



## Effects of ethanol and chitosan treatments on the quality and storage life of minimally processed pumpkin (*Cucurbita moschata* Duch)

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### ABSTRACT

**Purpose:** Pretreatments of ethanol and chitosan immersion were examined for their potential to maintain physiochemical attributes of fresh cut pumpkin. **Research method:** Fresh cut pumpkin cubes were dipped into different ethanol solutions (20%, 30%, 40%, 50%) or chitosan concentrations (0.5%, 1%, 1.5%). All samples were stored for 15 days at 10°C. **Main findings:** Among four concentrations being applied, the 30% ethanol sample (ET 30) sustained the highest sensory quality until the final day and effectively retained fruit firmness, total soluble solids, total phenolic content compared to the 20% ethanol treatment (ET 20) stored at the same condition. Chitosan application retained better content of carotenoid, phenolic compounds, firmness, and reduced weight loss compared to non – chitosan treatment but there was no significant difference among concentrations. As a result, overall quality index of the coated samples surpassed control ones, especially 1% chitosan. The coating did not affect total soluble solids and antioxidant capacity. **Limitations:** The investigations of antioxidant and cell wall degrading enzymes were absent to support for the study's results. **Originality/Value:** The combination of 30% ethanol and 1% chitosan suggested a possible application in practical context as it outperformed in maintaining the quality and prolonging storage time of the product up to 15 days at 10°C.

## INTRODUCTION

Pumpkin (*Cucurbita moschata* Duch) has become a popular crop as the vegetable is able to adapt to various types of soil and microclimate. As the consumption of ready-to-eat foods increases rapidly in crowded cities, pumpkin mostly comes in minimally processed forms. According to USDA, minimally processed products are the ones that undergo processing without being fundamentally altered.

The challenges of minimal processing, as it exposes internal tissues to surrounding, are facilitated water evaporation, enzymatic browning by polyphenol oxidase and microbial spoilage (Garcia & Barrett, 2002). The effects of starch – based coatings have been widely studied on pumpkin. However, polysaccharides-based coatings owe their effectiveness to the gas barrier properties. Nutritional depletion, therefore, cannot be hindered sufficiently. Furthermore, a thick coating layer can provide the commodity a micro-anaerobic condition, initiating fermentation and lead to rapid deterioration. On the other hand, low temperature and modified atmosphere packaging possibly leaves pumpkin chilling injuries and damaged texture.

Ethanol treatment is a classic method that has been used to solve common problems of fresh cut produce such as enzymatic browning, microbial spoilage (Gao et al., 2018). Effects of ethanol on endogenous enzymes such as cell wall degrading enzymes, hydrolases were expected in minimally processed pumpkin (MPP) as observed in sweet cherries (Bai et al., 2011). Ethanol was also used in extending the storage life of fresh-cut Chinese yam (Gao et al., 2018), indicated by least changes for O<sub>2</sub>, CO<sub>2</sub> in headspace package, and reducing physiological metabolism and preserving the surface of fresh-cut eggplant (Hu et al., 2010). Chitosan, derived from the deacylation of chitin, one of the most abundant polysaccharides in nature, can be an ideal coating material because of its ability to form film layer, hydrophilic nature and antimicrobial properties (Li & Yu, 2001). In the study of Suwannarak et al. (2015), chitosan 0.25% or 0.5% exhibited the most effective result for quality improvement and shelf life extension of the carved pumpkin, cantaloupe, and carrot. The addition of chitosan coating on minimally processed pumpkin was concluded to be efficient in minimizing water vapor, carotenoid degradation and microbial growth, hence, maintaining high quality of the vegetable for a longer period of time (Suwannarak et al., 2015).

The objective of this study was to assess the effects of different ethanol and chitosan concentrations on retarding weight loss, maintaining firmness, visual attributes, and preserving total soluble solids, total phenolic compounds, antioxidant capacity and carotenoid content of fresh cut pumpkin stored at 10°C.

## MATERIALS AND METHODS

### Materials

Fresh pumpkin fruits (*Cucurbita moschata* Duch) were collected from a farm in TienGiang province at intermediate level of maturity with no skin defects, uniform color, size and shape. Fruits were washed under running tap and peeled by sanitized knife. Seeds and sponge parts inside were removed completely and fruit flesh was cut into dice of 2 cm × 2 cm.

### Experimental design

#### *Experiment 1*

Ethanol treatment procedure followed the experiment of Gao et al. (2018). Pumpkin cubes would be immersed in ethanol solutions (Merck Chemicals Ltd., Darmstadt, Germany) of

20%, 30%, 40% and 50% for 2 minutes and subsequently soaked in 0.5% chitosan solution for 8 minutes. Control sample was submerged in distilled water instead of ethanol solution.

### Experiment 2

Chitosan solutions were prepared by dissolving chitosan powder (Sigma-Aldrich, St. Louis, MO, USA) in 0.5% acetic acid solution (Merck Chemicals Ltd., Darmstadt, Germany) at ratio 2:1 (Soares et al., 2018). Pumpkin was soaked into 30% ethanol determined from previous experiment before an 8 minute chitosan immersion. Control sample was immersed in 0.5% acetic acid. There was a group of no ethanol and chitosan dipping to intimidate supermarket condition. After coating steps, dry treated cubes in room condition and pack in styrofoam tray of 100 g portion, cover with PVC film and store at 10°C. Samples were analyzed at three day interval.

### Analytical methods

#### Determination of overall quality index

Different deterioration stages were assessed visually in scale of 5 with detail descriptions corresponding to subjective scores (Fig. 1) by trained personnel. The rating scale was developed based on verbal description of Cantwell and Suslow (2014).



**Fig. 1.** The overall rating chart of fresh cut pumpkin with photographs of pumpkin at different stages of visual deterioration corresponding to subjective scores and description.

**Determination of weight loss (%)**

Weight loss in %, was calculated as the ratio of the weight of the pumpkin portion at day of analysis to the initial weight of the coated portion and mathematically expressed as following formula (1):

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

Where  $W_i$  is the initial weight of coated sample and  $W_f$  is the weight of sample on analyzing day, determined using a top loading balance (TXB- 622L, Shimadzu Co, LTD., Japan) (Santos et al., 2016).

