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Influence of growing location, harvesting season and post-harvest storage time on carotenoid biosynthesis in orange sweet potato *(Ipomoea batatas)* tuber flesh

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Abstract

Carotenoid content in plants differs due to several factors such as cultivar, maturity, climate, locality and storage. Improving the nutritional values of sweet potato is an important breeding goal and understanding the regulation, genetics and inheritance of carotenoid biosynthesis are vital to achieve this. Environmental conditions can have a marked influence on the accumulation of carotenoids in sweet potato tubers. Little is known about the effects of location, post-harvest storage time and harvesting season particularly on carotenoid biosynthesis. Therefore, this study aimed to investigate the effects of growing location, harvesting season and storage time on carotenoid biosynthesis in orange sweet potato tuber flesh. The results showed that orange sweet potato tubers contained α-carotene and β-carotene in the first and second harvesting season (year 2011 and 2012), whereas lutein and zeaxanthin were detected only in the third harvesting season (year 2013). Analysis of carotenoid profiles of the orange sweet potato tubers grown in three different locations confirmed that the harvesting season had a major effect on the total carotenoid content and the individual carotenoid compounds. The post-harvest storage time of sweet potato tubers also appears to have distinct effects on carotenoid biosynthesis, the magnitude of the effects being dependent on the storage time, harvesting season and location. The results of this study will help to understand the effects of location, year of harvesting season and storage time on carotenoid accumulation in orange sweet potato tubers.

Introduction

Sweet potato *(Ipomoea batatas)* belongs to Convolvulaceae, a food plant of morning glory family. This plant is a native from Latin America and widely cultivated in tropical and subtropical areas (Davidson, 1999; Mark *et al.*, 2009; Seow-Mu *et al.*, 2011; Anuar and Wan, 2014). Sweet potato is one of the oldest vegetable known to man and it has been consumed since prehistoric times as evidenced by sweet potato relics dating back 10,000 years that have been discovered in Peruvian caves. Christopher Columbus brought sweet potatoes to Europe after his first voyage to the New World in 1492 (Mark *et al*., 2009; Seow-Mu *et al*., 2011; Anuar and Wan, 2014). Sweet potato crop ranks third of the world root and tuber crops production after potato and cassava (FAO Stat, 2008). World production has been estimated at 110 million tons per annum. Asia is the world's largest producer with 92.5 million tons (Woolfe, 1992; Bovell-Benjamin, 2007; FAO Stat, 2008). Sweet potato roots have remarkable pro-vitamin A quantities and they are one of the major food sources of carotenoids (Woolfe, 1992; Henkel, 1996). Recently, sweet potato is labeled as an "antidiabetic" food because of some animal studies in which sweet potato helped stabilize blood sugar levels and lowered insulin resistance (Kusano and Abe, 2000; Panda *et al*., 2006; Anuar and Wan, 2014). The orange fleshed sweet potato varieties provide the daily requirements of Vitamin A (Woolfe, 1992) and can be processed into juice or composite flours that can be used in making baked products and complementary foods (Hagenimana *et al*., 2001; Kuloba, 2013). Due to their high carotenoid content and yields, orange flesh sweet potatoes have also been used in several small-scale studies to increase vitamin A status (Haskell *et al*., 2004; Van Jaarsveld *et al*., 2005; Low *et al*., 2007). Sweet potato or locally known as *Keledek*, is one of the most important tuber crops for fresh consumption in Malaysia. It is traditionally grown for the fresh root market with a very small percentage being processed

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into traditional snacks such as *kerepek* (sweet potato crackers) or *cakar ayam* (fried sweet potato) (Siti Hasidah and Khatijah, 1994; Zaharah *et al*., 2004).

