# Stability of Carotenoid Composition in Orange Sweet Potato (*Ipomoea batatas*) Tuber Flesh Over Three Growing Seasons and 6 Months Storage Time

Rashidi Othman<sup>1,\*</sup>, Suhair Kamoona<sup>2</sup>, Irwandi Jaswir<sup>2</sup>, Parveen Jamal<sup>2</sup> and Farah Ayuni Mohd Hatta<sup>1</sup>

<sup>1</sup>International Institute for Halal Research and Training (INHART), Herbarium Unit, Department of Landscape Architecture, Kulliyyah of Architecture and Environmental Design, International Islamic University Malaysia, 53100 Kuala Lumpur, Malaysia

<sup>2</sup>Department of Biotechnology Engineering, Kulliyyah of Engineering, International Islamic University Malaysia, Jalan Gombak, 53100 Kuala Lumpur, Malaysia

**Abstract:** Considerable research interest has recently focused on the development of both transgenic and traditional breeding methods to increase total and individual carotenoid composition in sweet potatoes. Unfortunately little information is available on the influence of the environment on carotenoid profile stability in sweet potatoes especially growing seasons and storage. Therefore the aim of this study is to explore the composition and concentration of carotenoids in orange sweet potato tubers to enable their future enhancement through genetic manipulation over different growing seasons and storage time. Our findings revealed that orange sweet potato tubers contained  $\alpha$ -carotene and  $\beta$ -carotene in the first and second season, whereas in the third season only lutein and zeaxanthin were detected. Analysis of carotenoid profiles of the orange sweet potato tubers grown in three different seasons showed that the growing season had a major effect on the carotenoid compounds stability. Besides growing seasons, the level of total and individual carotenoids in orange sweet potato tuber flesh are strongly influenced and affected by storage conditions. The storage of tubers over 6 months period of time appears to have distinct effects on carotenoid content and composition. Results showed that storage from 1 to 6 months resulted in the accumulation of zeaxanthin and  $\beta$ -carotene and total carotenoid content.

Keywords: Orange sweet potato, abiotic factors, total carotenoid, storage, growing season, carotenoid stability.

#### INTRODUCTION

Although it ranks as the fifth most important food crop in the developing world (after rice, wheat, maize, and white potato), sweet potato (Ipomoea batatas) has been relatively neglected by the global agricultural research community [1.2]. In Malaysia, sweet potato is popular among local consumers, but studies focusing on its potential for pharmaceutical, cosmetic and other related products remain limited. There is an urgent need for research to evaluate the high nutritional value of diverse local sweet potato in Malaysia for use in pharmaceutical and food industries. Due to the natural variation in carotenoid composition, data obtained in local sweet potato cultivars in one country may not be relevant to sweet potatoes from other countries [3]. Therefore, it is important to study the carotenoids content in the most popular varieties of Malaysian sweet potato quantitatively and qualitatively and compare it with crops from other countries. Sweet potato roots have remarkable pro-vitamin A quantities and are one of the major food sources of carotenoids [4]. Besides acting as antioxidants, carotenoids compounds also provide sweet potatoes with their distinctive flesh colours such as white, cream, yellow, orange and purple [5]. Sweet potatoes are rich in dietary antioxidants, such as  $\beta$ -carotene [6]. The Recommended Dietary Allowance (RDA) for vitamin A is 1000 retinol equivalents, equal to 6 mg  $\beta$ -carotene, per day. Consuming 250 gm of this tuber flesh is sufficient to provide 50% of the Recommended Daily Allowance of Vitamin A. This consumption (47 µg/g dry weight) is even better than Golden Rice 2 with 31 µg/g dry weight of  $\beta$ -carotene [7,8]. Sweet potato is one of the most important tuber crops for fresh consumption in Malaysia. It is cheap and commonly available throughout the year [9]. Sweet potato tuber can also be commercially processed and used in the manufacturing of various food and non-food products such as flour, starch, health food, baby food and animal feed [2,10].

Carotenoid content in fruits and vegetables are influenced by many factors such as variety, level location, part of the plant utilised, environment conditions during agricultural production, post-harvest handling, processing, and storage conditions [11-13].

<sup>\*</sup>Address of correspondence to this author at the International Institute for Halal Research and Training (INHART), Herbarium Unit, Department of Landscape Architecture, Kulliyyah of Architecture and Environmental Design, International Islamic University Malaysia, 53100 Kuala Lumpur, Malaysia; Tel: +6012 6644772; Fax: +603 61964864; E-mail: rashidi@iium.edu.my

[13,14] reported that climate temperature could elevates carotenoid biosynthesis in fruits, and normally raises their carotenoids concentrations. According to [14,15] carotenoid biosynthesis may continue in plant organs, even after harvest. There is still lacking of data on carotenoids stability particularly during food processing and post-harvest handling, storage and climatic factors [16]. Therefore, this study aimed to explore the composition and concentration of carotenoids in orange sweet potato tubers to enable their future enhancement through genetic manipulation over different growing seasons and storage time.