**Determination of total soluble solids (%)**

Total soluble solids (TSS) was determined using refractometer (RX- 5000, Atago Co., LTD., Japan) at 25°C and the results were expressed as % Sucrose. Pumpkin flesh was homogenized with distilled water at the ratio of 1:5 (w/v). After centrifugation at 4000 rpm for 10 minutes, the supernatant was used for TSS analysis (Hernández-Muñoz et al., 2006).

**Determination of firmness (N)**

Fruit firmness was determined by digital fruit firmness tester according to Hernández-Muñoz et al. (2006). The result obtained from Digital Fruit Hardness Tester (FR- 5120, Lutron electronic enterprise Co., LTD., Taiwan) using 2mm tip was expressed in N unit.

**Extract preparation**

Extraction procedure followed the procedure of Nawirska-Olszańska et al. (2011). Specifically, 5 ml of sample was mixed with 25 ml of 80% methanol (Merck Chemicals Ltd., Darmstadt, Germany) (v/v) and sonicated for 30 minutes at room temperature. The extract was applied with centrifugation (UNIVERSAL 320R, Andreas Hettich GmbH & Co. KG, Germany) at 4000 rpm for 5 minutes at 4°C. The supernatant was then used for measurement of total phenolic compounds and antioxidant capacity.

**Determination of total phenolic content ( $\mu\text{g GAE g}^{-1}$ )**

Total phenolic content (TPC) was determined by Folin-Ciocalteu assay as described by Singleton and Rossi (1965). The absorbance was recorded at 760 nm with a UV-visible spectrophotometer (GENESYS 10 UV-Vis, Thermo Fisher Scientific, Inc., USA). Gallic acid was used to construct a calibration curve and results were expressed as  $\mu\text{g}$  of gallic acid equivalents per g pumpkin ( $\mu\text{g GAE g}^{-1}$ ).

**Determination of antioxidant capacity (%)**

DPPH assay was modified from a method of Lim et al. (2007). The absorbance was measured against a blank at 520 nm with a UV-Visible spectrophotometer. The percentage of free radical scavenging effect was calculated as (2):

$$\text{DPPH scavenging effect (\%)} = \left(1 - \frac{A}{A_0}\right) \times 100 \quad (2)$$

Where,  $A_0$  is the absorbance of the control solution and  $A$  is the absorbance of the DPPH solution containing sample extract at 520nm.

***Determination of total carotenoid content ( $\mu\text{g } \beta\text{-carotene g}^{-1}$ )***

Total carotenoid content was quantified using spectrophotometric analysis, as described by Rodriguez-Amaya (2001). Carotenoid was extracted using hexane (Merck Chemicals Ltd., Darmstadt, Germany) as the only solvent. First, 0.5 g sample was incubated in 15 minutes with 10 ml hexane, then, centrifuge the mixture at 4000 rpm for 15 minutes at 4°C. The absorbance was measured using a UV spectrometer at 450 nm. Carotenoid concentration was expressed as  $\mu\text{g } \beta\text{-carotene g}^{-1}$ .

***Statistical analysis***

All analyses were conducted in triplicate; the data were expressed as mean  $\pm$  standard deviation. To determine differences among treatments in each experiment, one way ANOVA and the least significant difference (Fisher's LSD) were used. Statistical analysis was carried out using Minitab software package (Version 18.0, Minitab Pty Ltd., Australia) with 95% level of confidence.

**RESULTS AND DISCUSSION****Effects of ethanol concentrations on the postharvest quality of fresh cut pumpkin*****Overall quality index***

Main indices for quality declination include translucent appearance, referred to as white blush, surface shriveling, decaying mold and off odor. High ethanol concentration was better at releasing carotenoid from cell components, thus enhance visual appeal of minimally processed fruits and vegetables (Homaida et al., 2017). However, such treatment also causes off odor as well as degradation of plasma membrane of vegetable tissue (Bai et al., 2011), leading to decreased sensory quality score. Those positive and negative effects occurred at the same time in different replicates, resulting in inconsistent scores of 40% ethanol treatment (ET 40) and 50% ethanol treatment (ET 50) (Table 1). Alcoholic off odor was noticed in 30% ethanol treated fresh cut lotus root slice (Gao et al., 2017) but was absent in 30% ethanol (ET 30) treated pumpkin. The result indicates that ET 30 was effective in preserving market appeal of fresh cut pumpkin.

***Weight loss***

The physiological weight loss of MPP found in this experiment is remarkably higher than the reported values from previous studies which were less than 5% (Cortez-Vega et al., 2014). The difference may source from packaging materials, PVC film used in this study possesses lower moisture retarding activity than previously used PE package (Kjeldsen, 1993). The fact that no significant difference observed in weight loss percentage ( $p = 0.43$ ) among treatments (Table 1) suggests that ethanol soaking generally had no effects on water loss of fresh cut pumpkin. Respiration rate, which is mainly responsible for the transpiration of fruits after harvesting, was also found not altered by ethanol treatment in tomato and fresh cut banana (Ritenour et al., 1997).

***Firmness***

High concentration ethanol immersion caused accelerated water loss, leading to increasing penetration force at first, then plant tissue senescence decreased the force (Table 1) (Cortez-Vega et al., 2014). On the fifteenth day, firmness of ET 30 samples was significantly higher than that of ET 20 and CO samples, proving the ability of ethanol to keep membrane rigid during storage time. Ethanol treatment was reported to effectively maintain firmness by Pesis

(2005) due to its effect on endogenous plant cell wall degrading enzymes such as pectinase, cellulase, polygalacturonase which dwell in cell lysosome. With a hydrophobic tail, ethanol can pass through the phospholipid bilayer of cell membrane without causing damage and impact on cell components, including lysosome, thereby limit the activity of cell degrading enzymes (Pesis, 2005).

### Total soluble solids

The sugar conversion in MPP which was induced by enzymes such as pectinase, invertase were delayed by ethanol until after day 6, later than reported in previous studies of MPP not treated with ethanol (Santos et al., 2016). This effect of ethanol treatment contradicted the conclusions of previous studies that ethanol treatments had no impact on total soluble solids (Plotto et al., 2006). However, this phenomenon can be explained by the ability of ethanol to disrupt linkages between solutes and plant matrices (Şahin & Şamlı, 2013), directly increases soluble solid content of sample.

