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Original Article

CHARACTERISATION OF CAROTENOID CONTENT IN DIVERSE LOCAL SWEET POTATO (*IPOMOEA BATATAS*) FLESH TUBERS

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ABSTRACT

Sweet potato (*Ipomoea batatas*) is one of the most important food crops in the world. Sweet potato is rich with carotenoids and pro-vitamin A. Carotenoids compounds are commonly found in fruits and vegetables and are responsible for yellow, orange, and red pigmentations. Carotenoids are antioxidants compounds with pharmaceutical and medicinal benefits. Carotenoids such as α-carotene and β-carotene react as provitamin A in the human body, while lutein and zeaxanthin are two major components of the macular pigment of the retina.

Objective: The objective of this study is to verify the high nutritional value of Malaysian sweet potatoes varieties by identifying and comparing their carotenoids content.

Methods: Spectrophotometry and high performance liquid chromatography (HPLC) analysis were used to identifying and comparing carotenoids content quantitatively and qualitatively in orange, yellow, purple and white Malaysian sweet potatoes flesh tuber.

Results: The results of this study showed that the highest total carotenoid content was in orange sweet potato followed by yellow, purple and white sweet potato. β-carotene was available in all types of sweet potato ranging from 91.95±2.05 µg/g DW in white sweet potato to 376.03±11.05 µg/g DW in orange sweet potato. Detectable levels of zeaxanthin were appeared with values 5.44±3.23 µg/g DW and 20.47±2.03 µg/g DW in yellow and white sweet potato, respectively. Lutein was available only in orange sweet potato at trace amount of 0.91±1.03 µg/g DW. Purple sweet potato contains only β-carotene (113.86±14.17 µg/g DW) with absence of other carotenoids.

Conclusion: Total and individual carotenoids content vary between the flesh of these local sweet potato varieties. The results from this study can festoon the pharmaceutical, food and cosmetic industries markedly.

Keywords: Sweet potato, α-carotene, β-carotene, Lutein, Zeaxanthin.

INTRODUCTION

Sweet potato (*Ipomoea batatas*) is one of the most important tuber crops for fresh consumption in Malaysia; it is cheap and commonly available throughout the year [1]. The sweet potato of the convolvulaceae family is a tuberous plant that grows in tropical and subtropical areas. Native from Latin America [2, 3], sweet potato is ranked the fifth among the world most important food crops, with more than 133 million tones of annual production [4]. Sweet potato tuber flesh can be either white, cream, yellow, orange, or purple [4, 5] but the most commonly grown and eaten are orange, white, and cream [6]. Sweet potatoes are an important staple crop in parts of Africa, Asia, and the Pacific, [4, 5]. Sweet potato roots have remarkable pro-vitamin A quantities and they are one of the major food sources of carotenoids [7, 4].

Carotenoids in plants, are the natural organic molecules with diverse and important biological actions an functions. Carotenoids participate in the process of photosynthesis as accessory pigments and also in protecting chlorophylls from photo damage. Many carotenoids are provitamin A-active, others act as antioxidants, and several have been associated with the prevention of cancer and other chronic diseases. Fruits and vegetables are an important source of carotenoids for the human diet; they provide about 70-90% of consumed carotenoids [8]. 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 [9, 10]. The major carotenoids important to humans are α-carotene, β-carotene, lycopene, lutein, zeaxanthin and β-cryptoxanthin [11, 12]. About 50 carotenoids are known to have a provitamin A activity [14] and they can be grouped into carotenes (non-polar) and xanthophylls (polar) [9, 13]. These compounds have biological properties of interest for humans, and pharmacological or nutritional properties. Carotenoids in particular, likewise vitamin C, vitamin E or polyphenols, have antioxidant properties [15, 16, 17]. Ingested with food, these compounds strengthen our natural defence against oxidative stress and thus prevent various chronic diseases such as cancer as well as cardiovascular diseases [18, 19]. Since they cannot be synthesized by human body, these pigments have to be supplemented through dietary intake [20].

In Malaysia, sweet potato is popular among local consumers, but there is an urgent need to determine their nutritional value and study their pharmacological properties. The aim of present study is to assess carotenoids content in local sweet potato to identify their potential utility for the pharmaceutical industry or other related industries.

MATERIALS AND METHODS

Sample preparation

Sweet potato samples were bought from local markets. Samples were hand-peeled, cut to reduce the size, freeze-dried (EYELA FDU-1100, Japan) for 72 hours, then the samples were ground into fine powder and kept at -20°C until further analysis. Fig. 1 shows the four types of sweet potato analyzed in the current study.

