Effects of Season and Storage Period on Accumulation of Individual Carotenoids in Pumpkin Flesh (Cucurbita moschata)

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Abstract: Carotenoids are antioxidants with pharmaceutical potential. The major carotenoids important to humans are α-carotene, β-carotene, lycopene, lutein, zeaxanthin, and β-cryptoxanthin. Some of the biological functions and actions of these individual carotenoids are quite similar to each other, whereas others are specific. Besides genotype and location, other environmental effects such as temperature, light, mineral uptake, and pH have been found affect carotenoid development in plant tissues and organs. Therefore, this research investigated the effects of the season and storage periods during postharvest handling on the accumulation of carotenoids in pumpkin. This study shows that long-term storage of pumpkins resulted in the accumulation of lutein and β-carotene with a slight decrease in zeaxanthin. The amounts of β-carotene ranged from 174.583 ± 2.105 mg/100g to 692.871 ± 22.019 mg/100g, lutein from 19.841 ± 9.693 mg/100g to 59.481 ± 1.645 mg/100g, and zeaxanthin from not detected to 2.709 ± 0.118 mg/100g. The pumpkins were collected three times in a year; they differed in that zeaxanthin was present only in the first season, while the amounts of β-carotene and lutein were the highest in the second and third seasons, respectively. By identifying the key factors among the postharvest handling conditions that control specific carotenoid accumulations, a greater understanding of how to enhance the nutritional values of pumpkin and other crops will be gained. Postharvest storage conditions can markedly enhance and influence the levels of zeaxanthin, lutein, and β-carotene in pumpkin. This study describes how the magnitudes of these effects depend on the storage period and season.

Key words: pumpkin, carotenoid, postharvest, storage, season

1 INTRODUCTION

Pumpkins belong to the family Cucurbitaceae and are commonly known as melons, gourds, or cucurbits, and include crops such as cucumbers, squashes (including pumpkins), luffas, melons, and watermelons. The family consists of nearly 100 genera and over 750 species¹⁻³⁰. Cucurbita is a genus of the gourd family that was first cultivated in America and is now used in many parts of the world¹⁻⁴⁰. According to data estimates generated by the Food and Agriculture Organization of the United Nations (FAO), the global pumpkin production in 2007 was over 20 million ton, primarily in China, India, Russia, the United States, and Egypt (FAOSTAT, 2008)⁷. In Malaysia, the production of Cucurbita moschata from 2004 to 2009 ranged from 3559 to 8058 metric ton per year (MOA, 2009)⁸⁰.

Carotenoids are organic pigments within the tetraterpenoid group. They are known to be bioactive and are beneficial for human health. They belong to a group of natural pigments that that act as antioxidants and play key roles in many important nutritional functions such as being converted into vitamin A. Many studies have reported that carotenoids can enhance immune responses and reduce the
risks of degenerative diseases such as cancer and cardiovascular disease because of their abilities to quench singlet oxygen atoms and react with free radicals found in the human body. Some of the carotenoids such as lutein and zeaxanthin can help prevent the degeneration of macular pigments, especially in the elderly. Consumption of carotenoid-rich foods has been related to the prevention of cancer, cardiovascular disease, and other degenerative processes that involve oxidative stress. According to the Recommended Nutrient Intake (RNI) for Malaysia (2005), the recommended dietary intake of vitamin A is about 500 μg/d retinol equivalent for women and 600 μg/d retinol equivalent for men. Carotenoid compounds are synthesized de novo only in plants and microorganisms. The major problems involved with the analysis of carotenoids include many factors that may affect the carotenoid composition in plants, such as season, locality, storage, and postharvest handling and processing procedures.

The demand for these compounds has increased during the past few years because research findings have shown that carotenoids have antioxidant properties that are beneficial to human health. Apart from the knowledge of their provitamin A activities, the other benefits of carotenoids, such as reducing the risks of cancer and other degenerative processes, including oxidative stress, have led to a tremendous expansion of the carotenoid research field. Recently, a number of studies have investigated the phenolic and carotenoid contents of vegetables and fruits. Most of the carotenoid research has focused on various aspects such as analysis and composition; chemical reactions, including synthesis; biotechnological elucidation; production; food product development; formulation and physico-chemical properties; stability and alteration during food processing and storage; kinetics; and mechanisms of degradation. However, most of the studies were centered on food product development, human health, and structure-function relationships.