Carotenoids are a group of fat-soluble pigments occurring widely in nature (Hanif *et al*.,2012). The carotenoids group include carotenes (non-polar) and xanthophylls (polar) (Rodriguez-Amaya, 1997; Aurelie, 2010). In nature, there are over 700 known carotenoids isolated from natural sources (Britton; 2008) and about 50 carotenoids are known to have a provitamin A activity (Lee *et al*., 1989). In plants, precursors of vitamin A called carotenoids are associated with cellular lipids and implanted in cellular structures (FAO/WHO Rome, 2002). Carotenoids are bioactive compounds that give benefits to human health. Carotenoids have been linked with the enhancement of the immune system and decreased risk of degenerative disease such as cancer, cardiovascular disease, age related muscular degeneration and cataract formation (Mathews-Roth, 1985; Bendich and Olson, 1989; Bendich, 1990; Krinsky, 1990; Gerster, 1991; Mathews-Roth, 1991; Ziegler, 1991; Byers and Perry, 1992; Bendich, 1994; Krinsky, 1994) due to their ability to quench singlet oxygen and react with free radicals found in human body (Krinsky, 1993). Some of the carotenoids such as lutein and zeaxanthin can help to prevent the degeneration of macular pigment especially in elderly (Moeller *et al*., 2000). Carotenoids have also been identified as a potential inhibitor of Alzheimer's disease (Zaman *et al*., 1992). Although carotenoids are synthesized only in the vegetal kingdom, these pigments have to be supplemented through dietary intake of human (Van den Berg, 2000; Hanif *et al*., 2012). Carotenoids content in plants are influenced by many factors such as variety, level of maturity, climate/geographic site of production (location), part of which the plant is being utilized, pH and type of soil, environmental conditions during agricultural production, post-harvest handling, processing, including storage conditions (Rodriguez-Amaya and Kimura, 2004). Being highly unsaturated, carotenoids are susceptible to isomerization and oxidation during processing and storage of foods (Rodriguez-Amaya, 1999). Carotenoid biosynthesis may continue in fruits, vegetables, and root crops, even after harvest, provided these plant materials are kept intact and not treated in any way that would inactivate the enzymes responsible for carotenogenesis. So, the post-harvest degradation of carotenoids appears to prevail, especially at high storage temperature and under conditions that favor wilting. According to Jing *et al*. (2007), seasonal changes have significant impact in plant nutritional value, including secondary metabolite such as carotenoid and flavonoid. The seasonal or annual changes are strongly correlated with rainfall, temperature and humidity of the locations of plantation. Besides, crop rotation could also affect the carotenoids content in plants. This is due to the decrease of inorganic and organic substances in the soil, thus impacted the soil fertility for plantations (Patricia, 2012). According to Azevedo and Rodriguez-Amaya (2005), carotenoid content is affected by climate alterations, with exposure to sunlight and higher temperatures increasing the biosynthesis of carotenoids but, at the same time, inducing photo-degradation, thus reducing their levels in plants. There is a very limited data on the effect of storage and harvesting season on individual carotenoid content especially in sweet potato tubers. Therefore, this study aimed to investigate the effects of environmental factors which comprise storage at 20°C for six months, five different growing locations and the effect of different harvesting seasons (year 2011, 2012 and 2013) on carotenoids composition in orange sweet potato tubers.

Materials and methods

Sample preparation

30 kg of orange sweet potato samples from Kelantan, Pahang, Terengganu, Selangor and Perak were obtained from Federal Agriculture Marketing Authority (FAMA), Selayang, Malaysia from three different harvesting seasons (2011, 2012 and 2013). All tubers were harvested from plots at full maturity following natural plant senescence and maintained at ambient temperatures for three weeks until grading, then placed in cool storage (air at 8°C). 5 kg of them were pooled and analyzed each month for sixth consecutive month for carotenoid analysis. All samples were hand-peeled and the remaining tuber tissue was cut into 5.0 mm slices. For each sample tuber tissue was pooled from three tubers, mixed, and a random 100 g FW sample was immediately frozen at -20^{o}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 analysis.

Storage time treatments

All tubers (2011 harvesting season) were stored in darkness at cool temperatures (air at 8°C) for 1 month up to 6 months.

