



# Effects of storage temperature on the quality of minimally processed pumpkin (*Cucurbita moschata* Duch) treated with ethanol and chitosan

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## ABSTRACT

**Purpose:** This investigation focused on the most suitable temperature for fresh-cut pumpkin with harmonization between benefits and drawbacks. **Research method:** Fresh-cut pumpkin cubes were pre-treated with 30% ethanol and 1% chitosan, then stored for 15 days at different temperatures (5 °C or T5, 10 °C or T10, 15°C or T15 and 25°C or T25). **Findings:** At refrigeration temperature (below 10 °C), fresh-cut pumpkin could maintain its overall visual quality until the end of storage duration (15 days). The difference in firmness and total carotenoid content between T5 and T10 suggested chilling injury occurrence when pumpkin was stored under 10°C. Besides, other nutritional parameters of these two treatments such as weight loss, total soluble solid content, total phenolic compounds and antioxidant capacity did not significantly differ from each other. Regardless of their nutritional composition, T15 and T25 became disqualified for consumption on day 3 and day 6, respectively due to dramatic shrinkage and microbial development. **Limitations:** Storage duration should be extended until all treatments reach unacceptable quality. The activity of cell wall degrading enzymes and antioxidant enzymes during storage should be investigated to support this study's findings. **Originality/Value:** The preservation of fresh-cut pumpkin can be elevated to 10 °C to avoid chilling damage without altering much of their nutritional value.

## INTRODUCTION

Pumpkin (*Cucurbita moschata* Duch) was among ten most produced crops of the world (FAO, 2014). The vegetable does not contribute much to global economy as it can be grown in various weather conditions but its importance to local trade and well-being of the population is undeniable (Sargent & Maynard, 2012).

Fresh-cut or minimally processed fruits and vegetables, as defined by International Fresh-cut Produce Association (IFPA), are products that have undergone preliminary steps to attain 100% usability (Jideani et al., 2017). Those steps add value to the produce in terms of convenience but at the same time limit its storage life by accelerating plant metabolism (de Oliveira Silva et al., 2012). During storage time, nutritional value of fresh-cut product should be retained although sensory properties, especially color in case of pumpkin, could be reduced and became unacceptable. Such limited time restricts the sale of pumpkin products. Nonetheless, a study into a critical parameter like storage temperature was absent to acquire a better understanding of minimally processed pumpkin (MPP) in its response to various temperatures.

No single approach can totally restrict the quality deterioration. The combination of different methods is common, including modified-atmosphere packaging (MAP) with temperature management. Another potential approach is edible coating which comprises of either polysaccharides (alginate, carrageenan, gums), protein (chitosan, gelatin) or lipid, waxes. The addition of chitosan coating on MPP 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). Another edible coating on fresh-cut vegetables is ethanol, which was reported to extend the storage life of mango (Plotto et al., 2006) as well as reduce physiological metabolism and preserving the surface of fresh-cut eggplant (Hu et al., 2010). The combination of chitosan and ethanol was applied to fresh-cut pumpkin by Huynh and Nguyen (2020) and exhibited better at preserving physiochemical properties.

According to a report by James et al. (2010), the control of storage temperature is the key factor in preserving quality along distribution chain and in retail. In fact, fresh-cut products are more perishable and susceptible to temperature deviation than their whole fruits counterparts with three major phenomena: microbial spoilage, tissue softening and surface browning. Temperature control is an effective approach to extend shelf life of fresh-cut produce (Clarke & Fraser, 2004) as plant cell metabolism and microbial growth are retarded at low temperature. However, chilling injury can occur at low temperature leading to accelerated fruit softening (Lana et al., 2005). The optimal temperature for the storage of whole fruit pumpkin was reported to be 13 °C – 15 °C and below 10 °C would allow space for chilling injury.

The objective of this study was to assess the effects of different storage temperatures 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 pre-treated with 30% ethanol and 1% chitosan.

## MATERIALS AND METHODS

### Materials

Fresh pumpkin fruits (*Cucurbita moschata* Duch) were collected at commercial 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 (Huynh & Nguyen, 2020).

### Experimental design

Pumpkin cubes were subjected to 30% ethanol and 1% chitosan coating prior to storage.

### Ethanol pretreatment

Ethanol treatment procedure followed the experiment of Gao et al. (2018). Pumpkin cubes would be immersed in 30% ethanol solution (Merck Chemicals Ltd., Darmstadt, Germany) for 2 minutes at ratio of 200 g pumpkin to 500 ml ethanol.

