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Identification of carotenoid composition in selected 'ulam' or traditional vegetables in Malaysia

[1]Fatimah, A. M. Z., [1]Norazian, M. H. and [2*]Rashidi, O.

[1]*Department of Pharmaceutical Chemistry, Kulliyyah of Pharmacy, International Islamic University Malaysia. Kuantan, Malaysia*
[2]*Department of Landscape Architecture, Kulliyyah of Architecture and Environmental Design, International Islamic University Malaysia, Kuala Lumpur, Malaysia*

Abstract: *Ulam* or traditional vegetables in Malaysia comprise more than 120 species representing various families ranging from groundcovers, shrubs to trees. The leaves, shoots, flowers, fruits, roots and rhizomes of the vegetables are eaten fresh as salad or cooked and are consumed to add variety and flavor to the diet, as well as for their health benefits. *Ulam* species are rich in carbohydrate, protein, mineral and vitamin. This study established that *ulam* species differ greatly with respect to types and concentrations of carotenoids in leaves. A total of 10 species were evaluated for quantitative and qualitative carotenoid composition through spectrophotometry and HPLC analysis. The main carotenoids identified in these selected *ulam* were lutein, neoxanthin, violaxanthin, zeaxanthin and β-carotene. The ratio of these carotenoids varies between species.

Keywords: Carotenoids, *ulam*, neoxanthin, violaxanthin, lutein, β-carotene

Introduction

Carotenoids are colored pigments that play vital role in the photosynthesis of plants, photosynthetic bacteria and algae. They can also be found in some of the non-photosynthetic bacteria, yeasts and crustaceans (Chandrika, 2009). They are mainly incorporated in the organisms as to provide protective mechanism towards oxidative damages caused by light and oxygen. Since they cannot be synthesized by human body, these pigments have to be supplemented through dietary intake (Van den Berg *et al*., 2000). Sparingly, the green leafy vegetables (GLVs) seem to be the best dietary sources for these phytochemicals (Devadas *et al*., 1980; Tee *et al*., 1991; Rodriguez-Amaya, 1997; Liu *et al*., 2007; Raju *et al.*, 2007).

Despite of their well known responsibilities for coloration and antioxidant properties, some carotenoids can also be sources for vitamin A activity (Britton *et al*., 1995). For this reason, they are merely pertinent compounds that have been pronounced to be correlated with vitamin A deficiency problem. Carotenoids are grouped into two classes depending on the presence of oxygen in their structures; xanthophylls (which contain oxygen) and carotenes (which the oxygen is absent). Reports have established that people who consume diets rich in carotenoids would live healthier and thus they are shielded from fatality due to chronic diseases (Seddon *et al*., 1994; Landrum *et al.*, 1997; Cunningham *et al*., 1998; Paiva *et al.*, 1999; Garcia-Closas *et al.*, 2004; Kopsell *et al.,* 2010).

Ulam refers to any vegetables usually eaten raw as salad in the Malaysian multiracial cultures. *Ulam* can also be cooked in dishes, come from various parts of the vegetable plants ranging from leaves to roots and rhizomes. Previous study have recorded that there are more than 100 plant species from various families, were consumed as *ulam* (Husain *et al.*, 2004). They are well known for improving one's health and also famous as they were claimed to exert anti-aging properties. Even though they are popular among local consumers, yet the availability of scientific findings on evaluation of their medicinal properties or specifically carotenoids activities are still lacking. Therefore, the aim of this research is to explore the composition and concentration of carotenoids in selected *ulam* species

Materials and Methods

Sample preparation

Edible parts of all *ulam* samples (Table 1) were freeze-dried for 72 hr, 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 Othman (2009), with some modifications. 0.1 g of each powdered sample 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 4ºC in the dark prior to analysis. To extract carotenoids an equal volume of hexane and distilled water was added to the combined supernatants. The

*Corresponding author.
Email: *rashidi@iium.edu.my*
Tel: +6012 6644772; Fax: +603 61964864

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.

Determination of total carotenoid content

Total carotenoid concentration was determined by spectrophotometry as described by Lewis *et al.* (1998). The dried carotenoid was resuspended in 300 µl of ethyl acetate and for determination of total carotenoid, 50 µl of the redissolved sample was then diluted with 950 µl chloroform for spectrophotometric analysis. 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 (Wellburn, 1994) in chloroform was applied to obtain the total carotenoid content as described below:

$$C_a = 10.91_A666 - 1.2A_{648}$$
$$C_b = 16.36_A648 - 4.57A_{666}$$
$$C_{x+c} = (1000_A480 - 1.42C_a - 46.09C_b)/202 \text{ (µg/mL)}$$

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 2 ml hexane with 0.1% butylated hydroxytoluene (BHT), followed by addition of 10% NaCl until phase separation was achieved. The extracts were washed with distilled water, dried under a gentle stream of oxygen-free nitrogen and re-suspended in ethyl acetate for spectrophotometry and HPLC analysis as described detail in Othman (2009).