Extraction of carotenoids

The extraction procedure essentially follows the methods described by Rashidi (2009), with some modification. 1.0 g of 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, sample was vortexed and centrifuged for 2 minutes at 13500 x 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 the sample was repeated until the supernatant or the tissue is colorless. The pooled supernatant were centrifuged to remove fine particles and then stored at -20ºC 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 then dried completely under a gentle stream of oxygen-free nitrogen. Vials/tubes were capped and sealed with parafilm to prevent oxidation and immediately stored at -20ºC until subsequent analysis.

Determination of total carotenoid content

Total carotenoid concentration of all sweet potato extracts were determined by spectrophotometry according to the method described by Rashidi (2009) and Lewis *et al*. (1998). The dried carotenoid was re-suspended 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 wavelengths λ; 480 nm, 648 nm, and 666 nm using Varian Cary 50 UV-Vis spectrophotometer. The Wellburn Equation (1994), in chloroform was applied to obtain the total carotenoid content as described below:

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

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

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

Where; 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 tuber was performed on an Agilent model 1200 series which comprises a binary pump with auto-sampler injector, micro vacuum degassers, thermostat column compartment and a diode array detector in accordance to Othman (2009). The column used was a ZORBAX Eclipse Plus C_{18} end capped 5µm, 4.6 x 250 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 is 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 and the injection volume was 10 µL. Carotenoid standards such as α-carotene, β-carotene, lutein and zeaxanthin were obtained from Sigma-Aldrich. Detection of individual carotenoids was confirmed by their spectral characteristics, absorption maximum and retention time. 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. The individual carotenoid concentration was expressed in terms of microgram per 1.0 g dry weight of freeze-dried matter (µg/g DW).

Results

Carotenoid content and location interactions for orange sweet potato

Orange sweet potato tuber samples from the 2011 harvesting season of five different localities grown at Kelantan, Pahang, Terengganu, Selangor and Perak were analysed for carotenoid content and composition to determine the effect of different locations on carotenoid accumulation. Analysis of variance established highly significant differences ($p < 0.0001$) between the five locations and their interaction for all the carotenoid pigments (Table 1). This clearly demonstrates that growing locations can have an important influence on the accumulation of carotenoids. The importance of the interaction components emphasises that the changes in carotenoid composition are complex and the responses are not consistent across localities. The major carotenoids identified in tubers derived from all five locations were lutein, zeaxanthin, α-carotene and β-carotene. However, the content of individual carotenoid compounds in orange sweet potato varied

Table 1. Analysis of total and individual carotenoid compounds (µg/g DW) of orange sweet potato (2011 harvesting season) grown at five different locations in Malaysia

Locality	Total Carotenoid (µg/g DW)	Lutein (µg/g DW)	Zeaxanthin (µg/g DW)	α-Carotene (µg/g DW)	β-Carotene (µg/g DW)
Kelantan	1331.15 ± 5.49	ND	13.01 ± 0.11	49.13 ± 0.02	1123.85 ± 0.04
Pahang	1139.33 ± 0.15	ND	ND	40.54 ± 0.01	1069.14 ± 8.49
Terengganu	938.08 ± 2.98	ND	ND	38.15 ± 0.32	773.03 ± 0.05
Selangor	649.90 ± 0.27	ND	ND	27.14 ± 0.23	529.60 ± 0.33
Perak	513.01 ± 4.07	ND	ND	22.49 ± 0.15	399.40 ± 3.62

ND – non-detectable, ($P<0.0001$)

Table 2. Analysis of total and individual carotenoid compounds (µg/g DW) of orange sweet potato grown at Terengganu at three different harvesting seasons (2011,2012 and 2013)

Harvesting season	Total Carotenoid (µg/g DW)	Lutein (µg/g DW)	Zeaxanthin (µg/g DW)	α-Carotene (µg/g DW)	β-Carotene (µg/g DW)
2011	938.08 ± 2.98	ND	ND	38.15 ± 0.32	733.03 ± 0.05
2012	482.82 ± 6.71	ND	ND	0.17 ± 0.01	467.15 ± 3.45
2013	405.01 ± 2.33	0.69 ± 0.25	111.83 ± 0.15	ND	290.12 ± 7.87