Extraction of carotenoids

The extraction procedure essentially follows the methods described by Othman [21], with some modification. 1.0 g of each powdered freeze-dried sample was weighed and rehydrated with 3 mL of distilled water, then extracted in 25 mL of acetone and methanol mixture (7:3) 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.

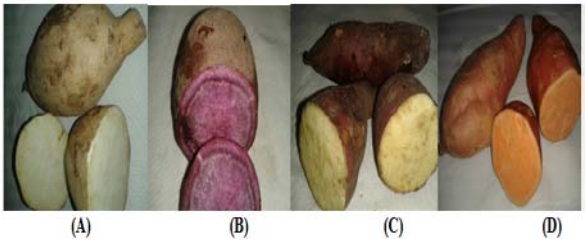


Fig. 1: Types of Malaysian sweet potato tuber with different flesh colours, A-Orange Flesh, B-Yellow Flesh, C-Purple Flesh, D-White Flesh

The extraction procedure for every sample was repeated until the supernatant or the tissue is colourless. 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 were added to the combined supernatants. The mixture was then allowed to separate under centrifugal force and the upper hexane layer was collected. The combined upper phase then dried completely under a gentle stream of oxygen-free nitrogen.

Determination of total carotenoid content

Total carotenoid concentration of all sweet potato extracts were determined by spectrophotometry according to the method described by Othman and Lewis [21, 22]. The dried carotenoid was resuspended in 300 µL of ethyl acetate for determination of total carotenoid content. 50 µL of the redissolved sample was then diluted with 950 µL chloroform for spectrophotometric analysis. The carotenoid-containing solutions were measured at three different wavelengths λ; 480 nm, 648 nm and 666 nm using Varian Cary 50 UV-Vis spectrophotometer. The Wellburn Equation [23] in chloroform was applied to obtain the total carotenoid content as described below:

$$C_a = 10.91A_{666} - 1.2A_{648} \quad (1)$$

$$C_b = 16.36A_{648} - 4.57A_{666} \quad (2)$$

$$C_{x+c} = (1000A_{480} - 1.42C_a - 46.09C_b)/202 \ (\mu g/ml) \quad (3)$$

C_a= concentration of carotenoid at 666 nm, C_b= concentration of carotenoid at 648 nm and C_{x+c} = total carotenoid concentration at 480 nm.

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 Othman and Morris [21, 24]. The column used was a ZORBAX SB-C_{18} end capped 5µm, 4.6x250 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 are 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 [21]. Detection of individual carotenoids was confirmed by their spectral characteristics, absorption maximum and retention time as described by Zaifuddin [25]. 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 were 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

Analysis of total and individual carotenoid content

Malaysian sweet potato flesh tubers ranging from orange, yellow, purple and white were selected for this study as shown in table 1 and fig. 1. These four types of different flesh colour exhibited highly significant differences in total and individual carotenoid content ($P < 0.0001$). Results revealed that the highest total carotenoid content was observed in the orange-fleshed sweet potato (389.22±2.18 µg/g DW) and followed by yellow-fleshed (138.96±7.54 µg/g DW). Purple and white fleshed accumulated almost the same amount at 116.28±1.80 µg/g DW and 115.18±5.71 µg/g DW respectively. There was strong relationship between total carotenoid content and the colour intensity of sweet potato tuber flesh. These results are in agreement with the previous reports where orange sweet potato cultivars were found richer in carotenoids and vitamin A value than yellow, cream and white sweet potato [26, 27].

Table 1: Total and individual carotenoid content (µg/g DW) in malaysian orange, yellow, purple and white sweet potato flesh tubers

Flesh colour	Lutein (µg/g DW)	Zeaxanthin (µg/g DW)	α-Carotene (µg/g DW)	β-Carotene (µg/g DW)	Total carotenoid (µg/g DW)
Orange	0.91±1.03	ND	16.16±0.02	365.03±11.05	389.22±2.18
Yellow	ND	5.44±3.23	8.61±1.98	117.00±3.12	138.96±7.54
Purple	ND	ND	ND	113.86±14.17	116.28±1.80
White	ND	20.47±2.03	3.3±1.32	90.95±2.05	115.18±5.71