### Total phenolic content

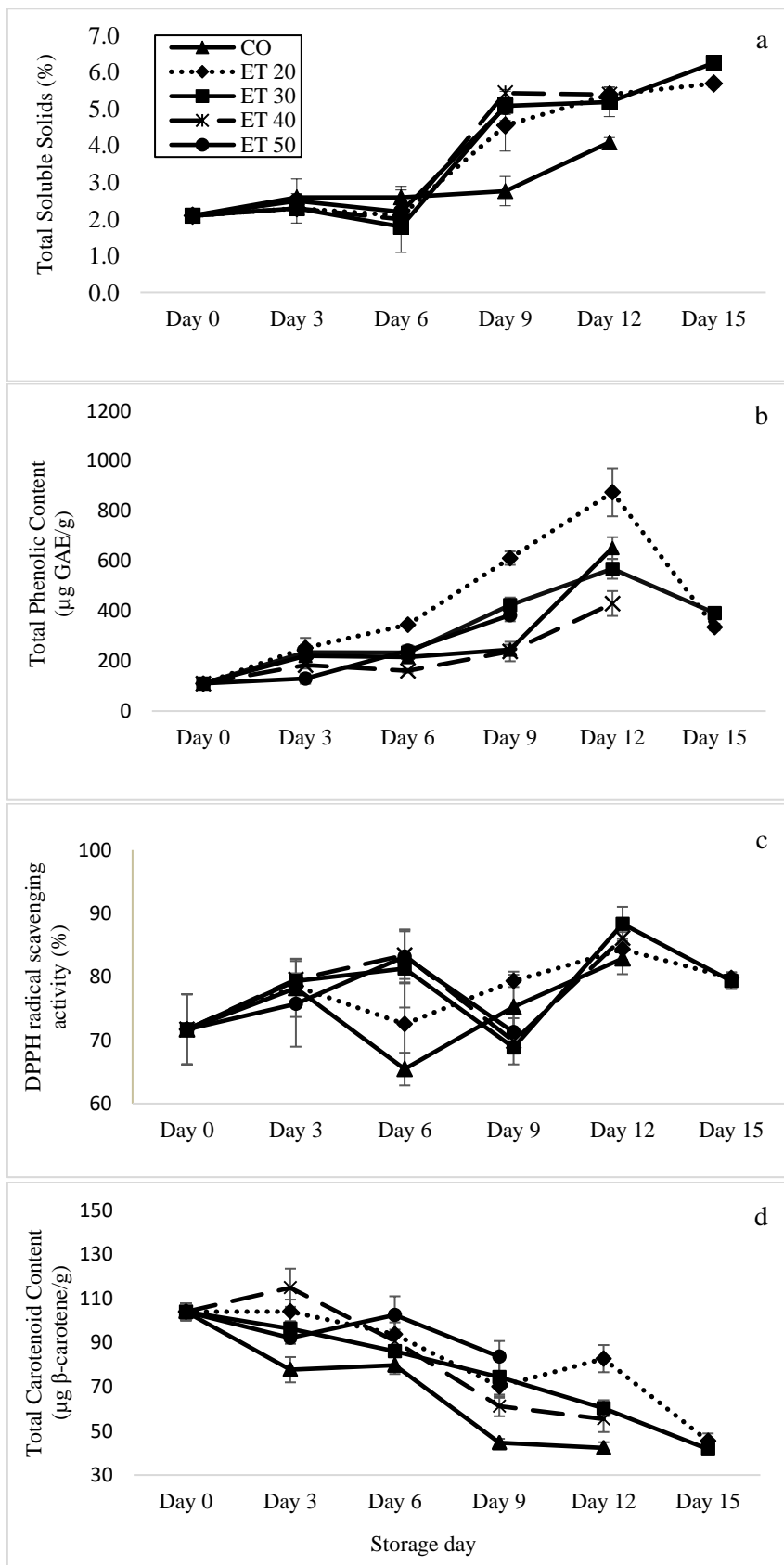
Generally, phenolic content of MPP increased significantly after fifteen days (Fig. 2b), attributable to the activity of phenylalanine ammonia lyase (PAL) enzyme and the extractability ethanol. The growing phase before day 12 was induced by cut damage, with PAL catalyzing the production of phenolic compounds through phenylpropanoid pathway (Halpin, 2004). The activity of polyphenol oxidase (PPO) enzyme was responsible for the phenolic metabolism, causing tissue browning or off color (Garcia & Barrett, 2002) in MPP during the final period. The ethanol pretreatment of MPP suggested a positive effect on phenolic accumulation as ET 30 retained the most TPC. The compatible polarity between phenolic compounds and ethanol accounted for this difference (Şahin & Şamlı, 2013).

**Table 1.** Overall quality score, weight loss percentage and firmness over storage time of minimally processed pumpkin of different ethanol treatments stored at 10°C

