

Research Article

CAROTENOID CONTENT IN DIFFERENT LOCALITY OF PUMPKIN (*CUCURBITA MOSCHATA*) IN MALAYSIA

^{1,2}NORSHAZILA, S., ¹IRWANDI, J., ³OTHMAN, R. AND ¹YUMI ZUHANIS, H. H.

¹Kulliyyah of Engineering, International Islamic University Malaysia, Jalan Gombak, 53100, Kuala Lumpur, Malaysia, ²Faculty of Food Technology, Universiti Sultan Zainal Abidin, Kuala Terengganu, Malaysia, ³International Institute for Halal Research and Training (INHART), Herbarium laboratory, Department of Landscape, Kulliyyah of Architecture and Environmental Design, International Islamic University Malaysia, Jalan Gombak, 53100, Kuala Lumpur, Malaysia.

*Email: irwandi@iium.edu.my

Received: 04 Feb 2014 Revised and Accepted: 03 Apr 2014

ABSTRACT

Pumpkin is believed to have health benefits due to its carotenoid content. Carotenoids are bioactive compounds with pharmaceutical potential. Carotenoids compound such as α -carotene and β -carotene react as provitamin A in human body, while lutein and zeaxanthin are two major components of the macular pigment of the retina. There are many extensive research has been done to study the benefit of these compounds to improve the nutritional value either for human consumption or commercialization purposes. The aim of this study is to identify the carotenoid content in pumpkin from five different localities in Malaysia. Carotenoid content in fruits and vegetables varies due to certain factors such as variety, level of maturity, climate or geographic site of production, part of the plant utilized, environment conditions during agricultural production, post-harvest handling, processing, and storage conditions. Based on these factors, measures could be taken to identify the individual carotenoid concentrations. In this study, pumpkins from Kelantan, Terengganu, Perak, Kedah and Melaka were analyzed. HPLC analysis was conducted to analyse the individual carotenoid in pumpkin. The individual carotenoids detected were α -carotene; which ranged from 1.26 mg/100g to 10.20 mg/100g, β -carotene; 29.16 mg/100g to 154.76 mg/100g and small amount of lutein were detected ranged from 0.22 mg/100g to 0.46 mg/100g. However lutein compound was not detected in pumpkin from Perak. The retinol equivalent was also calculated.

Keywords: Pumpkin, α -carotene, β -carotene, Lutein, Carotenoid, HPLC analysis

INTRODUCTION

Carotenoids are known as bioactive compounds that give benefits to human health. Many previous studies reported that carotenoid can enhance the immune response [1] and reduce the risk of degenerative diseases such as cancer and cardiovascular diseases [2,3,4] due to its ability to quench singlet oxygen and react with free radicals found in human body [5]. Some of the carotenoids such as lutein and zeaxanthin can help to prevent the degeneration of macular pigment especially in elderly [6]. The findings of the antioxidant activities and free radical-scavenging abilities in phenolics compound and their benefits to human health have increased the research interest in this field tremendously [7].

Scientifically, carotenoid can be obtained from the deep colour vegetables and fruits as they are rich with phenolics compounds including flavonoids, anthocyanins and carotenoids [8,9,10,11]. In pumpkins, the orange colour of the skin and flesh of the fruits are contributed by the carotenoid compounds, which is in line with some previous studies done by Azizah *et al.* [12]. Ben-Amotz and Fishler [13] reported that pumpkins consist of β -carotene and lycopene, while Murkovic *et al.* [14] reported that carotenoid compounds found in pumpkins was lutein, zeaxanthin, β -cryptoxanthin, and β -carotene.

Physically, pumpkins in Malaysia are from the species of *Cucurbita moschata* and *Cucurbita moschata* Duchesne. Locally, they are known as *labu manis* and *labu loceng* among the community. *Labu manis* is planted almost in every state in Malaysia, however *labu loceng* majorly came from Kedah. The different between them are the shape. *Labu manis* is sphere in shape while *labu loceng* look like a bell which is shown in Figure 1. They are varied in size and color; the young fruit is green while the older is pale yellow. The flesh thickness is around three centimeters and they have sweetish taste with a very good market compared to other species due to its size with an average of 1.4 kg per piece. The skin which is covered with wax facilitates the process of post harvest handling as they can be stored for more than 6 months after the harvesting process. These physical features allow farmers and wholesalers to plan the production and marketing of the crops [15]. In Malaysia, the production of pumpkin (*Cucurbita moschata*) from the year of 2004 to 2009 was estimated between 3559 up to 8058 tones metric per year [16]. Normally, they are widely used in Malaysia cuisine such as *masak lemak labu*, *labu sira* and *pengat labu*, while its white seeds are dried for 'kuaci' production. In east coast of Malaysia (Kelantan and Terengganu), pumpkins are preserved with sugars and serve as dessert.