Many studies have correlated the compositions of carotenoids with processing, packaging, and storage conditions. According to Rodriguez-Amaya et al., the carotenoid content of fruits and vegetables is affected by many factors such as variety, level of maturity, climate, geographic site of production, part of the plant utilized, environmental conditions during agricultural production, postharvest handling, processing, and storage conditions. Some of the factors mentioned above might affect the carotenoid biosynthesis in plants and microorganisms. Processes involved with carotenoid biosynthesis include desaturation, cyclization, hydroxylation, epoxidation, and epoxidefuranoxirane rearrangement. For this study, the effects of environmental factors, which included the storage temperature (20°C for six months) and the effect of harvesting in different seasons, on the accumulation of carotenoids in pumpkin were observed. Specifically, we focused on the effects of the season and storage period on the accumulation of individual carotenoids in pumpkin flesh. We expected that these two factors would exert significant influence on the carotenoid content of pumpkin flesh.

2 EXPERIMENTAL PROCEDURES
2.1 Sample preparation
Pumpkins were obtained from the Federal Agriculture Marketing Authority (FAMA), Selayang, Malaysia, which acquired the pumpkins from various places in Malaysia. The storage period samples were brought by FAMA from Terengganu, located on the east coast of Malaysia. For the seasonal samples, the pumpkins were obtained from Malacca. We divided the samples for the storage period and season as follows: ±30 kg of pumpkin were stored in a glass room at 20°C; from this sample, 5 kg was analyzed each month for six months. For the seasonal analysis, 5-kg samples was collected from FAMA every four months. All the samples were cut to reduce the size, and freeze-dried and extracted prior to analysis. According to the FAMA, the samples were collected and kept for 1–2 weeks in the store before distributing to the markets. For this study, one month of storage was counted after the day of collection from the FAMA.

2.2 Sample extraction
Extraction was conducted based on the method described by Othman, with some modifications. For each sample, 1.0 g of powdered freeze-dried pumpkin was weighed and rehydrated by adding 3 mL of distilled water; then, 25 mL of an acetone and methanol mixture (7:3, v/v) was mixed to allow an efficient solvent penetration. The solution was allowed to stand overnight in dark at room temperature. The next day, the samples were vortexed and centrifuged for 2 min at 10,000 rpm in a Thermo Scientific Sorvall Biofuge Primo R (Germany) centrifuge tube. This procedure was repeated until the samples became colorless. Subsequently, equal volumes of hexane and distilled water were added to the combined supernatants. The solutions were then allowed to separate, and the upper layers of hexane containing the carotenoids were collected and dried under a gentle stream of oxygen-free nitrogen. Vials/tubes were then capped and sealed with a parafilm to exclude oxygen and immediately stored at −20°C for further analysis.

2.3 High-performance liquid chromatography analysis
High-performance liquid chromatography analyses of carotenoids extracted from pumpkin were conducted as described by Othman and Norshazila et al., with some
modifications. The analyses were performed using an Agilent model 1100 series chromatograph comprising a binary pump with micro vacuum degassers, a thermostatted column compartment, an auto sample injector, and a diode array detector with minor alterations listed below. The column used was HPLC column: ZORBAX Eclipse XDB-C18, analytical 4.6×150 nm (5 micron) end-capped 5 μm. The solvents used were (A) acetonitrile: water (9:1, v/v) and (B) ethyl acetate. The solvent gradients were developed as follows: 0–40% solvent B (0–20 min), 40–60% solvent B (20–25 min), 60–100% solvent B (25–25.1 min), 100% solvent B (25.1–35 min) and 100–0% solvent B (35–35.1 min) at a flow rate of 1.0 mL/min. The column was allowed to re-equilibrate in 100% solvent A for 10 min prior to the next injection. The temperature of the column was maintained at 20°C. The injection volume was 10 μL. The carotenoid standards of zeaxanthin, lutein, and β-carotene were obtained from Sigma-Aldrich. Calibration curves were used to calculate the concentrations of the respective carotenoids in experimental samples. Compounds were identified by co-chromatography with standards and by elucidation of their spectral characteristics using a photodiode array detector. Detection for carotenoid peaks was in the range 350–550 nm. The individual carotenoid concentration was expressed in terms of milligrams per 100 g of the dry weight of freeze-dried matter (mg/100 g) of DW.