#### MATERIALS AND METHODS

#### **Sample Preparation**

*Ipomoea batatas* (keledek) or orange sweet potato tubers from Terengganu, Malaysia; were obtained from Federal Agriculture Marketing Authority (FAMA), Selayang, Malaysia. The skin of the tubers was removed with a peeler and the remaining tuber tissue was cut into 5 mm slices and immediately stored at -20°C. The tuber samples were freeze-dried for 7 days, after which the samples were ground into fine powder and stored at -80 °C until further analysis.

#### **Extraction of Carotenoids**

The extraction procedure essentially follows the methods described by [17]. Each powdered sample weighed 1.0 g was rehydrated with distilled water and extracted with a mixture of acetone and methanol (7:3) at room temperature until colorless. The crude extracted was then centrifuged for 5 min at 10 000 g and stored at 4°C in the dark prior to analysis. To extract carotenoids, an equal volume of hexane and distilled water was added to the combined supernatants. The solution was then allowed to separate and the upper layer containing the carotenoids was collected. The combined upper phase was then dried to completion under a gentle stream of oxygen-free nitrogen.

# Spectrophotometric Determination of Total Carotenoid Content

Total carotenoid concentration was determined by spectrophotometry as described by [17]. The dried carotenoid was resuspended in 300  $\mu$ l of ethyl acetate and for determination of total carotenoid, 50  $\mu$ l of the redissolved sample was then diluted with 950  $\mu$ l chloroform for spectrophotometric analysis. Carotenoid containing solutions were measured at three different wavelengths,  $\lambda$ : 480 nm, 648 nm and 666 nm using

Varian Cary 50 UV-Vis spectrophotometer. The Wellburn Equation [18] in chloroform was applied to obtain the total carotenoid content as described below:

$$C_a = 10.91A_{666} - 1.2A_{648}$$

 $C_{b} = 16.36A_{648} - 4.57A_{666}$ 

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

#### **HPLC Analysis of Carotenoids**

The HPLC analysis of carotenoids were performed on an Agilent model 1200 series comprised of a quarternary pump with autosampler injector, microdegassers, column compartment equipped with thermostat and a diode array detector. The column used was a ZORBAX Eclipse XDB-C<sub>18</sub> end capped 5 µm, 4.6x150 mm reverse phase column (Agilent Technologies, USA). The eluents used were (A) acetonitrile:water (9:1 v/v) and (B) ethyl acetate. The column separation was allowed via a series of gradient such 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<sup>-1</sup>. The column would be allowed to re-equilibrate in 100% A for 10 min prior to the next injection. The temperature of the column was maintained at 20°C. The injection volume is 10 µL each. Detection of individual carotenoids was made at the wavelengths of maximum absorption of the carotenoids in the mobile phase: neoxanthin (438 nm), violaxanthin (441 nm), lutein (447 nm), zeaxanthin (452 nm), β-carotene (454 nm), βcryptoxanthin (450 nm) and  $\alpha$ -carotene (456 nm). 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 milligram per 1.0 g dry weight of freeze-dried matter (µg/g DW).

#### **RESULTS AND DISCUSSION**

# Analysis of Carotenoid Content of Orange Sweet Potato Tubers in Response to Growing Seasons

Orange sweet potato flesh was analysed for total and individual carotenoid content from three growing seasons of 2011, 2012 and 2013. Analysis of variance

Growing season	Lutein (µg/g DW)	Zeaxanthin (µg/g DW)	α-carotene (μg/g DW)	β-carotene (μg/g DW)	Total carotenoid (μg/g DW)
2011	ND	ND	38.15±0.32	733.03±0.05	938.08±2.98
2012	ND	ND	0.17±0.1	467.15±3.45	482.82±6.71
2013	0.69±0.25	111.83±0.15	ND	290.12±7.87	405.01±2.33

Table 1:	Analysis of the Effect of Growin	g Season on Total and Individual Carotenoid in Oran	ge Sweet Potato Tubers

Data are expressed as means ±SD (n=3), ND: Not Detected, significantly different at p<0.0001.