Fig. 1: Pumpkin in Malaysia; left: *Cucurbita moschata* (*labu manis*) and right: *Cucurbita moschata* Duchesne (*labu loceng*) which is different in shape.

Many researchers reported that carotenoid content in fruits and vegetables are influenced by many factors such as variety, level of maturity, climate/geographic site of production (location), part of the plant utilized, environment conditions during agricultural production, post-harvest handling, processing, and storage conditions. Since there are many factors influencing the carotenoid content in pumpkin, the aim of this study is to get an overview of the carotenoid content quantitatively and qualitatively in this fruit from different locations in Malaysia.

MATERIALS AND METHODS

Sample Preparation

Pumpkins from Kelantan, Terengganu and Melaka were obtained from Federal Agriculture Marketing Authority (FAMA), Selayang, Malaysia, while pumpkins from Kedah and Perak were bought from the market. All of the pumpkins were *Cucurbita moschata* except the sample from Kedah, which was *Cucurbita moschata* Duchesne. The samples were cut to reduce size, freeze dried and ground prior to analysis.

Sample extraction

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 acetone and methanol mixture (7:3) was mixed to allow efficient solvent penetration and the solution was allowed to stand overnight in darkness at room temperature. The samples were vortexed and centrifuged for 2 minutes at 13 500 *g* (Thermo Scientific, Sorvall Biofuge Primo R, Germany) at the next day and the supernatant was transferred to a foil covered 50 ml centrifuge tube. This procedure was repeated until the samples became colourless. After that, an equal volume of hexane and distilled water was added to the combined supernatants. The solution was then allowed to separate and the upper ether layer containing the carotenoids was collected and dried under a gentle stream of oxygen-free nitrogen. Vials/tubes were then capped and sealed with parafilm to exclude oxygen and immediately stored at -20 °C for further analysis.

HPLC Analysis

The HPLC analysis of carotenoids extracted from pumpkin was performed on an Agilent model 1100 series comprised of a binary pump with autosampler injector, micro vacuum degassers,

thermostatted column compartment and a diode array detector according to Othman [17] with minor alterations listed below. The column used was a ZORBEX Eclipse SB - C18 end capped 5 μ m, 250 x 4.6 mm reverse phase column (Agilent Technologies, USA). The solvents used were (A) acetonitrile: water (9:1 v/v) and (B) ethyl acetate. The solvent gradient used 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⁻¹. 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 μ L.

Carotenoid standards of β -carotene, α -carotene and lutein were obtained from Sigma-Aldrich. Calibration curves were used to calculate the concentration of the respective carotenoids in experimental samples as described by Othman [17] and Norsnazila et al. [18]. The identity of individual carotenoids was confirmed by their spectral characteristics, absorption maximum and retention time as described by Britton et al. [19]. Compounds were identified by co-chromatography with standards and by elucidation of their spectral characteristics using a photo-diode array detector. Detection for carotenoid peaks was in the range of 350 to 550 nm. Individual carotenoid concentrations were calculated by comparing their relative proportions, as reflected by integrated HPLC peak areas, to the individual carotenoid content determined by spectrophotometry. The individual carotenoid concentration was expressed in terms of milligram per 100 g dry weight or freeze-dried matter (mg / 100g DW)

RESULTS AND DISCUSSION

Analysis of individual carotenoids by HPLC

Table 1 presents the individual carotenoid content in pumpkin from 5 locations in Malaysia. Carotenoid content was measured quantitatively and qualitatively by using High Performance Liquid Chromatography (HPLC). To assure the correct determination of carotenoids, spectrum of carotenoid detected in each samples were observed based on the retention time (RT) and UV-VIS spectrum recorded by the standard. From the results, the carotenoid profiles of pumpkin from different locations showed significant differences both in their qualitative and quantitative distribution which is consistent with the results reported by others [20,21,22,] for their samples.

