fluctuate during storage (Table 2b). It might be explained that the anthocyanin synthesis occurred in the initial stage of fruit storage via flavonoid and anthocyanin pathways (Ananga et al., 2013). TAC accumulated to the peak before it started the degradation stage correlating with cell breakdown of the fruit senescence process (Macheix & Fleuriet, 1990). As the cell was de-structured, the pigments, mostly anthocyanins, were degraded and reduced (Nunes et al., 2005). The effect of chitosan coating on delaying the decrease of anthocyanin content was recorded by Kerch et al. (2011). In overall, coating strawberry with 0.2% was the most effective one in maintaining TAC of fruit during storage.

It could be seen that the malondialdehyde (MDA) value in the uncoated sample stored at 2°C was significantly increased ($p < 0.05$) after 12 days of storage (Table 2c). However, coating strawberry with CS and NCS showed a better effect in delaying MDA production than the control treatment. The coating materials could be productive tools for preventing postharvest oxidative damage during cold storage, worked as a barrier to the oxygen responsible for lipid peroxidation, thereby maintaining the membrane integrity (Petrucciione et al., 2015).

Antioxidant capacity

After 12 days of storage, there were significantly higher in antioxidant capacity of coated fruits in compared with the uncoated one (Table 2d). Therefore, coating strawberry with CS and NCS could be suggested to maintain the antioxidant capacity in strawberries. This could be explained by the formation of a protective barrier on the surface of the fresh fruit, reducing water evaporation and inhibiting decline of antioxidant activity (Kou et al., 2014).

Polyphenol oxidase

Polyphenol oxidase activity of all samples increased during storage (Table 2e). The coating materials could act as a semi-permeable barrier against oxygen responsible for polyphenol oxidase reaction (Petrucciione et al., 2015). The inhibitory effect of chitosan coating on PPO activity was consistent with a previous study on sweet cherry (Pasquariello et al., 2015). After 15 days, coating strawberry with 1% chitosan showed the most effective on inhibiting the PPO activity ($p < 0.05$). Besides, in terms of NCS coating, coating fruit with 0.4% NCS greater remained the value of polyphenol oxidase after 21 days as compared with the 0.2% NCS one.

CONCLUSION

In the present study, chitosan and nano-chitosan working as physical barriers brought positive effects in preserving the strawberry quality. However, coating fruit with 0.2% NCS was the most effective treatment than the others on maintaining the overall quality index, firmness, total anthocyanin content, total soluble solid, L-ascorbic acid contents and antioxidant capacity of strawberries.

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CONFLICT OF INTEREST

The authors have no conflict of interest to report.

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