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POTENTIAL SOURCES FOR LIPID SOLUBLE FOOD COLORANTS FROM SELECTED MALAYSIAN TRADITIONAL VEGETABLES

(Sumber Pewarna Makanan Larut Lemak daripada Sayuran Tradisional Terpilih)

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Abstract

Colour is one important characteristic to food products as it dictates consumers' first perception on the foods' flavour and quality. In the current food industry, most of the colorants used were derived from synthetic sources. However, due to negative health impacts of the synthetic colorants, the urgency to find natural colorants and impose it to food products is of great importance. In this study, a group of plant pigments which are potentially introduced as natural food colorants were quantified from 24 species of local traditional vegetables (*ulam*), characterized as neoxanthin, violaxanthin, lutein, zeaxanthin, β-cryptoxanthin, α-carotene and β-carotene by using HPLC. It was shown that *Sauropus androgynus* contained the highest amount of neoxanthin, violaxanthin and β-cryptoxanthin at 142.40±3.57, 28.06±0.65 and 0.07±0.00 mg/g dry weight (DW), respectively. In contrast, highest content of lutein and α-carotene were observed in *Centella asiatica* at 16.53±0.97 and 2.14±0.12 mg/g DW, accordingly. Meanwhile, *Piper sarmentosum* contained the highest zeaxanthin level (123.45±12.3 mg/g DW) and *Oenanthe javanica* has the largest amount of β-carotene (3.09±0.06 mg/g DW). The extracted yellow-to-red lipid soluble pigments can be further developed into commercial food colorant to replace the synthetic colorants in the market thus improving social awareness towards natural products as well as strengthening the national economy.

Keywords: food colorant, traditional vegetables, ulam, natural colorants, carotenoids

Abstrak

Warna merupakan salah satu ciri penting kepada produk makanan yang membolehkan pengguna mengetahui tentang rasa dan kualiti makanan tersebut. Dalam industri makanan kini, kebanyakan pewarna yang digunakan adalah daripada sumber sintetik atau tiruan. Namun, pewarna sintetik yang diyakini mempunyai kesan yang negatif pada kesihatan,mendorong kepada pencarian pewarna alternatif daripada sumber semulajadi untuk digunakan di dalam penghasilan produk makanan .Di dalam kajian ini, sekumpulan pigmen tumbuhan yang berpotensi untuk diketengahkan sebagai pewarna semulajadi telah ditemui di dalam 24 spesies sayuran tradisi tempatan (ulam), dikenalpasti sebagai neoxanthin, violaxanthin, lutein, zeaxanthin, β-cryptoxanthin, α-carotene dan β-carotene melalui analisis HPLC. *Sauropus androgynus* didapati mengandungi kandungan neoxanthin, violaxanthindan β-cryptoxanthin yang amat tinggi iaitu masing-masing pada 142.40±3.57, 28.06±0.65 dan 0.07±0.00 mg/g berat kering (DW).Manakala *Centella asiatica* mempunyai kandungan lutein dan α-carotene yang tinggi iaitu pada16.53±0.97 dan 2.14±0.12 mg/g DW. *Piper sarmentosum* pula mempunyai kandungan zeaxanthin tertinggi (123.45±12.3 mg/g DW) manakala *Oenanthe javanica* mempunyai kandungan β-carotene tertinggi (3.09±0.06 mg/g DW). Pigmen larut lemak berwarna kuning merah yang telah diekstrak ini boleh dikembangkan lagi kepada pewarna makanan komersil untuk menggantikan pewarna sintetik di dalam pasaran dan dipercayai mampu memperbaiki kesedaran masyarakat terhadap produk semulajadi sekaligus menjana ekonomi negara.

Kata kunci: pewarna makanan, sayuran tradisional, ulam, pewarna semulajadi, carotenoid

Introduction

Development of foods with attractive colours has now become an important target in the food industries. This is due to the fact that the colours often dictate the consumers' first impression on the safety and quality of the food products eventually reflects their marketability [1,2]. For processed foods, the usage of colour additives is fairly critical to compensate the colour degradation during production and storage, to improve the coloration of naturally coloured foods, to reduce batch-to-batch variations as well as to impart colours onto uncoloured foods [1,3].