ND: Not Detected, significantly different at $p<0.0001$

The next step in this analysis was to determine whether colour pigmentation of sweet potato tuber flesh is associated with specific carotenoid compounds. HPLC analysis of individual carotenoid pigments detected at least four types of carotenoid peaks: lutein, zeaxanthin, α-carotene and β-carotene. As shown in table 1, lutein was detected only in orange flesh, zeaxanthin was highest in white flesh but absent in orange and purple flesh whereas α-carotene and β-carotene were detected in their highest levels both in orange-fleshed. Purple flesh was found to have only β-carotene however the other three were detected with at least three individual compounds. Interestingly it is noted that distribution of all individual carotenoid pigments for each group of sweet potato flesh colour were not similar even though predominated by α-carotene and β-carotene and that is confirmed by previous studies findings [30, 31, 32, 33, 34]. According to Aurelie [13], carotenoid content differs depending on the extraction method, the drying method and environmental factors. 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 carotenoids concentrations [28, 29]. In general,

deep-colored vegetables and fruits are known to be good sources of carotenoids [35-38].

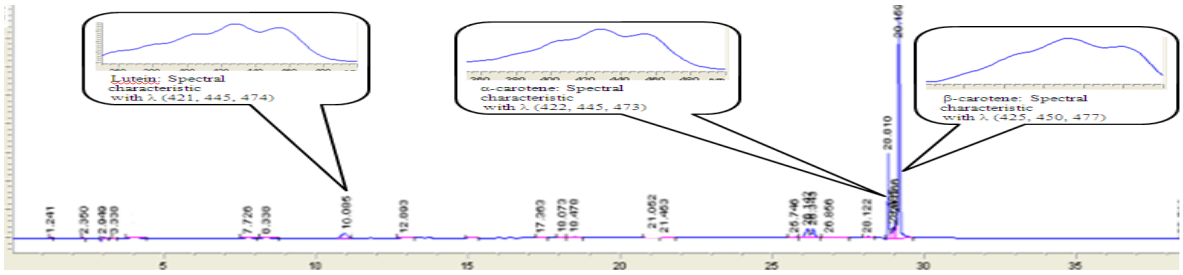
Fig. 2 showed HPLC chromatograms of carotenoids in orange, yellow, purple and white sweet potato flesh and their spectral characteristics. Most of the carotenoid compounds absorb maximally at three different wavelengths, resulting in three-peak spectra. The greater the number of conjugated double bonds, the higher the λmax values [31]. The retention time and the spectral characteristic can be used for individual carotenoid confirmation and each sweet potato sample detected by HPLC was compared to the previous study reported by Norshazila [31]. The retention time for individual carotenoids in sweet potato was; lutein at 10.669 minutes, zeaxanthin at 14.717 minutes, α–carotene at 28.810 minutes and β-carotene at 29.194 minutes.

Retinol equivalent (RE) value

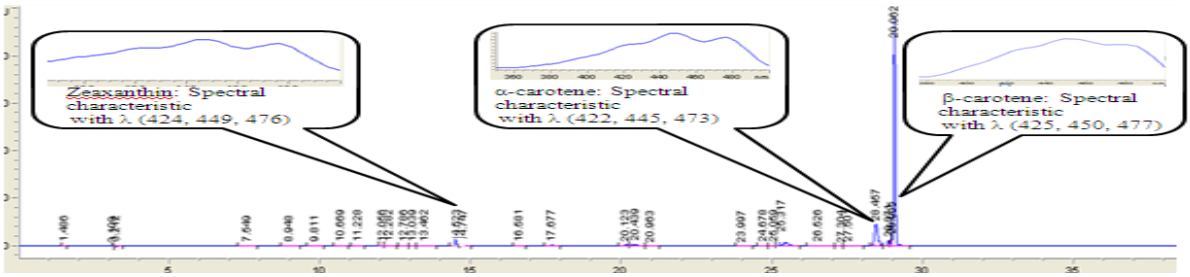
To express the vitamin A activities of α-carotene and β-carotene carotenoids in diets on a common basis, FAO [39] introduced the concept of retinol equivalent (RE) and established a following relationship among food sources of vitamin A, where 1 RE = l µg of retinol = 6 g of β-carotene or 12 µg of α-carotene. From this study, the pro-vitamin A in sweet potato samples include α–carotene and β–carotene will be converted enzymatically to retinol in the intestinal mucosa [40]. table 2 presents the RE values in different types of sweet potato of this study.