### Chitosan pretreatment

Chitosan solution at concentration of 1% was prepared by dissolving chitosan powder (Sigma-Aldrich, St. Louis, MO, USA) in 0.5% acetic acid solution (Merck Chemicals Ltd., Darmstadt, Germany) at 65 °C. Ethanol – treated pumpkin was soaked in chitosan solution for 8 minutes and drained at room condition (Soares et al., 2018).

Finally, treated cubes were packed in Styrofoam tray of 100g portion, covered with PVC film and stored at 5 °C (T5), 10 °C (T10) and 15 °C (T15). Control sample experienced the same pretreatments, then stored at 25 °C (T25). Samples were analyzed at three-day interval in triplicates.

### Analytical methods

#### *Determination of overall quality index*

Different deterioration stages were assessed visually on 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 & Suslow, 2014).

#### *Determination of weight loss (%)*

Weight loss was calculated by taking the weight of the pumpkin tray at day of analysis divided by 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<sup>-1</sup>). 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.



**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.

**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<sup>-1</sup>) 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 (µg GAE.g<sup>-1</sup>)**

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 µg of gallic acid equivalents per g pumpkin (µg GAE.g<sup>-1</sup>).

**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<sub>0</sub> 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 (µg β-carotene.g<sup>-1</sup>)**

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 µg β-carotene.g<sup>-1</sup>.

**Statistical analysis**

All analyses were conducted in triplicate; the data were expressed as mean ± standard deviation. Data were subjected to one-way ANOVA and the least significant difference (Fisher's LSD). Statistical analysis was carried out using Minitab software package (Version 18.0, Minitab Pty Ltd., Australia) with 95% level of confidence.

**RESULTS AND DISCUSSION****Overall quality index**

Room temperature and slightly acidic environment of coated pumpkin offered favorable condition for mold growing (Marriott et al., 2018) which caused T25 sample to be discarded from day 3. The sample of T15 could only last for 6 days before the commodity became shriveled with

excessive white blush and indications of microbial contamination. From this study, it can be inferred that the reported optimal temperature of 15 °C was more suitable for whole fruit pumpkin rather than the fresh-cut one because visual quality change may make the fresh-cut produce unmarketable before any nutrition change is noticed (Cortez-Vega et al., 2014). The employment of cold storage (5 °C and 10 °C) reduced the impairment of the visual aspect of MPP. As of T5 samples, they maintained at highest visual score after 6 days despite drastic increase of weight loss and sharp drop of carotenoid content. The correlation between weight loss and visual appeal is not consistent, especially for cucurbit family. For summer squash, weight loss threshold of marketability is as high as 24% (Sargent & Maynard, 2012). Then, a 12% drop distributed to plentiful 2 cm cubes of T5 and T10 samples was acceptable according to the rating chart (Fig. 1), especially in the absence of white blush. Regarding perceivable color and carotenoid content of MPP, a research by Soares et al. (2018) discovered that the difference in carotenoid content in agreement with color coordinates, was not noticeable by consumer. After day 6, T5 sample experienced insignificant change, suggesting effectiveness of the storage condition while T10 showed more features of senescence as a result of accelerated respiration rate (Omamor & Hamza, 2006).

### Weight loss

Table 1b shows the increasing trend of weight loss percentage of MPP preserved at 5 °C, 10 °C, 15 °C and 25 °C along storage duration. Weight loss percentage reported in this study is especially higher than previous research of Cortez-Vega et al. (2014) which remained under 5% through 12 storage days. The difference in variety can be attributed to this gap with respiration rate of tropical pumpkin significantly greater than that of pumpkin from other climate regions (Sargent & Maynard, 2012). From day 3, there was a discrete separation between group of T5, T10 and T15, T25. Both had gradual increases but the latter followed steeper pattern, T15 lost 21% of weight on day 6 whereas the former group reached the approximate number after 15 days. High temperature induced high respiration rate in fruits and vegetables, thereby, breaking down more organic reservation materials, which contributed to high weight loss in the commodities (Lee et al., 2017). The weight loss of MPP stored at different refrigeration temperatures (< 10 °C) was not significantly different from each other, however, 10 °C is more recommended for MPP as below this level, chilling injury may compromise the quality of pumpkin (Cantwell & Suslow, 2014).