Traditionally, the food colorants were extracted from minerals and plant pigments. However, the advancement in chemical techniques has led to a gradual replacement of the natural colorants by synthetically-produced dyes at relatively higher reproducible quality and lower production costs [4]. These artificial colorants offer several advantages compared to natural colorants as they are stable under wide range of light intensity, pH and oxygenic environment, uniform colouration and have minimum contamination by microbes [5]. Apart from that, the application of these man-made colorants in food industries has recently become a controversy due to their potential adverse effects in humans. Several studies have reported that the synthetic food colorants may become carcinogens, source of allergens and triggered hyperactive behaviour in children [6-8]. As a result, the consumers' preferences have shifted into the usage of safer natural food colorants for their food products.

In order to support the growing demand of natural colorants, plant pigments such as anthocyanins, betalains, carotenoids and chlorophylls potentially are among the best candidates to be explored and developed into commercial food colorants. They are not only important for their colours but also their health-promoting properties such as potent antioxidants, bactericidal, anti-viral, anti-mutagenic and anti-carcinogenic [9-15]. The sources for these pigments are in abundance in nature as they are produced by fruits, flowers and leaves of any edible plants. This includes our traditional vegetables or locally known as *ulam*, a group of more than 120 plant species which are consumed as fresh salad or cooked [16]. These *ulam* species were reported to be nutritious, full of health benefits and important sources for aroma and flavours [17-19]. In this study, we aimed to extract one of the pigment groups, collectively known as carotenoids from 24 selected species *ulam*. These pigments are sources for yellow, orange and red lipid soluble colorants that potentially can be further developed into commercial colorants, which are natural and *syariah* compliant.

Materials and Methods

Sample preparation

Edible parts of *ulam* samples (Table 1) were purchased from the local market (Pasar Tani Selayang Baru, Selangor) in 2011 and freeze-dried for 72 hours, after which the samples were ground into fine powder and kept at -20°C until further analysis.

Extraction of carotenoids

The extraction procedure essentially follows the methods described by Fatimah et al. [20], with some modifications. 0.1 g of each powdered sample was rehydrated and extracted with a mixture of acetone and methanol (7:3) at room temperature until became colourless. The crude extracted was then centrifuged for 5 min at 8500 rpm and stored at 4ºC in the dark prior to analysis. To extract carotenoids an equal volume of hexane and distilled water was added to the combined supernatants. Next, the solution was allowed to be separated 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.

Saponification

Samples were saponified with a mixture of acetonitrile and water (9:1) and methanolic potassium hydroxide solution (10% w/v). Base carotenoids were then extracted by addition of hexane pre-mixed with 0.1% butylated hydroxytoluene (BHT), followed by addition of 10% NaCl until phase separation was achieved. The extracts were washed, dried under a gentle stream of oxygen-free nitrogen and re-suspended in ethyl acetate for HPLC analysis as described detail in Othman [21].

HPLC analysis

Analysis of the saponified carotenoids were performed on an Agilent HPLC system model 1200 series comprised of a quaternary pump with auto-sampler injector, micro-degassers, column compartment equipped with thermostat and a diode array detector. The column used was a ZORBAX Eclipse XDB-C_{18} 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^{-1}. The column was 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), β-cryptoxanthin (450 nm) and α-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 peakareas. The total and individual carotenoid concentration would be expressed in terms of milligram per 1.0 g dry weight of freeze-dried matter (mg/g DW).

Results and Discussion

In this study, a group of 24 plant species which are commonly consumed as *ulam* by local folks were evaluated for their carotenoid profiles. The selected *ulam* species were: *Sauropus androgynus*, *Allium tuberosum*, *Centella asiatica*, *Oroxylum indicum*, *Ocimum basilicum*, *Piper sarmentosum*, *Oenanthe javanica*, *Ocimum americanum*, *Zea mays*, *Brassica chinensis*, *Lactuca sativa*, *Morinda citrifolia*, *Murraya koenigii*, *Piper betle*, *Apium graveolens*, *Cosmos caudatus*, *Pluchea indica*, *Polygonum minus*, *Anacardium occidentale*, *Euodia redlevi*, *Allium cepa*, *Ipomea batatas* and *Daucus carota*. There were seven types of carotenoids identified and quantified in this study particularly neoxanthin, violaxanthin, lutein, zeaxanthin, β-cryptoxanthin, α-carotene and β-carotene. These individual carotenoids were found in comparable amount and can be further categorized into several groups based on the number of carotenoids found in these plants, as tabulated in Table 1. From the results, *S. androgynus* has the highest total carotenoid concentration at 190.30±3.43 mg/g dry weight of freeze-dried matter (DW) while *D. carota* has the least carotenoids level at 1.31±0.01 mg/g DW. *S. androgynus* also was found to have the highest concentration of neoxanthin, violaxanthin and β-cryptoxanthin at 142.40±3.57, 28.06±0.65 and 0.07±0.00 mg/g DW, respectively. Meanwhile, the highest level of lutein and α-carotene were observed in *Centella asiatica* at 16.53±0.97 and 2.14±0.12 mg/g DW, accordingly. On the other hands, *Piper sarmentosum* contained high zeaxanthin content (123.45±12.3 mg/g DW) whereas *Oenanthe javanica* has the largest amount of β-carotene (3.09±0.06 mg/g DW) when compared to other species. These findings were comparable with other studies [15] and the variations may be influenced by several factors which can be further manipulated in the process of optimizing the application of carotenoids as food colorants such as temperature, light intensities and storage conditions [22-24].