3 RESULTS AND DISCUSSION

3.1 Evaluation of individual carotenoids by HPLC

Figure 1 shows the carotenoids detected in the pumpkin based on the season. The chromatograms showed two unknown carotenoids at the retention times of 10.3 and
findings, Christina et al. reported that the results varied in each season. In line with these results, the spectrum of carotenoids detected in each sample was observed based on the retention time (RT) and UV-VIS spectrum recorded by the standard. Table 1 presents the carotenoid contents in pumpkin from different seasons and Table 2 presents the carotenoid contents in pumpkin during six months of storage. To our knowledge, no equivalent data have been reported for carotenoid content in pumpkin based on the storage period and season in Malaysia.

In the present study, we found a significant difference in the carotenoid content among the pumpkins harvested in different seasons. The results show that zeaxanthin was detected only in the first season, while the highest amount of b-carotene was detected in the second season and lutein in the third season. From Table 1, we can also conclude that the results varied in each season. In line with these findings, Christina et al. reported that the amounts of carotenoids detected in pumpkin (Cucurbita maxima) were also different in each year of harvesting. This was probably because of factors such as climate change and farming practices, including fertilizer used, type of soil, water/irrigation provided, and postharvest handling. Soil fertilization is also a major factor that affects carotenoid biosynthesis in fruit. Instead of the factors mentioned above, the nutrient content in plants, including carotenoids, varies depending on many factors such as genetics, exposure to sunlight, rainfall, locality, type of soil, topography, season, and maturity, and type of fertilizer used. To support the statements above, Vera et al. reported that the carotenoid content in acerola fruits was found to be at its highest level when the fruits were harvested during the rainy season. Soil fertility increases during the rainy season. Soil fertility is defined as the capacity of the soil to provide plants with nutrients, water, and oxygen. This is in line with the results reported by Vera et al. In another study by Markus et al. on red peppers, it was found that carotenoid content was lower in the fruits harvested during periods of long sunshine, low rainfall, and high temperatures compared to those harvested in seasons with short sunshine periods, high rainfall, and low temperatures.

The results of the storage study showed that the b-carotene content in the pumpkin increased slightly in the second month of storage and tended to be stable during the following months. This result demonstrated that carotenoids probably undergo some sort of biosynthesis process during postharvest transportation or during the storage period. The enzyme system in the plants, which controls the carotenogenesis process, may increase the carotenoid content by carotenoid biosynthesis. Besides, another factor that probably affected the carotenoid content during the storage period was the ripening process, by which pumpkins mature gradually and ripen each month. Proportional to the storage period, the weight of pumpkin decreased and the color changed, becoming more intense, because of water loss. One of the main indicators that demonstrates the ripening and maturation processes in fruits is the color change. In the ripening process, a series of complex biochemical reactions are involved, such as

Table 1 Carotenoid content in pumpkin from different season.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Harvest Year</th>
<th>Zeaxanthin (mg/100g DW)</th>
<th>Lutein (mg/100g DW)</th>
<th>b– Carotene (mg/100g DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cucurbita moschata</td>
<td>Nov 2011</td>
<td>0.226 ± 0.002</td>
<td>42.530 ± 0.199</td>
<td>151.657 ± 3.891</td>
</tr>
<tr>
<td></td>
<td>Feb 2012</td>
<td>Not Detected</td>
<td>27.401 ± 2.221</td>
<td>414.153 ± 28.152</td>
</tr>
<tr>
<td></td>
<td>June 2012</td>
<td>Not Detected</td>
<td>48.585 ± 1.772</td>
<td>362.955 ± 15.836</td>
</tr>
</tbody>
</table>

Data are expressed as means_SD (n=3)

Table 2 Carotenoid content in pumpkin in different storage times.

