Australian Journal of Basic and Applied Sciences, 9(17) Special 2015, Pages: 60-63

ISSN:1991-8178

Australian Journal of Basic and Applied Sciences

Journal home page: www.ajbasweb.com

Identification of Total Carotenoids and β-Carotene Content In Different Flesh Tuber Colours of Local Sweet Potato (Ipomoea batatas) for Pharmaceutical Industry

[1]Suhair Kammona, [2]Rashidi Othman, [1]Irwandi Jaswir, [1]Parveen Jamal

[1]Department of Biotechnology Engineering, Kulliyyah of Engineering, International Islamic University Malaysia (IIUM), Jalan Gombak, 53100 Kuala Lumpur, Malaysia,
[2]International Institute for Halal Research and Training (INHART), Herbarium Unit, Department of Landscape Architecture, KAED, IIUM, Jalan Gombak, 53100 Kuala Lumpur, Malaysia

ARTICLE INFO

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

Keywords:
Sweet potato, β-carotene, total carotenoid, HPLC

ABSTRACT

Five varieties of sweet potato tuber in Malaysia, have been studied for their total carotenoid contents and βeta- carotene content through spectrophotometry and high performance liquid chromatography HPLC analysis. This study was conducted to compare carotenoids content in local orange, yellow, purple and white sweet potato tuber pulp. UV-spectrophotometry analysis revealed that the orange sweet potato tuber flesh had the highest both β-carotene content and total carotenoid concentrations comparing to other sweet potato tuber flesh colors. Malaysian orange sweet potato showed the highest values of total carotenoid content and β-carotene concentration while white sweet potato showed the lowest levels of total carotenoid content and beta-carotene. In general, results of this study revealed that carotenoid content can differ with type of sweet potatoes flesh tuber. This study aimed to evaluate the high nutritional value of local sweet potatoes in Malaysia and their potential use in Pharmaceutical Industry.

To Cite This Article: Suhair Kammona, Rashidi Othman, Irwandi Jaswir, Parveen Jamal., Identification of Total Carotenoids and β-Carotene Content In Different Flesh Tuber Colours of Local Sweet Potato (Ipomoea batatas) for Pharmaceutical Industry. ***Aust. J. Basic & Appl. Sci.,*** *9(17): 60-63, 2015*

INTRODUCTION

Fruits and vegetables are a rich source of carotenoids compounds. Studies have indicated that carotenoids have high free-radical scavenging activity, which helps to reduce the risk of chronic diseases, such as cardiovascular disease, cancer, and age related neuronal degeneration (Ames, 1993). Dietary antioxidants, such as carotenoids, are helpful in assisting the body to neutralize free radicals. Therefore, it is important to consume a diet high in antioxidants to reduce the harmful effects of oxidative stress. People who consume diets rich in carotenoids would live healthier and thus they are shielded from fatality due to chronic diseases (Seddon, 1994). β-carotene (vitamin A precursors), is a major carotenoid important to humans (Kopsell 2010, Khachik, 1997). Humans cannot synthesize carotenoids; therefore, fruits and vegetables are primary sources of carotenoids in human diets world-wide. (Kopsell 2010, van den berg 2000).

Sweet potato (Ipomea batatas) roots have remarkable pro-vitamin A quantities and they are one of the major food sources of carotenoids (Henkel 96, Woolfe 92). Besides acting as antioxidants, carotenoids compounds also provide sweet potatoes with their distinctive flesh colors (white, cream, deep yellow, orange and purple) (Woolfe,1992-1993, Bovell 2007). Sweet potatoes are rich in dietary antioxidants, such as b-carotene (Woolfe, 1993). Sweet potatoes grow well in tropical, subtropical, and temperate areas. Sweet potato flesh SPF can be white, cream, yellow, orange, or purple (Woolfe 1992, Bovell 2007). Climate temperature elevates carotenoid biosynthesis in fruits, and normally raises their carotenoids concentrations (Kreck 2006, Kimura 1991). Different types of sweet potatoes flesh tuber vary in their carotenoids content among them quantitatively and qualitatively (Azevedo 2002). Sweet potato is one of the most important tuber crops for fresh consumption in Malaysia, it is cheap and commonly available throughout the year (Siti Hasidah 94, A. Zaharah 2004). In Malaysia, sweet potato is popular among local consumers, but there is an urgent need for research to evaluate the high nutritional value of carotenoids and study their pharmacological properties. Thus, the objective of this research is to explore the carotenoids content in