ND – non-detectable, ($P<0.0001$)

with the location in which they were grown. This is indicative of the highly significant interactions between carotenoid composition and environment in the analysis of variance. The data from Table 1 showed that orange sweet potato grown in Kelantan contained the highest amount of total carotenoid (1331.15 µg/g DW) followed by Pahang, Terengganu, Selangor and Perak. Examination of carotenoid profiles of the orange sweet potato grown at different locations showed that the location had a major effect on the total and individual carotenoid contents. In addition, comparison of carotenoid profiles of these orange sweet potato tubers in five different locations also demonstrated tremendous fluctuation and variation in the individual carotenoids. For example, orange sweet potato from Kelantan contained relatively high concentration of β-carotene followed by α-carotene and zeaxanthin, but orange sweet potato from the four other locations contained only β-carotene and α-carotene. These data suggest that although location had an effect on the total carotenoid, they also had effect on the individual carotenoid content and carotenoid composition of the orange sweet potatoes in different localities.

Carotenoid content and harvesting season interactions for orange sweet potato grown in Terengganu

Orange sweet potatoes grown in Terengganu were analysed for total and individual carotenoid content from three harvesting seasons (2011, 2012 and 2013) to investigate the stability of their carotenoid profile. Analysis of variance exhibited highly significant differences ($p < 0.0001$) between the harvesting seasons and the individual carotenoid pigments (Table 2). This further reinforces that harvesting season can have a marked influence on the accumulation of carotenoids. The changes in carotenoid composition are complex and also change with harvesting season as well as growing locations as indicated by the interaction components. The total carotenoid content for all three seasons ranged from 938.08 µg/g DW in 2011 as compared to 482.82 to 405.01 µg/g DW in 2012 and 2013 respectively. Comparison of

Table 3. Analysis of the effect of storage time for six months at 8°C in the dark on carotenoid accumulation in orange sweet potato tubers.

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

ND – non-detectable, (P<0.0001)

carotenoid profiles of the Terengganu-grown orange sweet potato from the 2011 and 2013 seasons showed variations in the profiles of individual carotenoid compounds. For example, orange sweet potato in 2011 and 2012 accumulated mostly β-carotene and α-carotene, whereas in 2013 zeaxanthin and β-carotene were the major carotenoids with only traces of lutein.

Carotenoid content and storage time interactions for orange sweet potato grown in Terengganu

Statistical analysis revealed that there were highly significant differences ($p < 0.0001$) between storage time, carotenoid content, and all combinations of interactions (Table 3). The importance of the interaction components emphasises that the changes in carotenoid composition are complex and the responses to storage time are not consistent in orange sweet potato tubers. As demonstrated in Table 3, after harvest and six months' storage time, zeaxanthin, α-carotene and β-carotene were detected in all storage time at varying and generally low levels. Two exceptions were observed in zeaxanthin and α-carotene, in which not detected at the first and six months' storage time respectively. Lutein was detected only in second and third months of storage time at low levels. After extended storage time from one to six months, orange sweet potato tubers exhibited decreased in total carotenoid content, zeaxanthin, α-carotene and β-carotene as well as absence of lutein compound.

Discussion

Results of current study showed that the growing year of harvest significantly influenced the carotenoids content in orange sweet potato flesh. This finding is in agreement with previous research conducted by Norshazila *et al*. (2014) and Christina *et al.* (2008), where those studies revealed that the amount of carotenoids detected were vary in each year of harvesting seasons. According to Vera *et al.* (2005), carotenoids content in acerola fruits was found to be the highest level during rainy season. There are other environmental factors cause decrease in the carotenoids content such as sunshine periods, low rainfall, and high temperature (Markus *et al.*, 1999). 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 (Van and Prins, 1993). Presence of lutein and zeaxanthin in 2013 can be explained according to previous studies by Cunningham and Gantt (1998); Howitt and Pogson (2006) and Othman (2009) where they reported that the reactions involved to produce these two carotenoids is hydroxylation of α-carotene and β-carotene under a reversible reactions mediated by pH soil. Besides, Rockholm and Yamamoto (1996) added that organic matter could 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 other countries (Rodriguez Amaya *et al.*, 2006). During the six months' storage time, our findings revealed that there were changes in carotenoids content quantitatively and qualitatively. Both of total carotenoids and β-carotene content in orange sweet potato tubers increased slightly in the second month of storage, and this can be explained according to previous study by Rodriguez-Amaya (1997), in which attributed that increasing of carotenoids