	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
Overall quality index						
ET 50	5.00 ± 0.00 <sup>aA</sup>	4.33 ± 0.58 <sup>aB</sup>	3.33 ± 0.58 <sup>aB</sup>	2.67 ± 0.58 <sup>bB</sup>	ES	ES
ET 40	5.00 ± 0.00 <sup>aA</sup>	4.33 ± 0.58 <sup>aB</sup>	3.33 ± 0.58 <sup>aB</sup>	3.00 ± 0.00 <sup>bB</sup>	2.67 ± 0.58 <sup>aB</sup>	ES
ET 30	5.00 ± 0.00 <sup>aA</sup>	4.33 ± 0.58 <sup>aAB</sup>	3.67 ± 0.58 <sup>aBC</sup>	3.67 ± 0.58 <sup>aBC</sup>	3.33 ± 0.58 <sup>aC</sup>	3.00 ± 0.00 <sup>aC</sup>
ET 20	5.00 ± 0.00 <sup>aA</sup>	4.00 ± 1.00 <sup>aAB</sup>	4.33 ± 0.58 <sup>aAB</sup>	4.00 ± 0.00 <sup>aAB</sup>	3.33 ± 0.58 <sup>aBC</sup>	2.67 ± 0.58 <sup>abC</sup>
CO	5.00 ± 0.00 <sup>aA</sup>	4.33 ± 0.58 <sup>aAB</sup>	4.33 ± 0.58 <sup>aAB</sup>	4.00 ± 0.00 <sup>aB</sup>	3.00 ± 0.00 <sup>aC</sup>	ES
Weight loss (%)						
ET 50	0.00 ± 0.00 <sup>aB</sup>	1.98 ± 3.42 <sup>bB</sup>	10.44 ± 0.63 <sup>abA</sup>	13.54 ± 0.96 <sup>aA</sup>	ES	ES
ET 40	0.00 ± 0.00 <sup>aD</sup>	6.20 ± 0.34 <sup>aC</sup>	11.18 ± 1.68 <sup>aB</sup>	16.11 ± 0.61 <sup>aA</sup>	18.72 ± 0.61 <sup>aA</sup>	ES
ET 30	0.00 ± 0.00 <sup>aD</sup>	6.65 ± 0.86 <sup>aC</sup>	9.53 ± 1.42 <sup>bC</sup>	11.35 ± 2.85 <sup>aBC</sup>	15.81 ± 1.16 <sup>aB</sup>	22.77 ± 1.96 <sup>aA</sup>
ET 20	0.00 ± 0.00 <sup>aE</sup>	6.55 ± 0.35 <sup>aD</sup>	11.94 ± 0.90 <sup>aCD</sup>	19.16 ± 0.65 <sup>aAB</sup>	16.97 ± 7.14 <sup>aBC</sup>	24.88 ± 3.79 <sup>aA</sup>
CO	0.00 ± 0.00 <sup>aC</sup>	4.68 ± 0.84 <sup>abC</sup>	11.48 ± 0.90 <sup>aB</sup>	16.63 ± 6.76 <sup>aAB</sup>	21.35 ± 0.57 <sup>aA</sup>	ES
Firmness (N)						
ET 50	22.37 ± 2.71 <sup>aC</sup>	27.28 ± 3.42 <sup>abAB</sup>	31.59 ± 3.07 <sup>abA</sup>	23.91 ± 1.52 <sup>bBC</sup>	ES	ES
ET 40	22.37 ± 2.71 <sup>aC</sup>	31.62 ± 0.34 <sup>aA</sup>	27.90 ± 1.33 <sup>bcB</sup>	24.96 ± 0.20 <sup>bC</sup>	24.34 ± 1.69 <sup>aC</sup>	ES
ET 30	22.37 ± 2.71 <sup>aC</sup>	24.86 ± 0.86 <sup>cBC</sup>	32.24 ± 2.60 <sup>aA</sup>	26.98 ± 0.41 <sup>aB</sup>	21.99 ± 1.62 <sup>abC</sup>	27.38 ± 1.23 <sup>aB</sup>
ET 20	22.37 ± 2.71 <sup>aABC</sup>	24.33 ± 0.35 <sup>cA</sup>	22.90 ± 0.28 <sup>dABC</sup>	21.69 ± 0.41 <sup>cBC</sup>	20.55 ± 0.59 <sup>bC</sup>	23.75 ± 1.72 <sup>abB</sup>
CO	22.37 ± 2.71 <sup>aB</sup>	26.07 ± 0.84 <sup>bcA</sup>	26.13 ± 1.90 <sup>cdA</sup>	24.17 ± 0.32 <sup>abAB</sup>	23.65 ± 2.24 <sup>aAB</sup>	ES

Data was expressed as mean ± SD. Means in same column with different lowercase letters are not statically different at 5% significance. Equal capital letters in a row do not differ statically at 5% significance by Fisher's test.

ES: end of storage. ET 50: ethanol 50%; ET 40: ethanol 40%; ET 30: ethanol 30%; ET 20: ethanol 20%; CO: control samples.



**Fig. 2.** The changes in (a) total soluble solids; (b) total phenolic content; (c) antioxidant capacity; (d) total carotenoid content of different ethanol treatments stored at 10°C during 15 days.

### Antioxidant capacity

After 15 days, antioxidant capacity of samples from all treatments increased by 11% with no significant variation among treatment groups (Fig. 2c). The mechanism of plant coping with reactive oxygen species in response to postharvest stresses includes enzymatic and non – enzymatic detoxification (Toivonen, 2004). Enzymatic antioxidants consist of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), etc. located mostly in cell membrane, peroxisome and mitochondrion (Toivonen, 2004). However, the high dose application of ethanol on MPP could cause the rupture of plasma membrane and initiate phytotoxic effects on mitochondria (Li et al., 2018). Such effects hindered activities of antioxidant enzymes. Despite that, ethanol soaking delayed the drop of antioxidant capacity.

### Carotenoid content

Despite the 54% reduction in average value, carotenoid content in treated samples were remarkably higher than in the control throughout storage time (Fig. 2d). The destructive effect of ethanol on chromoplasts (Kulczynski & Gramza-Michalowska, 2019) caused the leakage of more color pigments, thus strongly enhanced visual appeal of displayed fresh cut pumpkin. Contradicting to this advantage, ethanol treatment may cause the leakage other cell components such as sugars, phospholipids due to its polarity feature and lead to lowered carotenoid selectivity (Takahashi et al., 2006). This fact explains the phenomenon of high ethanol treatments (ET 40, ET 50) not causing high retention of carotenoid comparing to low dose treatments.

**Table 2.** Overall quality score, weight loss percentage, firmness over storage time of minimally processed pumpkin of different chitosan treatments stored at 10°C