Corresponding Author: Suhair Kammona, Department of Biotechnology Engineering, Kulliyyah of Engineering, International Islamic University Malaysia (IIUM), Jalan Gombak,53100 Kuala Lumpur, Malaysia,
E-mail: rashidi@iium.edu.my,

different types of local sweet potatoes to determine their potential utility for pharmaceutical industry and other related industries.

2. *Experimental Procedure:*

2.1 Sample Preparation:

Malaysian orange sweet sample was obtained from Federal Agriculture Marketing Authority (FAMA), Selayang, Malaysia. while other local samples were bought from the market. Samples were cut to reduce the size and were freeze-dried for 72 hr, then the samples were ground into fine powder and kept at -20°C until further analysis.

2.2 Sample Extraction:

The extraction procedure essentially follows the methods described by (Othman, 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 subsequent analysis.

2.3 Determination of total carotenoid content (TCC):

Total carotenoid concentrations were determined by spectrophotometry according to the method described by (Othman, 2009). The dried carotenoid was re-suspended in 300 µL of ethyl acetate for determination of total carotenoid content. 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 Wellborn Equation (Wellborn 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.

2.4 Determination of individual carotenoid content by 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, 2009) 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 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 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, 2009. 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).

RESULT AND DISSCUSION

3.1 Total carotenoid content and β-Carotene concentration:

To compare the total carotenoids content in orange, yellow, purple and white sweet potato flesh tubers, the samples were analyzed by using UV-Vis spectrophotometer. Analysis of variance exhibited

highly significant differences (P < 0.0001) for carotenoids content in verities of local sweet potato flesh. Table 1 shows that the highest total carotenoids content was observed in the Malaysian orange sweet potato at 938.08±2.98 µg/g DW, followed by Indonesian orange sweet potato 405.07±7.65 µg/g DW and yellow sweet potato 122.96±7.54 µg/g DW. Purple sweet potato and white sweet potato show almost the same total carotenoids content 116.28±1.80 µg/g DW and 111.18±5.71 µg/g DW, respectively. These results are confirmed by previous studies findings, where they found that orange sweet potato cultivars are richer in carotenoids and vitamin A value than yellow, cream and white sweet potato (Pfander, 1992, Hagenimana, 1997, S.M. Hussein, 2014).

Table 1: Total carotenoid content (µg/g DW) and β-Carotene concentration (µg/g DW) in Sweet Potato Flesh Tubers of this study with their local name.

Sweet Potato Varieties	Local name	Σ carotenoid	β-caroten
Malaysian orange sweet potato	Keledek	938.084±2.98	773.03±0.05
Yellow sweet potato	Japanies sweet potato	122.96±7.54	118.00±3.12
Purple sweet potato	Keledek	116.28±1.80	107.86±14.17
White sweet potato	Keledek	111.18±5.71	103.90±2.05
Indonesian orange sweet potato	Keledek	405.07±7.65	291.07±11.51

