ISSN:1991-8178

Australian Journal of Basic and Applied Sciences

Journal home page: www.ajbasweb.com

Effect of Locality on Carotenoids Stability in Malaysian Orange Sweet Potato (Ipomoea batatas) Tuber Flesh

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ARTICLE INFO

Article history:
Received 12 March 2015
Accepted 28 April 2015
Available online 2 May 2015

Keywords:
carotenoids, sweet potato, locality, HPLC, β-carotene, zeaxanthin.

ABSTRACT

Background: Carotenoids are bioactive antioxidant pigments with pharmaceutical potential. Carotenoids 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. Due to its high carotenoids and pro-vitamin A content, sweet potato (Ipomoea batatas) is believed to have many nutritional and medicinal benefits. The carotenoid content in sweet potato or as locally known Keledek in Malaysia were determined using spectrophotometry and high performance liquid chromatography HPLC analysis. Objective: This study aimed to assess the carotenoids content stability in five different localities in Malaysia (Kelantan, Pahang, Terengganu, Selangor and Perak) of orange sweet potato tuber. All analyses were carried out in triplicate. Results: Results of this study revealed that the orange sweet potato (OSP) from Kelantan showed the highest total carotenoids content. For individual carotenoids, β-carotene ranged from 1123.85±0.04 µg/g to 399.4±3.62 µg/g DW. Zeaxanthin was detected only in Kelantan OSP 13.01±0.00 µg/g DW. Carotenoids profiles for orange fresh tubers from five Malaysian localities revealed marked differences in individual carotenoids composition. Conclusion: Generally, location or geographic site of crop can be considered as an important factor that affects carotenoids content in sweet potato flesh tuber.

To Cite This Article: Suhair Kammona, Rashidi Othman, Irwandi Jaswir, Parveen Jamal., Effect of Locality on Carotenoids Stability in Malaysian Orange Sweet Potato (Ipomoea batatas) Tuber Flesh. ***Aust. J. Basic & Appl. Sci.,*** *9(17): 40-45, 2015*

INTRODUCTION

Carotenoids are antioxidants with pharmaceutical potential and have attracted the interest of researchers from diverse fields including, biochemistry, biology, food science and technology, medicine, pharmacy, and nutrition for more than a century. Carotenoids are widely distributed natural pigments responsible for the yellow, orange, and red colors of fruits, roots, flowers, fish, invertebrates, and birds (Rodriguez-Amaya, 1997 and Fatimah Azzahra *et al*., 2014). The carotenoids group include carotenes (non-polar) and xanthophylls (polar) (Rodriguez-Amaya, 1997 and Aurelie, 2010). The major carotenoids important to humans are α-carotene, β-carotene, lycopene, lutein, zeaxanthin and β-cryptoxanthin (Olmedilla *et al*., 1994 and Khachik *et al*., 1997). Vitamin A deficiency is a major public health issue in developing countries; with children and pregnant/lactating women the most vulnerable (Aurelie, 2010). About 50 carotenoids are known to have a provitamin A activity (Lee *et al*., 1989). Since crotenoids cannot be synthesized by human body, these pigments have to be supplemented through dietary intake (Van den Berg *et al*., 2000). In plants, precursors of vitamin A called carotenoids are associated with cellular lipids and implanted in cellular structures (FAO/WHO, 2002).

Sweet potato (Ipomoea batatas) roots are one of the major food sources of carotenoids (Henkel, 1996 and Woolfe, 1992). Sweet potato is one of the world's most important food crops, with annual production exceeding 134 million tons in more than 9 million hectares (Kenneth, 2009 and FAO 1997). Cultivated in more than 100 countries, sweet potato ranks third of the world root and tuber crops production after potato and cassava (FAOstat, 2008). Sweet potatoes are a nutritious food, low in fat and protein, but rich in carbohydrate. Tubers and leaves are good sources of antioxidants (Teow *et al*., 2007). Sweet potato flesh SPF can be white, cream, yellow, orange, or purple (Woolfe, 1992 and Bovell-Benjamin, 2007), with orange, white, and cream the most commonly grown and eaten (Sandhill

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Preservation Centre, 2010). Sweet potato SP is one of the most important tuber crops for fresh consumption in Malaysia and its cheap and commonly available throughout the year. It is traditionally grown for the fresh root market with a very small percentage being processed into traditional snacks such as kerepek (sweet potato crackers) or cakar ayam (fried sweet potato) (Siti Hasidah and Khatijah, 1994). Because of their high carotenoid content and good yields, orange-fleshed sweet potato OFSP have also been used in several small-scale studies to increase VA status (Van Jaarsveld *et al*., 2005).