	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
Overall quality index						
CH 0.5	5.00 ± 0.00 <sup>aA</sup>	4.67 ± 0.58 <sup>aA</sup>	4.33 ± 0.58 <sup>abA</sup>	4.33 ± 0.58 <sup>aA</sup>	3.33 ± 0.58 <sup>aB</sup>	3.00 ± 0.00 <sup>aB</sup>
CH 1	5.00 ± 0.00 <sup>aA</sup>	4.33 ± 0.58 <sup>abB</sup>	4.00 ± 0.00 <sup>abBC</sup>	4.00 ± 0.00 <sup>abBC</sup>	3.67 ± 0.58 <sup>aC</sup>	4.00 ± 0.00 <sup>bBC</sup>
CH 1.5	5.00 ± 0.00 <sup>aA</sup>	3.33 ± 0.58 <sup>bBC</sup>	3.67 ± 0.58 <sup>bB</sup>	3.33 ± 0.58 <sup>bBC</sup>	2.67 ± 0.58 <sup>aC</sup>	ES
ND	5.00 ± 0.00 <sup>aA</sup>	4.67 ± 0.58 <sup>aAB</sup>	4.67 ± 0.58 <sup>aAB</sup>	4.33 ± 0.58 <sup>aAB</sup>	3.33 ± 1.16 <sup>aBC</sup>	ES
AA	5.00 ± 0.00 <sup>aA</sup>	3.33 ± 0.58 <sup>bB</sup>	2.67 ± 0.58 <sup>cB</sup>	ES	ES	ES
Weight loss (%)						
CH 0.5	0.00 ± 0.00 <sup>aE</sup>	5.66 ± 1.02 <sup>aD</sup>	9.76 ± 1.40 <sup>abC</sup>	11.29 ± 0.29 <sup>bC</sup>	14.14 ± 0.11 <sup>bB</sup>	20.41 ± 0.53 <sup>aA</sup>
CH 1	0.00 ± 0.00 <sup>aF</sup>	6.33 ± 1.32 <sup>aE</sup>	9.84 ± 1.30 <sup>abD</sup>	12.36 ± 1.52 <sup>abC</sup>	15.91 ± 1.68 <sup>bB</sup>	18.85 ± 1.04 <sup>aA</sup>
CH 1.5	0.00 ± 0.00 <sup>aE</sup>	5.29 ± 0.48 <sup>aD</sup>	9.11 ± 0.78 <sup>bC</sup>	11.69 ± 0.93 <sup>bB</sup>	15.17 ± 1.10 <sup>bA</sup>	ES
ND	0.00 ± 0.00 <sup>aE</sup>	6.17 ± 0.08 <sup>aD</sup>	10.97 ± 0.79 <sup>aC</sup>	13.79 ± 0.70 <sup>aB</sup>	19.01 ± 0.95 <sup>aA</sup>	ES
AA	0.00 ± 0.00 <sup>aC</sup>	6.46 ± 1.28 <sup>aB</sup>	10.42 ± 0.41 <sup>abA</sup>	ES	ES	ES
Firmness (N)						
CH 0.5	26.98 ± 1.02 <sup>aA</sup>	21.88 ± 0.44 <sup>cCD</sup>	24.11 ± 0.55 <sup>aB</sup>	21.10 ± 0.40 <sup>aCD</sup>	20.39 ± 0.28 <sup>aD</sup>	22.02 ± 1.28 <sup>aC</sup>
CH 1	26.98 ± 1.02 <sup>aA</sup>	26.33 ± 1.31 <sup>aA</sup>	24.99 ± 1.48 <sup>aAB</sup>	24.11 ± 0.55 <sup>bB</sup>	23.81 ± 1.15 <sup>bB</sup>	24.96 ± 1.25 <sup>bAB</sup>
CH 1.5	26.98 ± 1.02 <sup>aAB</sup>	23.62 ± 2.35 <sup>bcC</sup>	26.23 ± 2.16 <sup>aABC</sup>	28.03 ± 0.93 <sup>bA</sup>	24.73 ± 1.03 <sup>bBC</sup>	ES
ND	26.98 ± 1.02 <sup>aA</sup>	25.12 ± 1.77 <sup>abA</sup>	20.65 ± 1.09 <sup>bB</sup>	17.84 ± 0.45 <sup>dC</sup>	20.09 ± 0.59 <sup>aB</sup>	ES
AA	26.98 ± 1.02 <sup>aA</sup>	21.62 ± 0.20 <sup>cB</sup>	12.54 ± 0.69 <sup>cC</sup>	ES	ES	ES

Data was expressed as mean ± SD. Means in same column with different lowercase letters are not statically different at 5% significance. Equal capital letters in a row do not differ statically at 5% significance by Fisher's test.  
ES: end of storage; CH 0.5: chitosan 0.5%; CH 1: chitosan 1%; CH 1.5: chitosan 1.5%; ND: no dipping, AA: acetic acid.



## Effects of chitosan coating on the postharvest quality of fresh cut pumpkin

### *Overall quality index*

Samples soaked in acetic acid were the soonest to be discarded due to watery appearance, sour odor after six days of storage. It is reported that acetic acid in contact with plant could cause rapid desiccation and facilitate food deterioration (Roos & Drusch, 2015). On the contrary, chitosan coating significantly delayed the decrease in visual sensory score (Table 2). The main trait that dropped the quality score of chitosan treatments was surface white blush, also concerned in minimally processed carrot (Bolin & Huxsoll, 1991). The removal of epidermal outer layer of mature plants initiates the formation of another protective layer, causing the milky white appearance (Bolin & Huxsoll, 1991). With the increase of chitosan concentration, the level of whitening was heightened due to polysaccharides nature of chitosan, leaving white color once dried (Arnon-Rips et al., 2019). No dipping treatment (ND) retained good color throughout storage time but sour smell was detected from day 12, threatened its commercial acceptance. In the view of market acceptability, the chitosan 1% (CH 1) treatment was the most favored.