Table 1: Individual Carotenoid of Pumpkin based on Location in Malaysia

Locality	Species	β -carotene (mg / 100g)	α -carotene (mg / 100g)	Lutein (mg / 100g)
Kelantan	<i>Cucurbita moschata</i> (<i>Labu manis</i>)	154.76 + 2.75	3.28 + 0.05	0.24 + 0.00
Terengganu	<i>Cucurbita moschata</i> (<i>Labu manis</i>)	77.17 + 9.32	10.20 + 1.02	0.46 + 0.04
Perak	<i>Cucurbita moschata</i> (<i>Labu manis</i>)	29.16 + 0.52	1.26 + 0.04	ND
Melaka	<i>Cucurbita moschata</i> (<i>Labu manis</i>)	75.79 + 1.63	3.51 + 0.07	0.23 + 0.00
Kedah (<i>Labu loceng</i>)	<i>Cucurbita moschata</i> Duchesne (<i>Labu loceng</i>)	29.27 + 6.44	1.70 + 0.72	0.22 + 0.01

ND: Not Detected

From this study, site of plantation influenced the individual carotenoid in pumpkin; this factor might be due to the temperature of the location, type of soil, fertilizer used for the crops and post-harvest handling. The value of β -carotene in pumpkin from Kelantan was the highest followed by Terengganu. However, the highest content for α -carotene was detected in pumpkin from Terengganu. For lutein compound, the average of its concentration was around 0.22 mg / 100g - 0.46 mg / 100g dry weight. However, this compound was not detected in pumpkin from Perak. Climate temperature influence the carotenoid content in fruits, where elevated tropical climates accommodate the carotenoid biosynthesis, with fruits produced in this type of climates normally contains higher carotenoid concentrations [23,24].

According to Harris [25], nutrient content in freshly harvested edible plants are varied, and the factors that contribute to this circumstances are genetics, exposure to sunlight, amount of rainfall, topography, type of soils, location, season, fertilization of soils and maturity. Meanwhile, a research conducted by Rodriguez-Amaya and Mieko [26], reported that among the factors that affected the type and quantity of carotenoid in vegetables and fruits are the i) variety of the species, ii) location of the plantation, iii) stage of maturation, iv) part of the plant utilized, v) post harvest handling, vi) processing, vii) environmental and viii) storage condition. In conjunction with these factors, location of plantation plays important roles in carotenoid production in plants. Location of plantation can be associated with environmental factors such as type of soil and climate.

Based on previous study showed in Table 2 [27,28,29], pumpkins from different species and locations provided different profiles of carotenoid content. The results showed those carotenoids content in pumpkins were varies even though

they were come from same species. From the study, localities of the pumpkins indicated that location of plantation is one of the significant factors that affected the carotenoid content quantitatively and qualitatively.

Table 2: Comparison of individual carotenoid compounds detected in previous studies

Samples	Cultivar / Location	Carotenoid(s) detected in previous study
<i>Cucurbita maxima</i>	Bischofsmu tze, Germany	^a lutein, β - carotene
	Golden Nuggets, Germany	^a antheraxanthin, lutein, zeaxanthin, β - carotene
	Halloween, Germany	^a lutein, zeaxanthin, β - carotene
	Hokkaido I, Germany	^a antheraxanthin, lutein, zeaxanthin, β - carotene
	Hokkaido II, Germany	^a antheraxanthin, lutein, zeaxanthin, β - carotene
<i>Cucurbita pepo</i>	Malaysia	^b lutein, α - carotene, β - carotene
	Sweet Lightning, Germany	^a lutein, β - carotene
<i>Cucurbita moschata</i>	Muscade de Provence, Germany	^a α - carotene, β - carotene
<i>Cucurbita moschata (Duch)</i>	Butternuts, Germany	^a lutein, α - carotene, β - carotene
	Brazil	^c α - carotene, β - carotene

^aChristina, K. et al., (2008)

^bTee et al.,(1991)

^cLucia Maria Jaeger de Carvalho et al., (2012)

Figure 2 showed the HPLC chromatograms of carotenoids in pumpkin from Kelantan and their spectral characteristic. Most of the carotenoid compounds absorb maximally at three different wavelengths, resulting in three-peak spectra. The greater the number of conjugated double bonds, the higher the λ_{max} values [30]. Besides of the retention time, the spectral characteristic

can be used for individual carotenoid confirmation. From this study, the carotenoid spectral characteristic from pumpkin detected by HPLC was compared to previous study reported by Rodriguez-Amaya [30]. The retention time for lutein was at 15.8 minutes, α - carotene was at 29.1 minutes and β - carotene was at 29.3 minutes.