All individual carotenoids originate from phytoene and type of carotenoids in plants is determined by desaturation, isomerization, cyclization, hydroxylation and epoxidation. The significant branch point in carotenoid biosynthesis is the cyclization of lycopene. The introduction of two β rings to lycopene can lead to the formation of β-carotene and on another branch point the formation of α-carotene take place with both ε-ring and β ring present. Neoxanthin, violaxanthin and zeaxanthin are derived from the β-carotene pathway, whereas lutein is derived from the α-carotene pathway [25]. In 24 selected ulam species, two species were found to have six types of individual carotenoids with a relatively high concentration of neoxanthin. Three species were detected to have five carotenoid pigments and again with a relatively high concentration of neoxanthin. Six species were observed with 4 types of individual carotenoids, of which 4 species were detected with high neoxanthin and only 2 species with high zeaxanthin. A group of two and three types of pigments accumulated merely lutein and only 2 species with high zeaxanthin.

This result demonstrates that carotenoid composition and accumulation level vary with ulam species. The identification of such a genetic basis to significant levels of carotenoid within 24 selected ulam species has provided

the mechanism to manipulate carotenoid levels using both ulam species and molecular such as metabolomic strategies. Various regulatory factors in the carotenoid biosynthesis pathway may be responsible for the genetic differences in carotenoid content in plant species. It seems that genetic factors play an important role in grouping these 24 selected ulam species into five categories. Selection of the right species with the right capability to accumulate carotenoids will determine whether or not certain genetic manipulation strategies for carotenoid biosynthesis will succeed. Understanding the mechanism that controls carotenoid biosynthesis and exploring the diversity of carotenoid compounds in a wide range of Malaysian traditional vegetables will contribute greatly as potential natural lipid soluble food colorant or dye sources for *halal* market such as in pharmaceutical, nutraceutical, cosmeceutical, food and beverage industries.

Table 1. Distribution of total and individual carotenoids in 24 species of *ulam*

Species	**Total Carotenoid (mg/g DW)**	**Neoxanthin (mg/g DW)**	**Violaxanthin (mg/g DW)**	**Lutein (mg/g DW)**
Species with 6 types of individual carotenoids				
S. androgynus	190.30±3.43	142.40±3.57	28.06±0.65	15.57±0.32
A. tuberosum	24.61±1.00	13.95±0.75	2.98±0.24	5.00±0.38
Species with 5 types of individual carotenoids				
C. asiatica	130.61±15.03	96.10±11.4	13.45±2.68	16.53±0.97
O. indicum	100.78±2.45	81.79±2.70	4.36±0.12	13.12±0.31
O. basilicum	95.28±3.25	65.16±3.22	17.97±0.50	9.66±0.96
Species with 4 types of individual carotenoids				
P. sarmentosum	161.36±12.72	24.06±4.63	nd	12.58±1.28
O. javanica	144.48±4.93	115.55±4.09	11.06±0.70	14.80±0.44
O. americanum	108.79±6.35	74.62±3.30	24.12±2.63	9.27±0.35
Z. mays	61.53±5.55	nd	nd	1.54±0.03
B. chinensis	27.00±2.73	18.75±1.84	nd	6.51±0.63
L. sativa	15.14±0.65	10.15±0.69	1.87±0.09	2.58±0.07
Species with 3 types of individual carotenoids				
M. citrifolia	57.11±1.94	nd	nd	5.20±0.00
M. koenigii	51.48±2.60	nd	nd	4.04±0.14
P. betle	18.30±0.08	nd	nd	15.49±0.10
A. graveolens	14.35±0.14	nd	nd	11.53±0.09
C. caudatus	12.59±0.27	nd	nd	9.60±0.32
P. indica	10.66±0.39	nd	nd	5.76±0.17
P. minus	7.40±0.38	nd	nd	4.16±0.11
Species with 2 types of individual carotenoids				
A. occidentale	14.20±0.29	nd	nd	12.46±0.55
E. redlevi	11.65±0.14	nd	nd	10.39±0.15
A. cepa	6.90±0.04	nd	nd	4.83±0.02
I. batatas	1.34±0.06	nd	nd	nd
D. carota	1.31±0.01	nd	nd	0.72±0.00