### *Weight loss*

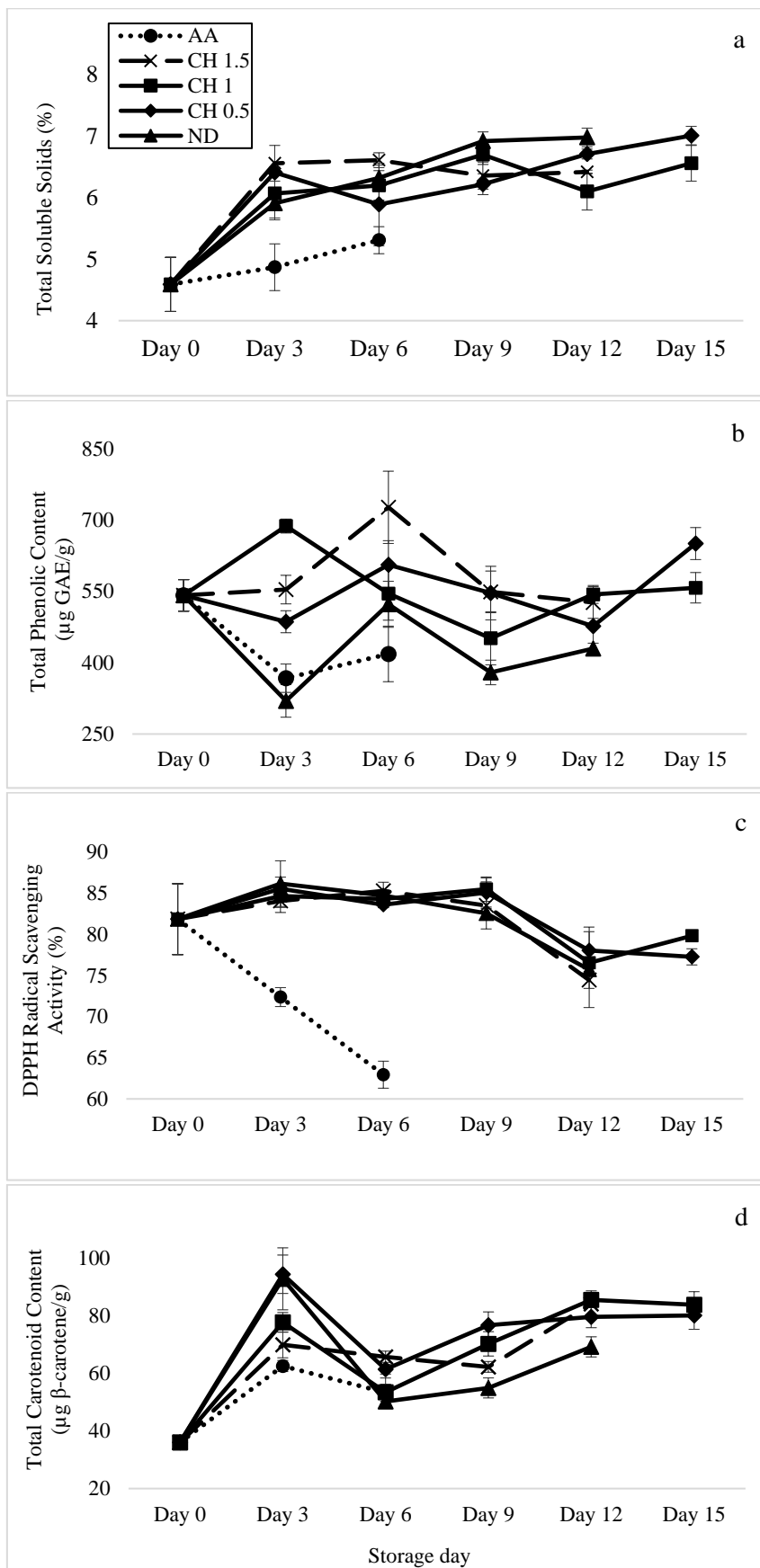
By forming a semi permeable barrier on the surface of minimally processed fruits and vegetables, chitosan retards transpiration rate, slows water loss and texture degradation (Li & Yu, 2001). However, no statistical disparity was recorded when increasing chitosan concentration (Table 2). A research by Soares et al. (2018) showed increased coating incorporation in higher chitosan concentrations, but no difference in water content was observed after a 16 day storage. The fact that water vapor barrier properties of hydrophilic chitosan film decreased remarkably with time (Arnon-Rips et al., 2019) can explain these observations. Control sample treated with acetic acid (0.5% v/v) behaved similarly to ND sample suggested that the addition of acetic acid in solubilizing chitosan did not have any unusual effects on the fresh cut pumpkin.

### *Firmness*

It can be inferred from Table 2 that firmness retention in MPP was obtained by sufficient chitosan coatings of 1% and 0.5%. The coating matrix lowered Lipoxygenases reactivity, which catalyzes the oxidation of plant plasma membrane by limiting the presence of oxygen in cell, thereby preserving the membrane integrity (Tian et al., 2004). On the other hand, the acidic environment created by acetic acid facilitated the deoxygenation of polyunsaturated fatty acids, subsequently damage cell membrane (Harwood & Moore Jr, 1989), made AA samples unmarketable from day 6. The ND samples, despite being acceptable until day 12, required so low penetration force that may imply an irreversible damage of cell outer layer by senescence (Simon, 1974). Eventually, CH 1 proved to be the best treatment to preserve MPP firmness.

### *Total soluble solids*

The content of TSS increased significantly after 15 days of storage for all treatments with the highest value belonged to ND group (Fig. 3a). The extractability of ethanol observed in the previous experiment was overshadowed by cell wall disassembly in ND which led to the leakage of cell components, in agreement with previous studies conducted on mango, banana and strawberry (Kittur et al., 2001; Petriccione et al., 2015). The amount of soluble solids in MPP remarkably inclined during storage period, consistent with result of Santos et al. (2016).



**Fig.3.** The changes in (a) total soluble solids; (b) total phenolic content; (c) antioxidant capacity; (d) total carotenoid content of different chitosan treatments stored at 10°C during 15 days.

### ***Total phenolic content***

The total phenolic content of chitosan – treated groups experienced various fluctuations and ended up statistically the same with initial value (Fig. 3b). High oxygen in the surrounding environment promoted phenolic compounds depletion, especially oxidation by PPO in uncoated samples (Pareek, 2016). Chitosan was proved to trigger defense response in vegetative tissue by activating PAL enzyme, the key enzyme in phenol synthesis pathway (Romanazzi et al., 2017). Such chitosan – induced effect successfully preserved phenolic content in fresh cut pumpkin cubes. The high phenolic content of MPP at the end of storage time suggested that the commodities remained in high quality, cell breakdown due to senescence during storage did not occur yet.

### ***Antioxidant capacity***

There was no significant difference in antioxidant capacity among treatment groups except for AA treatment, until the final day of storage (Fig. 3c). The samples applied with acetic acid soaking immediately decreased in the free radical scavenging ability after day 0 as the result of improved lipoxygenase (LOX) activity in acidic environment, causing cell rupture and increased membrane free radical (Engwa, 2018). The antioxidant capacity of MPP in this study was not correlated with total phenolic content suggesting that antioxidant activity of MPP depending much on antioxidant enzymes (superoxide dismutase, peroxidase, and catalase ) and non – phenolic compounds (ascorbic acid, glutathione) (Mittler, 2002). The application of chitosan coating was reported to partially inhibit the activity of POD enzyme in fresh cut litchi by Zhang and Quantick (1997), hence limited the radical scavenging capacity of chitosan coated MPP.