Many previous studies revealed that carotenoids content in vegetables and fruits are strongly influenced by many factors such as variety, part of which the plant is being utilized, climate/geographic site of production (location), level of maturity, environmental conditions during agricultural production, post-harvest handling, processing, including storage conditions (Rodriguez Amaya, 2004). Since locality of of plantation is one of the most important factors that influenced carotenoids content in sweet potato flesh and although there are many previous studies reported on the correlations between locations and carotenoids content quantitatively and qualitatively, but there is no data on carotenoids content in sweet potato flesh from various locations in Malaysia. This study aimed to get an overview of carotenoids composition quantitatively and qualitatively in the Malaysian orange sweet potato from different locality and to determine nutritional qualities of Malaysian to overcome and combat the Vitamin A Deficiencies VAD and enhance the possibility of exploitation for the Malaysian sweet potatoes in the pharmaceutical industry on a broad global scale.

Experimental Procedure:

Sample preparation:

Orange sweet potato OSP samples were obtained from Federal Agriculture Marketing Authority (FAMA), Selayang, Malaysia, which was brought in from Terengganu, that is located at the East Coast of Malaysia whereas others orange sweet potato samples from Kelantan, Terengganu, Pahang, Perak and Selangor were bought from the local markets. Samples were hand-peeled, cut to reduce the size and were freeze-dried (EYELA FDU-1100, Japan) for 72 hr, then the samples were ground into fine powder and kept at -20°C until further analysis.

Sample extraction:

The extraction procedure essentially follows the methods described by Rashidi (2009), with some modification. 1 g of each powdered freeze-dried sample was weighed and rehydrated with 3 mL of distilled water, then extracted in 25 mL of acetone:methanol mixture (7:3) (v:v) containing calcium carbonate. The samples were mixed well and left overnight in darkness at room temperature. The following day, each sample was vortexed and centrifuged for 2 minutes at 13500 g (Thermo Scientific, Sorvall Biofuge Primo R, Germany) and the supernatant was collected and transferred to a foil covered 50 mL centrifuge tube. The extraction procedure for every sample was repeated until the supernatant or the tissue is colorless, but at this time, without additional calcium carbonate. The pooled supernatant were centrifuged to remove fine particles and then stored at -20 ºC in the dark prior to analysis. Then, equal volume of hexane and distilled water to the combined supernatants. The mixture was then allowed to separate under centrifugal force and the upper hexane layer was collected. The procedure (without addition of distilled water) was done until the hexane layer seemed colorless. The combined upper phase would be dried completely under a gentle stream of oxygen-free nitrogen. Vials/tubes were then be capped and sealed with parafilm to prevent oxidation and immediately stored at -20 ºC until subsequentanalysis.

Determination of total carotenoid content (TCC):

Total carotenoid concentration of all sweet potato extracts were was determined by spectrophotometry according to the method described by Rashidi (2009). The dried carotenoid was re-suspended in 300 µL of ethyl acetate for determination of total carotenoid content (TCC). 50 µL of the re-dissolved sample was then diluted with 950 µL chloroform for spectrophotometric analysis. The steps of extraction and re-suspension were repeated at least three times for each sample. The carotenoid-containing solutions were measured at three wavelengths λ; 480 nm, 648nm, and 666nm using Varian Cary 50 UV-Vis spectrophotometer. The Wellburn Equation (Wellburn, 1994), in chloroform was applied to obtain the total carotenoid content as described below:

Ca= 10.91A666 - 1.2A648 ..... (1)

Cb= 16.36A648 – 4.57A666 .....(2)

Cx+c = (1000A480 – 1.42Ca – 46.09Cb)/202 (µg/ml)…..(3)

Wheres; Ca= concentration of carotenoid at 666 nm, Cb= concentration of carotenoid at 648 nm, and Cx+c = total carotenoid concentration at 480 nm.