Table 2: Total retinol equivalent (RE) activity in four different types of sweet potato flesh.

Flesh colour	Orange	Yellow	Purple	White
Total re	60.84	19.5	18.98	15.16



(A)



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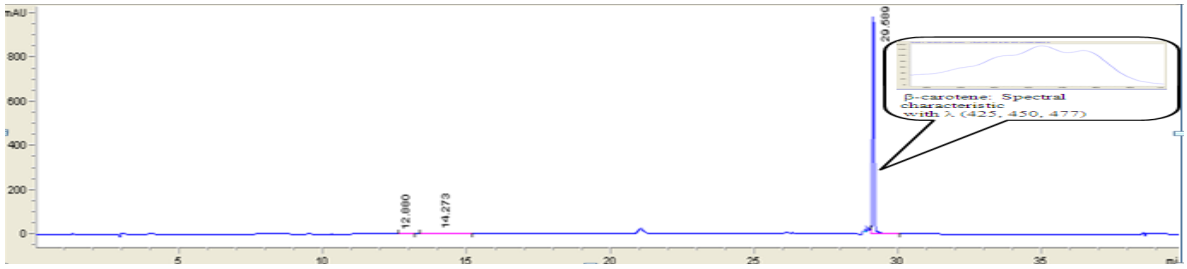
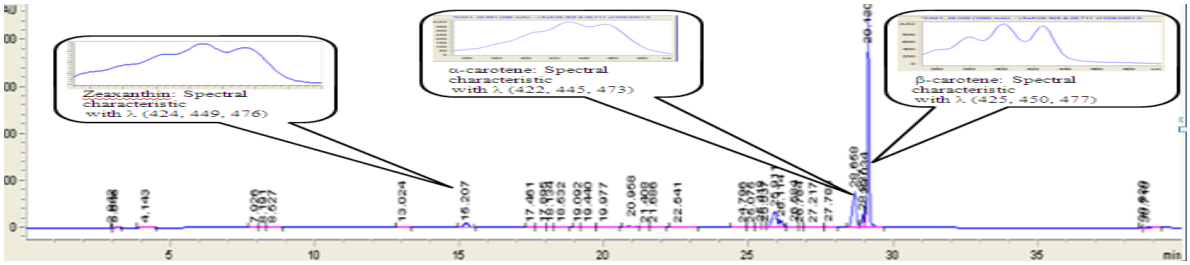


(C)



(D)

Fig. 2: HPLC chromatograms and retention time of individual carotenoids in different types of sweet potato flesh, A-Orange, B-Yellow, C-Purple, D-White

RE for orange sweet potato in the current study were higher than RE that was reported in the previous study by Scotta [40], suggesting that Malaysian sweet potatoes are an excellent source of provitamin A. The recommendation vitamin A intake for children is 375-500 µg/day whereas for adults is 500-600 µg/day as reported in previous studies [31, 41, 42]. From the present study, the RE value that can be obtained from 1g DW sweet potato is ranged from 15.16 µg RE to 60.84 µg of RE. It was found that around 6 g DW of orange sweet potato or 25 g DW of other types of sweet potato can provide appropriate and sufficient quantity of vitamin A in the daily food. These results revealed that carotenoids from orange-fleshed sweet potato are highly vitamin A active and in agreement with Jalal [43].

The carotenoid composition of different Malaysian sweet potato flesh tubers showed significant differences in their qualitative and quantitative distribution which in consistent with the results reported by others [44-47]. Moreover, due to the natural variation in carotenoid composition, data obtained in sweet potato cultivars in Malaysia may not be relevant to sweet potatoes from other countries [48]. Total and individual carotenoid concentrations in sweet potato are influenced by many factors such as temperature of the location, fertilizer used, 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 [29].

CONCLUSION

This study provided new information on total and individual carotenoids composition in the most popular, available and cheapest variety of Malaysian sweet potato and quantified their nutritional values and their importance to overcome and combat the Vitamin A Deficiencies (VAD). Due to their bright color, non poisonous nature, rich nutrition, safe and health care function, carotenoids from local sweet potato are recommended for applications in pharmaceutical, food and cosmetic industries locally and globally. Since there are many factors influence carotenoid content in plants such as post-harvest handling, locality, variety, season, temperature and storage, therefore, more study need to be done to identify the key factors that influence the total and individual carotenoid in local sweet potato in order to enhance their productivity and nutritional quality.

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CONFLICT OF INTERESTS

Declared None

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