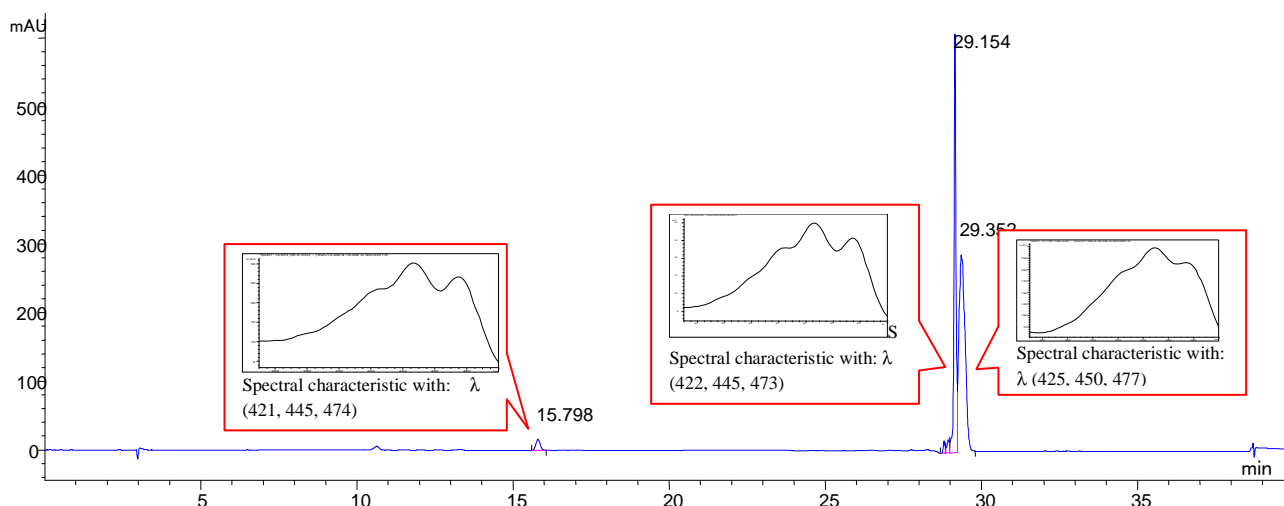


Fig. 2: The example of HPLC chromatograms of carotenoids in pumpkin from Kelantan; (a) lutein – RT: 15.8 minutes (b) α-carotene – RT 29.1 minutes, and (c) β-carotene – RT 29.3 minutes and their spectral characteristic.

RE value of pumpkin based on locality

Vitamin A activities of β- and α-carotene were calculated in terms of retinol equivalents (RE) based on the *in vivo* conversion factor proposed by WHO (1982) [31], where 1 RE = 1 μg of retinol = 6 g of β-carotene or 12 μg of α-carotene. From this study, the pro-vitamin A in pumpkin which includes α - carotene and β - carotene will be

converted enzymatically to retinol in the intestinal mucosa [32]. The recommendation nutrient intake for men and women age from 19 to 65 years old is 500 - 600 μg per day [33]. From this study, the RE value that can be obtained from 1g of dry weight pumpkin is around 49.65 μg RE to 260.66 μg of RE. From this result, we can conclude that pumpkin at least 2.5g dry weight of pumpkin can provide more than 100% RE which is sufficient for the daily needs.

Table 3: The Retinole Equivalents (RE) value of pumpkin from different locations

Cultivars (locations)	Kelantan	Terengganu	Kedah	Perak	Melaka
Retinol Equivalents (μg/g) of DW	260.66	137.11	50.20	49.65	129.24

CONCLUSION

Carotenoid from pumpkin was successfully extracted from five locations in Malaysia. The individual carotenoids detected were α -carotene, β -carotene and small amount of lutein. However lutein compound was not detected in pumpkin from Perak. From these details, we can conclude that pumpkin from different locations provide different values of carotenoid qualitatively and quantitatively. More research can be expanded to study the other factors that influence carotenoid profile in pumpkin to enhance the production of pharmaceutical and nutraceutical product from pumpkin which is cheap and abundantly planted in Malaysia.

ACKNOWLEDGMENT

We would like to thank the IIUM herbarium lab staff for the facilities provided and ERGS12-016-0016 grant for the financial support.

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