Data are expressed as means_SD (n=3)
Significantly different at $p<0.0001$

β-carotene content was measured quantitatively and qualitatively by using High Performance Liquid Chromatography HPLC. To assure the correct determination of carotenoids, spectrum of b-carotenoid detected in each samples were observed based on the retention time (RT) and UV-VIS spectrum recorded by the standard. Table 1 shows the b-carotene was found in all samples in this study, and it ranged from 103.90±2.05µg/g DW in white sweet potato to 773.03±0.05 µg/g DW in Malaysian orange sweet potato. β-carotene in Indonesian orange sweet potato was detected in high level 291.07±11.51µg/g DW. White sweet potato and purple sweet potato were convergent somewhat in the concentrations of b-carotene 103.90±2.05 µg/g DW and 107.86±14.17 µg/g DW, respectively. High values of b-carotene in orange sweet potato pulp in this study, is confirmed with previous studies (Burgos 2001, Almeida 1992, Takahata 1993), where they reported that a positive correlation was observed between intensity of colorations of the sweet potatoes and the b-carotene content. In general, deep-colored vegetables and fruits are known to be good sources of carotenoids (Qian, 2004, Sass, 2005, Cieslik, 2006, Lucia, 2012). In this study, b-carotene is predominating other carotenoids compounds and that is confirmed by previous studies findings (Chaoyang, 2011, Lucia, 2012). Tropical climate elevate carotenoid biosynthesis, therefore, it is normal that Malaysian fruits and vegetables contain higher carotenoids concentrations (Rodriguez, 2004, Kimura 1991).

Conclusion and recommendation:

This study provide an overview of carotenoids composition and their nutritional value in the most popular, available and cheapest variety of Malaysian sweet potato and can be used 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.

REFERENCES

A. Zaharah, S.L. Tan, A.M. Abdul Aziz and B. Ibrahim, 2004. Performance evaluation of sweetpotato clones through multi-locational trials. J. Trop. Agric. and Fd. Sc., 32(2): 147–153.

Almeida-Muradian, L.B. and M.V.C. Penteado, 1992. Carotenoids and provitamin A value of some Brazilian sweet potato cultivars (Ipomoea batatas Lam.). Rev. Farm. Bioquím. Univ. S. Paulo, 28:145-154.

Ames, B.M., M.K. Shigena, T.M. Hagen, 1993. Oxidants, antioxidant and the degenerative diseases of aging. Proceedings of the National Academy of Sciences, 90: 7915–7922.

Azevedo-Meleiro, C.H. and D.B. Rodriguez-Amaya, 2002. Determination of the Carotenoids of Cucurbitaceae Fruit Vegetables by HPLC-DAD and HPLC-MS. Presentation at the IV Brazilian meeting on Chemistry of Food and Beverages, Campinas, Brazil.

Bovell-Benjamin, A., 2007. Sweet potato: A review of its past, present and future role in human nutrition. Advances in Food and Nutrition Research, 52: 1-59.

Britton, G., UV/Visible Spectroscopy, 1995. In Britton G, Liaaen-Jensen S, Pfander H (eds). Carotenoids: Spectroscopy, vol 1B. Birkhauser Verlag, Basel, 13-63.

Burgos Gabriela, Rossemary Carpio, Cynthfia Sanchez, Sosa Paola, Porras Edouardo, Jorge Espinoza and Wolfagang Grunerberg, 2001. A color chart to screen for high beta-carotene in Orange fleshed sweet potato breeding. International Potato Center;Cairo, Egypt,.

Chaoyang Li, Earl S. Ford, M.P.H. Guixiang Zhao, Lina, S. Balluz, M.P.H. Wayne, H. Giles, Simin Liu, M.D. ScD, 2011. Serum α-Carotene Concentrations and Risk of Death Among US Adults. The Third National Health and Nutrition

Examination Survey Follow-up Study, 171-6.

Cieslik, E., A. Greda and W. Adamus, 2006. Contents of polyphenols in fruit and vegetables. Food Chemistry, 94: 135–142.

Hagenimana, V., E. Carey, S.T. Gichuki, M.A. Oyunga and J.K. Imungi, 1997. Carotenoid contents in fresh, dried and processed sweetpotato products (Upcoming in Ecol Food Nutr.

Henkel Co., 1996. Recent research on carotenoids. Lagrange, Illinois.

Khachik, F., C.J. Spangler, J.C. Smith, L.M. Canfield, A. Steck and H. Pfander, 1997. Identification, quantification and relative concentrations of carotenoids and their metabolites in human milk and serum. Anal. Chem., 69: 1873-1881.