established highly significant differences (P < 0.0001) between total carotenoid, individual carotenoids, the seasons, and all combinations of interactions (Table 1). This clearly demonstrates that growing season is one of the key factors that can have an influence on the stability of carotenoids. As shown in Table 1, in 2011 growing season, only  $\alpha$ -carotene and  $\beta$ -carotene were detected at 38.15±0.32 µg/g DW and 733.03±0.05 µg/g DW respectively. The same individual carotenoids were detected in 2012 growing season but with different concentrations, where  $\alpha$ -carotene was detected in a trace amount (0.17±0.1  $\mu$ g/g DW) and  $\beta$ -carotene at 467.15±3.45 µg/g DW. Lutein and zeaxanthin were not detected in orange sweet potato flesh samples in 2011 and 2012 growing seasons. In 2013 growing season, lutein was detected in trace amount (0.69±0.25 µg/g DW) whereas zeaxanthin and  $\beta$ -carotene were detected at 111.83±0.15 µg/g DW and 290.12±7.87  $\mu g/g$  DW respectively. Interestingly  $\alpha$ -carotene was absent in orange sweet potato flesh in 2013 growing season. Total carotenoid content appeared in varying amounts during these three years of harvesting. Total carotenoid did not represent sum up of total individual carotenoids in this study due to estimation and not based on the standards as in HPLC. The highest total caroteneoid content was observed in 2011 growing season and the lowest was in year 2013 growing season with values of 938.08±2.98 µg/g DW and 405.01±2.33 µg/g DW respectively.

Results of current study shows that the growing seasons significantly influenced the carotenoids content in orange sweet potato tuber flesh. This finding is in agreement with previous research conducted by [19] and [20], where those studies revealed that the amount of carotenoids detected were vary in each year of growing seasons. According to [21], carotenoids content in acerola fruits was found to be the highest during other level rainy season. There are environmental factors cause decrease in the carotenoids content such as daylight periods, low rainfall, and high temperature [22]. These circumstances strongly correlated with the fertility of the soil, where the rainy season would increase the soil

fertility, thus provide the plants with the essential nutrients, water and oxygen [23]. Presence of lutein and zeaxanthin in 2013 growing season can be explained according to previous studies of [17,24,25], where they reported, that the reactions involved to produce these two carotenoids is hydroxylation of acarotene and  $\beta$ -carotene under a reversible reactions mediated by pH soil. Besides, [26] explained, organic matter can increase the acidity of the soil and pH which would affect the epoxidation and de-epoxydation reactions of carotenoid in the xanthophyll cycle. Moreover, due to the natural variation in carotenoid composition, data obtained in sweet potato cultivars in Malaysia may not be relevant in another, as reported by [28]. Therefore a notable differences were observed between the three growing seasons whereby the accumulation of  $\alpha$ -carotene and  $\beta$ -carotene in 2011 and 2012 growing seasons and the presence of zeaxanthin in 2013 growing season.

# Analysis of Carotenoid Content of Orange Sweet Potato Tubers in Response to 6 Months Storage Time

Table **2** presents the total and individual carotenoids content in Malaysian orange sweet potato tubers post harvesting during six months of storage at temperature 20°C. There was a highly significant difference (p <0.0001) in total and individual carotenoid content during six months storage time. As demonstrated in Table **2**, after harvest and one month storage time, only αcarotene and β-carotene were detected at 38.15±0.32 µg/g DW and 773.03±0.05 µg/g DW respectively.

After second and third months of storage time lutein, zeaxanthin,  $\alpha$ -carotene and  $\beta$ -carotene were detected with relatively high concentration of  $\beta$ -carotene and low level of zeaxanthin. During the forth and fifth months of storage time, lutein was absence and finally after sixth month of storage time both lutein and  $\alpha$ -carotene were absence. Both of total carotenoids and  $\beta$ -carotene content in orange sweet potato flesh increased slightly in the second month of storage, and this can be explained according to

Months of storage	Lutein (µg/g DW)	Zeaxanthin (μg/g DW)	α-carotene (µg/g DW)	β-carotene (μg/g DW)	Total Carotenoid (µg/g DW)
1 <sup>st</sup> month	ND	ND	38.15±0.32	773.03±0.05	938.08±2.98
2 <sup>nd</sup> month	0.47±0.41	24.17±0.35	0.29±0.16	924.15±3.53	969.52±6.64
3 <sup>rd</sup> month	0.14±0.02	5.65±0.15	0.21±0.12	704.12±5.62	718.82±2.03
4 <sup>th</sup> month	ND	6.45±0.01	0.12±0.08	521.84±2.85	531.11±1.11
5 <sup>th</sup> month	ND	2.02±0.03	0.06±0.01	289.52±1.20	295.92±10.97
6 <sup>th</sup> month	ND	9.54±1.05	ND	104.28±4.93	117.58±3.89

 Table 2: Analysis of the Effect of Storage Time for 6 Months at 20°C in the Dark on Total and Individual Carotenoid in Orange Sweet Potato Tubers