### Firmness

Maintaining firmness is a crucial objective of every preservation technique to maintain the marketability of fresh-cut produce (Lana et al., 2005). The firmness of T5, T10 and T15 experienced a sudden drop on day 3 as a physiological response to processing stress (Ghidelli & Perez-Gago, 2018). Samples of T25 were exceptions, whose firmness rose on day 3 caused by intense moisture loss forming tough outer layer. On later days, the penetration force fluctuated downwards as a result of accumulating weight loss and metabolic activity (Sargent & Maynard, 2012). At the end of storage time, T10 recovered to approximately the initial value, suggesting that the activity of cell membrane degrading enzymes were suppressed by appropriate temperature (Huber et al., 2001). The decrease in firmness of T5 treatment could be a result of chilling injury effect, which damaged cell organelles and released various enzymes such as pectinase, cellulase (Jackman et al., 1992).

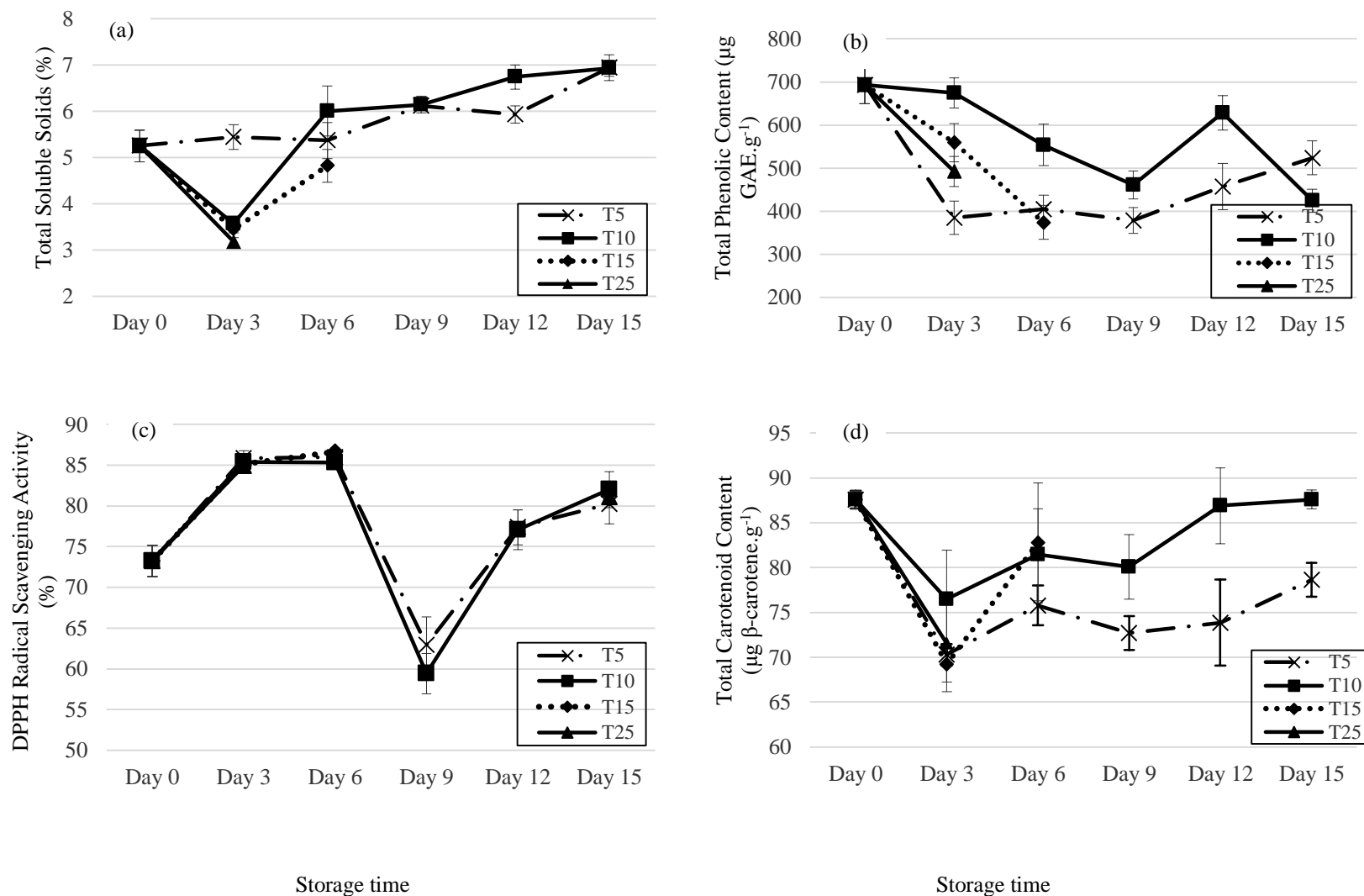


**Table 1.** Overall quality score, weight loss percentage and firmness over storage time of minimally processed pumpkin of different ethanol treatments stored at 10 °C