Kimura, M., D.B. Rodriguez-Amaya, S.M. Yokoyama, 1991. Cultivar Differences and Geographic Effects on the Carotenoid Composition and Vitamin A Value of Papaya. Lebens Wissen Technol, 24: 415-418.

Kopsell, D.A. and D.E. Kopsell, 2010. Carotenoids in vegetables: Biosynthesis, occurrence, impacts on human health, and potential for manipulation. In Watson, R.R. and Preedy, V. R. (Eds). Bioactive foods in promoting health: Fruits and vegetables, 645-662.

Elsevier Inc., M., Kreck, P. Ku¨ rbel, M. Ludwig, P.J. Paschold and H. Dietrich, 2006. Identification and Quantification of Carotenoids in Pumpkin Cultivars (Cucurbita maxima L) and Their Juices by Liquid Chromatography with Ultraviolet-Diode Array Detection. Journal of Applied Botany and Food Quality, 80: 93–99.

Lucia, Maria, J.D.C., B.G. Patricia, L.D.O.G. Ronoel, P. Sidney, H.F.D.M. Pedro, 2012. Total Carotenoid Content, α-Carotene and β- Carotene, of Landrace Pumpkins (Cucurbita Moschata Duch): A Preliminary Study. Food Research International, 47: 337– 340.

Othman, R. Ph.D., 2009. Thesis. Biochemistry and genetics of carotenoids composition in potato tubers, PhD thesis, Lincoln University.

Pfander, H., 1992. Carotenoids: An overview. Methods Enzymol., 213: 3-13.

Qian, J.Y., D. Liu and A.G. Huang, 2004. The Efficiency of Flavonoids in Polar Extracts of Lycium Chinense Mill Fruits as Free Radical Scavenger. Food Chemistry, 87: 283–288.

Rodriguez Amaya, D.B. and M. Kimura, 2004. HarvestPlus Handbook for Carotenoid Analysis. Copyright HarvestPlus, Washington DC., 63.

Hussein, S.M., I. Jaswir, P. Jamal and R. Othman, 2014. Carotenoid Stability and Quantity of Different SPF Color over Postharvest Storage Time. Advances in Environmental Biology; ISSN:1995-0756 EISSN: 1998-1066.

Sass-Kiss, A., J. Kiss, P. Milotay, M.M. Kerek and M. Toth-Markus, 2005. Differences in Anthocyanin and Carotenoid Content of Fruits and Vegetables. Food Research International, 38: 1023–1029.

Seddon, J.M., U.A. Ajani, R.D. Sperduto, R. Hiller, N. Blair, T.C. Burton, 1994. Dietary carotenoids, vitamins A, C and E, and advanced age-related macular degeneration. Journal of American Medical Association, 272(18): 1413-1420.

Siti Hasidah, N. and I. Khatijah, 1994. Food uses of tuber crops. Proc. National seminar on tuber crop production and utilization, 5–7.

Kuantan Tan, S.L., ed., Serdang: MARDI, UPM and Malays Soc. Hort. Sci., 184–96.

Takahata, Y., T. Noda and T. Nagata, 1993. HPLC determination of β-carotene content in sweetpotato cultivars and its relationship with color value. Japanese J. Breed, 43: 421-427.

Van den Berg, H., R. Faulks, H. Fernando Granado, J. Hirshberg, B. Olmedilla, G. Sandmann, S. Southon and W. Stahl, 2000. Review: The potential for the improvement of carotenoids levels in foods and the likely systemic effects. J. of the Science of Food and Agr., 80: 880-912.

Wellburn, A.R., 1994. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution.. Journal of Plant Physiology, 144: 301-313.

Woolfe, J., 1993. Sweet potato: An untapped food resource. Cambridge: Cambridge Univ.Press.

Woolfe, J.A., 1992. Post harvest procedures: Sweet Potato an Untapped Food Source. Cambridge, UK: Cambridge University Press, 643.