nd – non-detectable, differences are significant at $p < 0.0001$

Table 1 (continued). Distribution of total and individual carotenoids in 24 species of *ulam*

Species	Zeaxanthin (mg/g DW)	β-cryptoxanthin (mg/g DW)	α-carotene (mg/g DW)	β-carotene (mg/g DW)
Species with 6 types of individual carotenoids				
S. androgynus	nd	0.07±0.00	1.36±0.42	2.84±0.37
A. tuberosum	nd	0.06±0.00	0.75±0.08	1.86±0.27
Species with 5 types of individual carotenoids				
C. asiatica	nd	nd	2.14±0.12	2.39±0.06
O. indicum	nd	nd	0.38±0.03	1.12±0.03
O. basilicum	nd	nd	0.53±0.11	1.95±0.24
Species with 4 types of individual carotenoids				
P. sarmentosum	123.45±12.3	nd	nd	1.27±0.29
O. javanica	nd	nd	nd	3.09±0.06
O. americanum	nd	nd	nd	0.78±0.16
Z. mays	58.87±5.38	0.05±0.00	1.06±0.14	nd
B. chinensis	nd	0.04±0.00	nd	1.68±0.26
L. sativa	nd	nd	nd	0.54±0.02
Species with 3 types of individual carotenoids				
M. citrifolia	52.08±0.25	nd	nd	1.31±0.01
M. koenigii	45.74±2.32	nd	nd	1.69±0.28
P. betle	nd	0.07±0.00	nd	2.74±0.10
A. graveolens	nd	0.06±0.00	nd	2.76±0.09
C. caudatus	nd	nd	1.56±0.16	1.43±0.05
P. indica	nd	nd	1.92±0.16	2.98±0.31
P. minus	nd	nd	0.71±0.08	2.53±0.25
Species with 2 types of individual carotenoids				
A. occidentale	nd	nd	nd	1.74±0.28
E. redlevi	nd	nd	nd	1.30±0.03
A. cepa	nd	nd	nd	2.07±0.02
I. batatas	nd	nd	0.38±0.09	0.97±0.06
D. carota	nd	nd	nd	0.60±0.01

nd – non-detectable, differences are significant at $p < 0.0001$

Every individual carotenoid possesses a unique yellow to red colours as well as their health-promoting values. Lutein and zeaxanthin are yellow coloured carotenoids which have been reported to be the essential components of retina, thus potentially the best candidates to protect humans from eye diseases [26], while the reddish orange β-carotene is a potent antioxidant which was reported to reduce visceral and subcutaneous fat mass [27]. This carotenoid also has double potential to be converted into vitamin A in the intestines as compared to other carotenoids [28]. In order to produce a different palette of food colorants, these carotenoids may be either isolated as a single compound or served as a mixture of few carotenoids. Because of the lengthy double bond in their chemical structures, they are highly hydrophobic and therefore these carotenoids are suitable candidates for the production of

natural fat-soluble yellow, orange and red nutritious dyeing materials [10,11,29,30]. However, before these pigments can be commercially available in the consumer market, further investigations such standardisation and development of food-grade *halal* colorants need to be done.

Conclusion

In this study, neoxanthin, violaxanthin, lutein, zeaxanthin, β-cryptoxanthin, α-carotene and β-carotene which are the potential natural and *halal* food colorants have been extracted from 24 ulam species. These targeted phytochemical compounds can be introduced as individual ingredients or as a mixture of few carotenoids for the production of food colorant. The phytochemical extraction, purification and manipulation of postharvest storage condition which are *syariah* compliant can be applied prior to commercialisation into the *halal* market. The significant outcome of this research will be new findings for new *halal* natural sources of food colorants.

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