Determination of individual carotenoid content HPLC Analysis:

The HPLC analysis of carotenoids extracted from sweet potato was performed on an Agilent model 1100 series comprised of a binary pump with auto-sampler injector, micro vacuum degassers, thermostatted column compartment and a diode array detector according to Rashidi (2009) and Morris *et al*. (2004) with minor alterations listed below. The column used was a ZORBEX Eclipse SB - C18 end capped 5 µm, 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 gradientused 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 20oC. The injection volume was 10 µL. Carotenoid standards of α-carotene, β-carotene, lutein and zeaxanthin were obtained from Sigma-Aldrich. Calibration curves were used to calculate the concentration of the respective carotenoids in experimental samples as described by Othman. Detection of individual carotenoids was confirmed by their spectral characteristics, absorption maximum and retention time as described by Britton (1995). 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 total carotenoid content determined by spectrophotometry. The total and individual carotenoid concentration would be expressed in terms of microgram per 1.0 g dry weight of freeze-dried matter (µg/g DW).

RESULTS AND DISCUSSION

Determination of total and individual carotenoid content:

Total carotenoid content (TCC) was measured quantitatively by using UV-VIS spectrophotometer. Throughout the present study, all the procedures were done under dim light condition to minimize photo-isomerization and photo-oxidation of the carotenoids since they possess highly conjugated double bonds in their structures. The use of oxygen-free nitrogen gas for storage of samples is also essential as to avoid oxidation of the phyto chemicals by atmospheric loss.

Table 1 presents the total and individual carotenoid content measured in (µg/g) DW dry weight in Malaysian orange sweet potato flesh; from five different locality in Malaysia; Kelantan, Pahang, Terengganu, Selangor and Perak. Table 1 shows that the highest total carotenoids content was observed in the orange sweet potato OSP from Kelantan at 1331.15±0.49 µg/g DW., followed by Pahang and Terengganu OSP with values (1139.33±0.15 and 938.90±0.22) µg/g DW, respectively. This result is confirmed by the findings of previous study by Suhair *et al*. (2014), where they revealed that the Malaysian orange sweet potato was the best in its content of carotenoids. The lowest total carotenoids content TCC that was detected in Perak OSP sample with value of 513.01±.0.07 (µg/g) DW. Many previous studies demonstrated that regular consumption of sweet potato (OFSP) significantly increased vitamin A status in children (Rodriguez Amaya and Kimura 2004, Low *et al*., 2007 and Aurelie, 2010) Malaysian orange sweet potato flesh shows high content of carotenoids comparing to a previous study was done by Aurélie (2010), where the values of total carotenoids content in orange sweet potato was 294.5 and 250.3 µg/g DW form Uganda and UK, respectively.

Table 1: Total & individual carotenoid content (µg/g DW) in Malaysian Orange Sweet potato from five different sites; Kelantan, Pahang, Selangor, Perak and Terengganu.

Locality	Total Carotenoid (µg/g DW)	Zeaxanthin (µg/g DW)	α-carotene (µg/g DW)	β-Carotene (µg/g DW)
Kelantan	1331.15±5.49	13.01±0.11	49.13±0.02	1123.85±0.04
Pahang	1139.33±0.15	ND	40.54±0.01	1069.14±8.49
Terengganu	938.084±2.98	ND	38.15±0.32	773.03±0.05
Selangor	649.90±0.27	ND	27.14±0.23	529.60±0.33
Perak	513.01±4.07	ND	22.49±0.15	399.40±3.62

tected, significantly different at $p<0.0001$ (n=3)

The findings of current study improved that carotenoids content differs depending on the extraction method, the drying method and environmental factors, these findings are comparable with Aurélie (2010). Climate temperature influence the carotenoids content in fruits, where elevated tropical climates accommodate the carotenoids biosynthesis, with fruits produced in this type of climates normally contains higher carotenoids concentrations (Kreck *et al*., 2006 and Kimura *et al*., 1991).

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. For distribution of individual carotenoids, it's noted that β-carotene and α-carotene are predominating other carotenoids compounds (Chaoyang *et al*., 2011). In general it was found that orange sweet potato from Kelantan was the species with highest total an individual carotenoids content. Orange sweet potato samples from Kelantan and Pahang were convergent somewhat in the concentrations of β-carotene and α-carotene 1123.85±0.04 µg/g DW and 49±0.02 µg/g DW, respectively. In current study, a different intensity of the orange color was observed in orange sweet potato from different locality. Also, β-carotene content

varies between the orange sweet potato samples that is confirmed by the findings of previous studies (Burgos *et al*., 1997, Almeida-Muradian and Penteado 1992, Takahata *et al*. 1993), where they reported that a positive correlation was observed between intensity of colorations of the sweet potatoes and the β-carotene content. Deep-colored vegetables and fruits are known to be good sources of carotenoids (Qian *et al*., 2004, Sass-Kiss *et al*., 2005, Trappey *et al*., 2005 and Cieslik *et al*. 2006).