### ***Carotenoid content***

The protection of chitosan against carotenoid depletion could be due to the selective permeability of chitosan – acetic complex. The coating layer formed by chitosan dissolved in acetic solution gives high permeability to oxygen but sufficiently low absorption and release activity to carbon dioxide (Tian et al., 2004). Such properties limited the contact of carotenoids with oxygen, a potent oxidizing agent, hence retained significantly higher carotenoid content in coated samples (Fig. 3d) (Kulczynski & Gramza-Michalowska, 2019). The result of this study is consistent with previous researches of chitosan application on MPP (Soares et al., 2018), sliced mango (Plotto et al., 2006).

## **CONCLUSIONS**

The results of this study show a promising application of ethanol and chitosan on fresh cut produce, especially pumpkin. The treatment of 30% ethanol maintained higher overall quality index as well as preserved the most of physiochemical attributes of MPP, including firmness, total soluble solids and total phenolic content, despite its side effects in interrupting cell membrane. The coating of 1% chitosan pretreated with 30% ethanol not only acted as a physical barrier but also an interactive outer skin that helped to protect plant cell against aging and retain more nutrients.

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**Conflict of Interest**

The authors have no conflict of interest to report.

**REFERENCES**

- Arnon-Rips, H., Porat, R., & Poverenov, E. (2019). Enhancement of agricultural produce quality and storability using citral-based edible coatings; the valuable effect of nano-emulsification in a solid-state delivery on fresh-cut melons model. *Food Chemistry*, 277, 205-212. <https://doi.org/10.1016/j.foodchem.2018.10.117>
- Bai, J., Plotto, A., Spotts, R., & Rattanapanone, N. (2011). Ethanol vapor and saprophytic yeast treatments reduce decay and maintain quality of intact and fresh-cut sweet cherries. *Postharvest Biology and Technology*, 62(2), 204-212. <https://doi.org/10.1016/j.postharvbio.2011.05.010>
- Bolin, H. R., & Huxsoll, C. C. (1991). Effect of Preparation Procedures and Storage Parameters on Quality Retention of Salad-cut Lettuce. *Journal of Food Science*, 56(1), 60-62. <https://doi.org/10.1111/j.1365-2621.1991.tb07975.x>
- Cantwell, M., & Suslow, T. V. (2014). Pumpkin and winter squash: Recommendations for maintaining postharvest quality. <http://postharvest.ucdavis.edu/>
- Cortez-Vega, W. R., Brose Piotrowicz, I. B., Prentice, C., & Dellinghausen Borges, C. (2014). Influence of different edible coatings in minimally processed pumpkin (*Cucurbita moschata* Duch). *International Food Research Journal*, 21(5), 2017-2023.
- Engwa, G. A. (2018). Free radicals and the role of plant phytochemicals as antioxidants against oxidative stress-related diseases. In *Phytochemicals: source of antioxidants and role in disease prevention*, (Toshiki Asao and Md Asaduzzaman eds). IntechOpen, ebook, 49-73. <https://doi.org/10.5772/intechopen.76719>
- Gao, J., Luo, Y., Turner, E., & Zhu, Y. (2017). Mild concentration of ethanol in combination with ascorbic acid inhibits browning and maintains quality of fresh-cut lotus root. *Postharvest Biology and Technology*, 128, 169-177. <https://doi.org/10.1016/j.postharvbio.2016.12.002>
- Gao, J., Zhu, Y., & Luo, F. (2018). Effects of ethanol combined with ascorbic acid and packaging on the inhibition of browning and microbial growth in fresh-cut Chinese yam. *Food Science and Nutrition*, 6(4), 998-1005. <https://doi.org/10.1002/fsn3.647>
- Garcia, E., Barrett, D. M., & (2002). Preservative treatments for fresh-cut fruits and vegetables. In *Fresh-cut fruits and vegetables: science, technology, and market*. (Olusola Lamikanra ed). CRC Press.
- Halpin, C. (2004). Investigating and manipulating lignin biosynthesis in the postgenomic era. *Advances in Botanical Research*, 41, 63-106.
- Harwood, J., & Moore Jr, T. S. (1989). Lipid metabolism in plants. *Critical Reviews in Plant Sciences*, 8(1), 1-43. <https://doi.org/10.1080/07352688909382269>
- Hernández-Muñoz, P., Almenar, E., Ocio, M. J., & Gavara, R. (2006). Effect of calcium dips and chitosan coatings on postharvest life of strawberries (*Fragaria x ananassa*). *Postharvest Biology and Technology*, 39(3), 247-253. <https://doi.org/10.1016/j.postharvbio.2005.11.006>
- Homaida, M. A., Yan, S., & Yang, H. (2017). Effects of ethanol treatment on inhibiting fresh-cut sugarcane enzymatic browning and microbial growth. *LWT- Food Science and Technology*, 77, 8-14. <https://doi.org/10.1016/j.lwt.2016.10.063>
- Hu, W., Jiang, A., Tian, M., Liu, C., & Wang, Y. (2010). Effect of ethanol treatment on physiological and quality attributes of fresh-cut eggplant. *Journal of the Science of Food and Agriculture*, 90(8), 1323-1326. <https://doi.org/10.1002/jsfa.3943>
- Kittur, F., Saroja, N., & Tharanathan, R. (2001). Polysaccharide-based composite coating formulations for shelf-life extension of fresh banana and mango. *European Food Research and Technology*, 213(4-5), 306-311. <https://doi.org/10.1007/s002170100363>
- Kjeldsen, P. (1993). Evaluation of gas diffusion through plastic materials used in experimental and sampling equipment. *Water Research*, 27(1), 121-131. [https://doi.org/10.1016/0043-1354\(93\)90202-S](https://doi.org/10.1016/0043-1354(93)90202-S)