content 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 β-carotene 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 µg/g DW, and β-carotene content decreased from 924.15±3.53 µg/g DW to 104.28±4.93 µg/g DW. Total carotenoids and β-carotene content decreased up to 50% in 60 days of storage as well as from the second month to the fourth month of storage. To support these findings, Rosa *et al.* (2011) articulated that losses of 50% total carotenoids and β–carotene occurred in the sweet potato flour after 50-days of storage. Also, this result is in agreement with previous study by Carvalho *et al.* (2013), in which studied the composition of carotenoids, chlorophyll derivatives, over different ripening stage. The profile of pigments in ripening raspberries changes drastically, with a dramatic decrease of β-carotene and chlorophyll derivatives. α–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 Rodriguez-Amaya (1997), carotenoids in dehydrated stage 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 was appeared in the second month (0.47±0.41 µg/g DW) and decreased in the third month to (0.14±0.02 µg/g DW), this finding is in agreement with Carvalho *et al.* (2013), in which reported that the xanthophyll lutein has also decreased but not to the same extent of β-carotene. Then, lutein disappeared in the next following months. Zeaxanthin was not detected in the first month of storage of current study and was fluctuated for the whole six months. It is presumed that from these results, β-carotene was converted to zeaxanthin and β-cryptoxanthin through the hydroxylation process (Rodriguez-Amaya and Kimura, 2004).

According to Othman (2009), there was a significant change in the carotenoids content at different stages of maturity in potato tubers; lutein and β-carotene were present in the first four weeks of storage time, while neoxanthin, violaxanthin, lutein, and β-carotene were detected after 12 months of storage times. Compared to the carotenoids content in the Malaysian orange sweet potato flesh based on the effect of postharvest storage in current study, appearance of zeaxanthin in the second month along with the decline in the content of β-carotene and lutein. The mechanism for this reaction is not well understood and still remains an enigma. The ripening process is an important factor that affected carotenoids content during the storage period. During the storage period, the weight of samples decreased and their color changed to more intense orange color due to water loss. According to Jay *et al*. (2010), color changes were the main indicators that demonstrate the ripening and maturation processes in fruits. In the ripening process, a series of complex biochemical reactions are involved, such as hydrolysis of starch, production of carotenoids, anthocyanins, and phenolics, and the formation of volatile compounds (Rodriguez-Amaya, 1999). In addition to the factors discussed above, other factors that might affect the stability of carotenoid compounds; the type and physical form of the carotenoid compound, the presence of oxygen and metals in the matrix, exposure to the light and heat, and type of the food matrix (Rodriguez-Amaya; 2001; Norshazila *et al*., 2014) due to the double bonds in the carbon chains of carotenoids compound. The presence of double bonds in carotenoid carbon chains had caused this compound to be more sensitive to chemical reactions such as oxidation and isomerization (cis-trans), and normally formed during food processing and during post-harvest handling. These reactions will lead to the degradation and alteration of carotenoids compound in plants (Rodriguez-Amaya, 1999; Rao and Rao, 2007). Thus, the stability and concentration of carotenoids compound in plants differ greatly. Generally, losses of carotenoids during food storage are somewhat conflicting, but carotenoid degradation is known to increase with the destruction of the food cellular structure, increase of surface area or porosity, length and severity of the processing conditions, storage time and temperature, transmission of light and packaging permeability to O_2. Being highly unsaturated, carotenoids are susceptible to isomerization and oxidation during processing and storage of foods (Rodriguez-Amaya, 1999).

Conclusion

It can be concluded that accumulation of carotenoids in orange sweet potato tuber differs quantitatively and qualitatively based on the growing location, harvesting season and storage time. Determination of the key factors among the

above-mentioned factors that control carotenoids accumulation will provide a greater understanding to enhance the nutritional values of sweet potato.

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