Zeaxanthin compound was detected only in orange sweet potato from Kelantan 13.01±0.11 µg/g DW. In current study, the value of zexanthin found is considered to be an excellent compared with zeaxanthin content in a prior study (Sajilata *et al*., 2008), where they investigated zeaxanthin in a wide range of fruits and vegetables, and found out the maximum level of zeaxanthin was in corn 5. 28 µg/g DW. A previous study on lutein and zeaxanthin demonstrated the acceptable Daily Intake (ADI) for zeaxanthin of 0 to 2 mg/kg body weight (Sajilata *et al*., 2008, and JECFA, 2006). α-carotene compound was detected in all orange sweet potato samples, it ranged from 22.49±0.15 µg/g DW to 49.13±0.02 µg/g DW in Perak and Kelantan OSP, respectively. Values of a-carotene in this study was confirmed by previous study of Lucia *et al*. (2012). The individual carotenoid in orange sweet potato is influenced by site of plantation and this factor might be due to temperature of the location, fertilizer used for the crops, type of soil, exposure to sunlight, amount of rainfall and post-harvest handling. Tropical climate elevate carotenoid biosynthesis, therefore, it is normal that Malaysian fruits and vegetables contain higher carotenoids concentrations (Cavalcante and Rodriguez-Amaya. 1992, Kimura *et al*., 1991).

Figure 1 represents the HPLC chromatograms of carotenoids in orange sweet potato from Kelantan and their spectral characteristic.

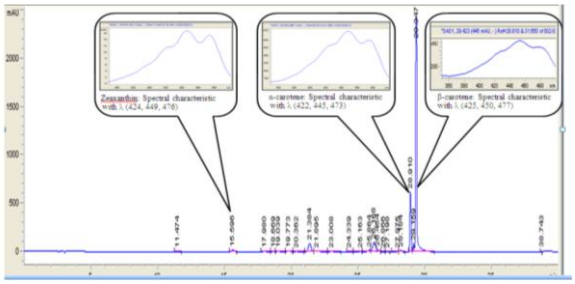

Fig. 1: Example of HPLC chromatograms of carotenoids in orange sweet potato from Kelantan; (a) zeaxanthin at RT: 15.596, (b) α-carotene at RT: 28.909 , (c) β-carotene at RT: 29.349 minutes and their spectral characteristic.

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 λmax values (Rodriguez-Amaya, 2001). The retention time and the spectral characteristic can be used for individual carotenoid confirmation. From current study, the carotenoid spectral characteristic from orange sweet potato detected by HPLC was compared to previous study reported by Rodriguez-Amaya (2001). The retention time for individual carotenoids were; zeaxanthin at 15.596 minutes, α–carotene at 28.910 minutes and β – carotene at 29.917 minutes.

From all these results, the carotenoid profiles of orange sweet potato from different sites show significant differences both in their qualitative and quantitative distribution which is consistent with the results reported by Arima and Rodriguez-Amaya (1988), and (Azevedo and Rodriguez-Amaya, 2002) for their samples. Moreover, due to the naturalvariation in carotenoids composition, data obtained in sweet potato cultivars in Malaysia may not be relevant in another, as reported by Rodriguez-Amaya *et al*. (2006).

Conclusion:

Orange sweet potato from different locality in Malaysia vary qualitatively and quantitatively in their carotenoids content according to the location or geographic site of crop. α-carotene and β-carotene were detected in all orange sweet potato samples from different locality in Malaysia, zeaxanthin was detected only in orange sweet potato from Kelantan. In conclusion, □-carotene represents the dominant component in the orange sweet potato. Due to the low cost and availability throughout the year, Malaysian orange sweet potato is considered to be a sustainable source of carotenoids to enhance pharmaceutical industry inside and outside Malaysia.

ACKNOWLEDGMENT

The authors would like to express their sincere appreciation to Ministry of Education (MOHE) and to International Islamic University Malaysia (IIUM) for the Research Grant RACE14-001-0007 and MIRGS13-01-002-0004.

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