<table>
<thead>
<tr>
<th>Storage time</th>
<th>Zeaxanthin (mg/100g DW)</th>
<th>Lutein (mg/100g DW)</th>
<th>b– Carotene (mg/100g DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st month</td>
<td>Not Detected</td>
<td>19.841 ± 9.693</td>
<td>174.583 ± 2.105</td>
</tr>
<tr>
<td>2nd month</td>
<td>2.709 ± 0.118</td>
<td>59.481 ± 1.645</td>
<td>692.871 ± 22.019</td>
</tr>
<tr>
<td>3rd month</td>
<td>1.667 ± 0.178</td>
<td>54.279 ± 3.408</td>
<td>485.358 ± 36.815</td>
</tr>
<tr>
<td>4th month</td>
<td>2.182 ± 0.035</td>
<td>41.529 ± 0.225</td>
<td>418.750 ± 5.832</td>
</tr>
<tr>
<td>5th month</td>
<td>0.593 ± 0.006</td>
<td>50.481 ± 0.749</td>
<td>555.183 ± 4.419</td>
</tr>
<tr>
<td>6th month</td>
<td>2.033 ± 0.065</td>
<td>55.458 ± 0.941</td>
<td>479.064 ± 14.820</td>
</tr>
</tbody>
</table>

Data are expressed as means_SD (n=3)
as hydrolysis of starch, production of carotenoids, anthocyanins, and phenolics, and the formation of volatile compounds\textsuperscript{30}. Later stages of the ripening process increase the amount of sugar by the activities of enzymes such as α-amylase, β-amylase, and starch phosphorylase\textsuperscript{41}. According to Pandya and Rao\textsuperscript{35}, the amounts of total carotenoids in pumpkin increased by more than onefold at the preripened stage and further increased by more than fourfold during the ripening stage. However, the lycopene content decreased slightly from the mature to the preripened stages and increased by more than threefold during the ripening stage.

We could not detect zeaxanthin in the first month of storage. The β-carotene content was found to increase in the second month, while lutein tended to be stable from the second to the sixth month. From these results, we assumed that β-carotene converted to zeaxanthin during the second month. β-Carotene can be converted to β-cryptoxanthin and zeaxanthin through the hydroxylation process\textsuperscript{30}. According to Othman\textsuperscript{36}, there were significant changes in the carotenoid contents at different stages of maturity in potato tubers; lutein and β-carotene were present in the first four weeks of storage, while neoxanthin, violaxanthin, lutein, and β-carotene were detected after 12 months. Compared to the carotenoid content in pumpkin based on the affect of storage times in this study, zeaxanthin was detected in the second month along with the increase in the amounts of β-carotene and lutein. However, the mechanism for this reaction is still not well understood and extensive studies should be performed to elucidate this mechanism.

In addition to the factors discussed above, other factors that might affect the stability of carotenoid compounds are the type and physical form of the carotenoid compound, presence of oxygen and metals in the matrix, exposure to light and heat, and type of the food matrix\textsuperscript{30}. Because double bonds are common in the carbon chains of carotenoid compounds, they are prone to chemical reactions such as oxidation and isomerization (cis-trans) during food processing and storage; these reactions occur more often if the carotenoid compounds are exposed to light, heat, acids, or oxygen. This leads to degradation and decay of the color and biological activity in plants\textsuperscript{21,40}. With regard to isomerization, trans-isomers are more stable and abundantly found in foods, while cis-isomers are normally formed during food processing\textsuperscript{40}. Hence, the stability and concentration of carotenoids in plants vary greatly.

4 CONCLUSION

This study determined the effects of the season and storage period on the carotenoid content of pumpkin (\textit{Cucurbita moschata}) during postharvest handling and provided new information on the carotenoid profiling of pumpkin from Malaysia. From the results, we concluded that carotenoid accumulation in pumpkin varied, both qualitatively and quantitatively, based on the season and storage period. The differences in the carotenoid contents measured during the six-month study period showed that the amounts of β-carotene and lutein in the pumpkin increased in the second month and tended to decrease or remain stable in the following months. This might be due to the ripening process and probably there was also some conversion of β-carotene to zeaxanthin. It was also found that the pumpkins were stable during the six months of storage; moreover, to maintain the quality and quantity of the carotenoid content, the most suitable storage temperature was 20°C. Thus, it can be concluded that the knowledge of the content and stability of carotenoids in foods is essential to evaluate the nutritional value of these compounds. Even though the topic of carotenoids is discussed often and vigorously among researchers worldwide and there are many publications in the field, the data for carotenoid compounds in the vegetable of Malaysia, specifically for pumpkins, are still inadequate. A comprehensive study of these compounds should be done, especially in the context of Malaysia.

ACKNOWLEDGMENT

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