	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
(a) Overall quality index						
T5	5.00 ± 0.00 <sup>aA</sup>	5.00 ± 0.00 <sup>aA</sup>	5.00 ± 0.00 <sup>aA</sup>	4.33 ± 0.58 <sup>aB</sup>	4.33 ± 0.58 <sup>aB</sup>	4.00 ± 0.00 <sup>aB</sup>
T10	5.00 ± 0.00 <sup>aA</sup>	5.00 ± 0.00 <sup>abB</sup>	4.33 ± 0.58 <sup>bAB</sup>	4.00 ± 1.00 <sup>aBC</sup>	4.00 ± 0.00 <sup>aBC</sup>	3.33 ± 0.58 <sup>aC</sup>
T15	5.00 ± 0.00 <sup>aA</sup>	4.33 ± 0.58 <sup>aA</sup>	3.00 ± 0.00 <sup>cB</sup>	ES	ES	ES
T25	5.00 ± 0.00 <sup>aA</sup>	2.33 ± 0.58 <sup>bA</sup>	ES	ES	ES	ES
(b) Weight loss (%)						
T5	0.00 ± 0.00 <sup>aA</sup>	6.50 ± 0.96 <sup>aB</sup>	11.66 ± 1.18 <sup>aC</sup>	14.47 ± 1.06 <sup>aC</sup>	13.64 ± 0.67 <sup>aC</sup>	24.30 ± 2.27 <sup>aD</sup>
T10	0.00 ± 0.00 <sup>aA</sup>	7.89 ± 1.29 <sup>aB</sup>	11.58 ± 1.11 <sup>aC</sup>	14.13 ± 1.03 <sup>aCD</sup>	17.91 ± 0.88 <sup>bDE</sup>	21.13 ± 1.93 <sup>aE</sup>
T15	0.00 ± 0.00 <sup>aA</sup>	13.56 ± 1.34 <sup>bB</sup>	22.51 ± 1.58 <sup>bC</sup>	ES	ES	ES
T25	0.00 ± 0.00 <sup>aA</sup>	15.39 ± 1.98 <sup>bB</sup>	ES	ES	ES	ES
(c) Firmness (N)						
T5	26.98 ± 1.02 <sup>aA</sup>	22.64 ± 0.87 <sup>aB</sup>	22.74 ± 0.90 <sup>aB</sup>	22.21 ± 0.75 <sup>aB</sup>	22.28 ± 1.08 <sup>aB</sup>	20.52 ± 0.67 <sup>aB</sup>
T10	26.98 ± 1.02 <sup>aA</sup>	22.18 ± 0.25 <sup>aC</sup>	23.55 ± 1.99 <sup>aBC</sup>	25.87 ± 1.61 <sup>bAB</sup>	24.44 ± 0.49 <sup>bABC</sup>	25.84 ± 0.40 <sup>bAB</sup>
T15	26.98 ± 1.02 <sup>aA</sup>	23.10 ± 0.98 <sup>aB</sup>	25.35 ± 2.21 <sup>aAB</sup>	ES	ES	ES
T25	26.98 ± 1.02 <sup>aA</sup>	28.62 ± 1.65 <sup>bA</sup>	ES	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. T5: temperature 5 °C; T10: temperature 10 °C; T15: temperature 15 °C; T25: temperature 25 °C.



**Fig. 2.** The changes in (a) total soluble solids; (b) total phenolic content; (c) antioxidant capacity; (d) total carotenoid content of different storage temperature during 15 days. The data were expressed as mean  $\pm$  standard deviation with 95% level of confidence.