Data are expressed as means ±SD (n=3), ND: Not Detected, significantly different at p<0.0001.

previous study by [28], where he attributed that increasing of carotenoids content to the carotenogenesis which might continue in intact fruits, vegetables, and root crops after harvest, and carotenoids are 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 during the storage times and this circumstance was also reflected by storage conditions. Both of total carotenoids and βcarotene content decreased dramatically from the second month to the sixth month of storage, total carotenoids content decreased from 969.52±6.64 µg/g DW to 117.58±3.89  $\mu$ g/g DW, and  $\beta$ -carotene content decreased from 924.15±3.53 µg/g DW to 104.28±4.93  $\mu$ g/g DW. Quantity of total carotenoids and  $\beta$ -carotene content decreased to 50% from the second month to the fourth month of storage time. [10] articulated that losses of 50% total carotenoids and  $\beta$ -carotene occurred in the sweet potato flour after 50-days of storage. [27] also observed similar results where the profile of pigments in ripening raspberries changes drastically, with a dramatic decrease of β-carotene and chlorophyll derivatives. a-carotene decreased from 38.15±0.32 µg/g DW to not detected, in the first month till the six month storage time, respectively. According to [28], carotenoids in dehydrated products are more likely to undergo degradation during storage because the greater surface area and porosity increase their exposure to oxygen and light.

Lutein was not detected in the first month of storage, it appeared in the second month ( $0.47\pm0.41$  µg/g DW) and decreased in the third month to 0.14±0.02 µg/g DW. This finding is in agreement with [27], where the xanthophyl lutein was observed also decreased but not to the same extent of  $\beta$ -carotene. Then, lutein disappeared in the next following months.

Zeaxanthin was not detected in the first month of storage of current study, then, this compound was fluctuated for the whole six months. Except the first month, the highest appearance for zeaxanthin was in the second month (24.17±0.35 µg/g DW) and then decreased in the third month to  $5.65\pm0.15$  µg/g DW. In the fourth month, zeaxanthin again raised to  $6.45\pm0.01$  µg/g DW, meanwhile, in the fifth month, zeaxanthin fell to  $2.02\pm0.03$  µg/g DW and raised to  $9.54\pm1.05$  µg/g DW the sixth month. From these results, it is presumed that  $\beta$ -carotene converted to zeaxanthin and  $\beta$ -cryptoxanthin through the hydroxylation process [26]. These phenomena can be explained as followed as stated by [29-32]:

- any lutein chemically destroyed by, for example, reaction with potentially damaging oxidants was replaced at a faster rate than the other major carotenoids;
- (ii) the activity of enzymes and candidate enzymes (carotenoid cleavage dioxygenases) that catabolize the β-carotene-derived carotenoids such as zeaxanthin was greater than those utilizing lutein as their substrate;
- the carotenoid sequestering structures formed in the tubers may also help to slow down carotenoid degradation especially in the intense yellow flesh tubers;
- (iv) due to induction mechanism such as physical changes like sprouting and subsequent dehydration, concentration and induced stress.

Another point is according to [30] the increase and decrease in particular carotenoid compounds is most probably due to low stability of specific carotenoid compounds. Furthermore, stimulation of antioxidant synthesis such as carotenoid is known to occur with stress, which may have increased at the end of the storage period due to dehydration [33,34,35]. Activation of systemic host defence systems upon pathogen infection is also known to involve stress response genes, which can also involve responses to oxidative stress [36]. Physical changes such as sprouting and dehydration are believed to be responsible for this phenomenon as well [30]. Therefore the response of orange sweet potato tubers to such environments appeared to be very important and the magnitude of these changes was found to be highly genotype dependent.

#### CONCLUSION

Besides cultivar and growing season, the levels of total carotenoids, zeaxanthin, lutein,  $\alpha$ -carotene and  $\beta$ carotene in orange sweet potato tubers are strongly influenced and affected by post harvest storage conditions. Examination of carotenoid profiles of the orange sweet potato tubers grown in three different seasons showed that the season had a major effect on the total carotenoid content and the individual carotenoid compounds. High concentrations of βcarotene was found in the first and second seasons, whereas high concentrations of zeaxanthin and  $\beta$ carotene were found in the third season. Meanwhile, the storage of orange sweet potato tubers over a sixmonth period of time appears to have distinct effects on carotenoid biosynthesis, the magnitude of the effects being dependent on the time of storage. Results show that storage for 1 till 6 months resulted in the accumulation B-carotene and zeaxanthin with a concomitant decreased of lutein, *a*-carotene and total carotenoid content. Determination the key factors among the growing seasons and postharvest handling conditions that control carotenoids accumulation, will provide a greater understanding to enhance the nutritional value of orange sweet potato.

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