- Kulczynski, B., & Gramza-Michalowska, A. (2019). The Profile of secondary metabolites and other bioactive compounds in *Cucurbita pepo* L. and *Cucurbita moschata* Pumpkin Cultivars. *Molecules*, 24(16). <https://doi.org/10.3390/molecules24162945>
- Li, H., & Yu, T. (2001). Effect of chitosan on incidence of brown rot, quality and physiological attributes of postharvest peach fruit. *Journal of the Science of Food and Agriculture*, 81(2), 269-274. [https://doi.org/10.1002/1097-0010\(20010115\)81:2<269::aid-jsfa806>3.0.co;2-f](https://doi.org/10.1002/1097-0010(20010115)81:2<269::aid-jsfa806>3.0.co;2-f)
- Li, M., Li, X., Li, J., Ji, Y., Han, C., Jin, P., & Zheng, Y. (2018). Responses of fresh-cut strawberries to ethanol vapor pretreatment: improved quality maintenance and associated antioxidant metabolism in gene expression and enzyme activity levels. *Journal of Agricultural and Food Chemistry*, 66(31), 8382-8390. <https://doi.org/10.1021/acs.jafc.8b02647>
- Lim, Y., Lim, T., & Tee, J. (2007). Antioxidant properties of several tropical fruits: A comparative study. *Food Chemistry*, 103(3), 1003-1008. <https://doi.org/10.1016/j.foodchem.2006.08.038>
- Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science*, 7(9), 405-410. [https://doi.org/10.1016/s1360-1385\(02\)02312-9](https://doi.org/10.1016/s1360-1385(02)02312-9)
- Nawirska-Olszańska, A., Biesiada, A., Sokół-Lętowska, A., & Kucharska, A. Z. (2011). Content of bioactive compounds and antioxidant capacity of *Pumpkin puree* enriched with Japanese quince, cornelian cherry, strawberry and apples. *Acta Scientiarum Polonorum Technologia Alimentaria*, 10(1), 51-60.
- Pareek, S. (2016). *Fresh-cut Fruits and Vegetables: Technology, Physiology, and Safety*. Taylor & Francis, US & UK: CRC Press.
- Pesis, E. (2005). The role of the anaerobic metabolites, acetaldehyde and ethanol, in fruit ripening, enhancement of fruit quality and fruit deterioration. *Postharvest Biology and Technology*, 37(1), 1-19. <https://doi.org/10.1016/j.postharvbio.2005.03.001>
- 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>
- Plotto, A., Bai, J., Narciso, J., Brecht, J., & Baldwin, E. (2006). Ethanol vapor prior to processing extends fresh-cut mango storage by decreasing spoilage, but does not always delay ripening. *Postharvest Biology and Technology*, 39(2), 134-145. <https://doi.org/10.1016/j.postharvbio.2005.09.009>
- Ritenour, M., Mangrich, M., Beaulieu, J., Rab, A., & Saltveit, M. (1997). Ethanol effects on the ripening of climacteric fruit. *Postharvest Biology and Technology*, 12(1), 35-42. [https://doi.org/10.1016/S0925-5214\(97\)00031-8](https://doi.org/10.1016/S0925-5214(97)00031-8)
- Rodriguez-Amaya, D. B. (2001). *A guide to carotenoid analysis in foods*. Washington (Vol. 71): ILSI Press.
- Romanazzi, G., Feliziani, E., Baños, S. B., & Sivakumar, D. (2017). Shelf life extension of fresh fruit and vegetables by chitosan treatment. *Critical Reviews in Food Science and Nutrition*, 57(3), 579-601. <https://doi.org/10.1080/10408398.2014.900474>
- Roos, Y. H., & Drusch, S. (2015). *Phase transitions in foods*. Academic Press.
- Şahin, S., & Şamlı, R. (2013). Optimization of olive leaf extract obtained by ultrasound-assisted extraction with response surface methodology. *Ultrasonics Sonochemistry*, 20(1), 595-602. <https://doi.org/10.1016/j.ultsonch.2012.07.029>
- Santos, A. R., Da Silva, A. F., Amaral, V. C., Ribeiro, A. B., de Abreu Filho, B. A., & Mikcha, J. M. (2016). Application of edible coating with starch and carvacrol in minimally processed pumpkin. *Journal of Food Science and Technology*, 53(4), 1975-1983. <https://doi.org/10.1007/s13197-016-2171-6>
- Simon, E. (1974). Phospholipids and plant membrane permeability. *New Phytologist*, 73(3), 377-420. <https://doi.org/10.1111/j.1469-8137.1974.tb02118.x>
- Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16(3), 144-158.
- Soares, A. d. S., Ramos, A. M., Vieira, É. N. R., Vanzela, E. S. L., de Oliveira, P. M., & Paula, D. d. A. (2018). Vacuum impregnation of chitosan-based edible coating in minimally processed

- pumpkin. *International Journal of Food Science and Technology*, 53(9), 2229-2238. <https://doi.org/10.1111/ijfs.13811>
- Suwannarak, J., Phanumong, P., & Rattanapanone, N. (2015). Combined effect of calcium salt treatments and chitosan coating on quality and shelf life of carved fruits and vegetables. *Chiang Mai University Journal of Natural Sciences*, 14, 269-284. <https://doi.org/10.12982/cmujns.2015.0088>
- Takahashi, M., Watanabe, H., Kikkawa, J., Ota, M., Watanabe, M., Sato, Y., SATO, N. (2006). Carotenoids extraction from Japanese persimmon (*Hachiya-kaki*) peels by supercritical CO<sub>2</sub> with ethanol. *Analytical Sciences*, 22(11), 1441-1447. <https://doi.org/10.2116/analsci.22.1441>
- Tian, S. P., Jiang, A. L., Xu, Y., & Wang, Y. S. (2004). Responses of physiology and quality of sweet cherry fruit to different atmospheres in storage. *Food Chemistry*, 87(1), 43-49. <https://doi.org/10.1016/j.foodchem.2003.10.014>
- Toivonen, P. M. (2004). Postharvest storage procedures and oxidative stress. *Horticultural Science*, 39(5), 938-942. <https://doi.org/10.21273/hortsci.39.5.938>
- Zhang, D., & Quantick, P. C. (1997). Effects of chitosan coating on enzymatic browning and decay during postharvest storage of litchi (*Litchi chinensis* Sonn.) fruit. *Postharvest Biology and Technology*, 12(2), 195-202. [https://doi.org/10.1016/S0925-5214\(97\)00057-4](https://doi.org/10.1016/S0925-5214(97)00057-4)