**Total soluble solids**

Total soluble solid contents of MPP subjected to different storage temperature are shown in Fig. 2a. Among the temperatures studied, both T5 and T10 were effective in enhancing TSS value of fresh-cut pumpkin after 15 days ( $p < 0.05$ ). After 3 days, TSS value of T10, T15 and T25, significantly declined probably due to accelerated respiration and metabolism after processing. Meanwhile, the sugar content of s T5 was maintained on day 6 and then risen up until the end of storage time. Such stability can be explained by enhancing effect of low temperature on the conversion of starch to sugar by improving associated gene expression (Gil & Beaudry, 2020). Subsequent increase in soluble solids content claimed the positive effects of storage temperature in lowering the depletion of carbohydrate (Ayala-Zavala et al., 2004). The result of this study is consistent with the finding of Lee et al. (2017).

**Total phenolic content**

The storage of MPP resulted in a sharp decrease ( $p < 0.05$ ) of phenolic content after 15 days regardless of the temperature employed (Fig. 2b). The loss or increase of phenolic content during storage time depends not only on postharvest conditions but also on cultivar and maturity stage (Gajewski et al., 2008). The abrupt decrease of phenolic content on day 3 of T5, T15 and T25 was comprehensible and common in fresh-cut produce in response to processing stress (Jideani et al., 2017). Meanwhile, T10 maintained at the same level. The immediate decline in total phenolic content could be caused by the activity of polyphenol oxidase (PPO) on cut surface or the degradation of cell structure during senescence stage (Akyol et al., 2016). It can be seen that high temperature (T15, T25) induced great loss in total phenolic compound due to early senescence on day 3 and day 6.

**Antioxidant capacity**

Samples preserved at various temperatures followed the same changing pattern for the entire storage duration with no significant difference among applied temperatures (Fig. 2c). Generally, DPPH scavenging activity of MPP increased after 15 days ( $p < 0.05$ ) from 73.24% to 81.12% despite the reduction in TPC value, implying that free radical scavenging activity of MPP was mostly decided by antioxidant enzymes rather than phenolic compounds. Similar results were previously confirmed by Yasar et al. (2014) in the study on enzyme activities of several pumpkin species. The sharp decrease in AC value on day 9 might be a technical error rather than a result of physiological phenomenon. Refrigeration temperatures (5 °C and 10 °C) successfully enhanced antioxidant capacity of fresh-cut pumpkin due to the favorable condition they provided for antioxidant enzymes (Yang et al., 2018).

**Total carotenoid content**

The change in total carotenoid content in of MPP stored at various temperatures during preservation time is presented in figure 2d with significant differences recorded between T5 and T10 treatments. The amount of this phytochemical declined sharply on day 3, which may be caused by processing stress with a great amount of carotenoids exposing to oxygen and light, led to the oxidation and isomerization of carotenoids (Zdunic et al., 2016). Such depletion affected visual color of sample and more importantly, biological activity of carotenoids that work as potent antioxidants (de Carvalho et al., 2017). T10 treatment retained statistically higher amount of carotenoid of MPP compared to T5. At the end of storage time, the carotenoid content of T10

sample was approximate to the content on day 0 ( $87.60 \mu\text{g } \beta\text{-carotene.g}^{-1} \text{ FW}$  and  $87.56 \mu\text{g } \beta\text{-carotene.g}^{-1} \text{ FW}$ , respectively). At lower storage time, T5 was expected to reduce respiration rate, protect color pigment against oxidation better than T10 (Lee et al., 2017). However, the lower amount of carotenoid in T5 suggested the occurrence of chilling injury which caused the loss of cell compartments, especially chromoplast, storage place of carotenoid (Lukatkin & Anjum, 2014). Hence,  $10^\circ\text{C}$  proved to be the best temperature for carotenoid retention in MPP.

## CONCLUSION

Despite both thresholds of  $5^\circ\text{C}$  and  $10^\circ\text{C}$  did not cause remarkably changes in pumpkin's visual score, weight loss, soluble solids content and antioxidant capacity, differences in firmness and carotenoid content revealed that temperature of  $5^\circ\text{C}$  might have caused irreversible chilling injury to the commodity. Therefore, this study suggests that  $10^\circ\text{C}$  is appropriate for storage of the fresh-cut pumpkin treated with ethanol and chitosan in terms of nutritional value and visual acceptance, which is important to consumer's preference. Further studies should focus on the package materials for the minimally processed products as they can help to prevent the chilling injuries and partly extend the products' shelf life.

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## Conflict of interest

The authors have no conflict of interest to report.

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