HPLC analysis

The HPLC analysis of saponified carotenoids were performed on an Agilent model 1200 series comprised of a quarternary pump with autosampler injector, micro-degassers, column compartment equipped with thermostat and a diode array detector, in accordance to Morris *et al.* (2004). The column used was a ZORBAX Eclipse XDB-C18 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 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. Carotenoid standards violaxanthin and neoxanthin were isolated from *Lactuca sativa* (salad) by open column chromatography as described by Othman (2009), whereas β-carotene, lutein and zeaxanthin were obtained commercially from Sigma-Aldrich. 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) and β-carotene (454 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 microgram per 1.0 g dry weight of freeze-dried matter (µg/g DW).

Results and Discussion

A total of ten *ulam* species (Table 1) were evaluated for their total and individual carotenoid profile in particular violaxanthin, neoxanthin, lutein, zeaxanthin and β-carotene.

Table 1. List of *ulam* samples

Common Name	Botanical Name
Beko	*Oroxylum indicum*
Beluntas	*Pluchea indica*
Cekur manis	*Sauropus androgynus*
Daun selom	*Oenanthe javanica*
Kaduk	*Piper sarmentosum*
Mengkudu	*Morinda citrifolia*
Pegaga	*Centella asiatica*
Pucuk gajus	*Anacardium occidentale*
Tenggek burung	*Euodia redlevi*
Ulam raja	*Cosmos caudatus*

To date, there is no report published on carotenoid content for *beko*, *beluntas* and *tenggek burung*. Therefore, the results from this study for these three samples would provide new information regarding the carotenoids that can be found in the said plant species. Throughout the study, all the procedures were done under dim light condition to minimise photo-isomerisation and photo-oxidation of the carotenoids since they possess highly conjugated double bonds in their structures. The use of oxygen-free nitrogen gas for storage of samples is also essential as to avoid oxidation of the phytochemicals by atmospheric oxygen which would result in individual carotenoid loss. Since the chlorophylls were excluded from our major concern, therefore they are not quantified

Table 2. Distributions of total and individual carotenoid content (µg/g DW) in diverse range of *ulam* species

Local name	Botanical name	Total Carotenoid (µg/g DW)	Neoxanthin (µg/g DW)	Violaxanthin (µg/g DW)	Lutein (µg/g DW)	β-Carotene (µg/g DW)
Tenggek burung	*Euodia redlevi*	98.09 ± 3.97	ND	ND	37.51±1.09	60.58±1.23
Ulam raja	*Cosmos caudatus*	101.20 ± 5.62	ND	ND	37.44±0.50	63.76±1.01
Pucuk gajus	*Anacardium occidentale*	108.21 ± 1.41	ND	ND	38.20±0.41	70.01±1.09
Kaduk	*Piper sarmentosum*	111.14 ± 22.72	ND	ND	51.89±0.97	59.25±5.48
Beluntas	*Pluchea indica*	169.12 ± 25.01	ND	55.26±0.92	14.88±0.32	98.98±3.25
Pegaga	*Centella asiatica*	174.16 ± 24.79	33.60±8.75	83.98±6.86	18.69±0.57	37.88±4.93
Beko	*Oroxylum indicum*	176.39 ± 10.88	ND	75.91±17.17	51.09±0.24	49.39±0.36
Mengkudu	*Morinda citrifolia*	235.36 ± 11.02	130.90±11.4	34.03±11.91	42.93±1.94	27.50±2.85
Daun selom	*Oenanthe javanica*	250.43 ± 16.35	ND	ND	70.44±1.94	179.99±1.20
Cekur manis	*Sauropus androgynus*	358.90 ± 21.43	93.27±4.54	117.63±15.10	51.59±1.11	96.40±7.71

**ND – non-detectable*

Table 3. Comparison of individual carotenoid compounds of 8 *ulam* species detected in this studies and previous studies

Local Name	Carotenoid(s) detected in this studies	Carotenoid(s) detected in previous studies
Cekur manis	neoxanthin, violaxanthin, lutein and β-carotene	Lutein, β-carotene and zeaxanthin (Tee *et al.*, 1991; Liu *et al.*, 2007; Mohd Salleh, 2005)
Daun selom	lutein and β-carotene	β-carotene (Rodriguez-Amaya, 1997)
Kaduk	lutein and β-carotene	β-carotene (Speek *et al.*, 1988)
Mengkudu	neoxanthin, violaxanthin, lutein and β-carotene	Lutein and β-carotene (Tee *et al.*, 1991)
Pegaga	neoxanthin, violaxanthin, lutein and β-carotene	β-carotene and zeaxanthin (Rodriguez-Amaya, 1997; Speek *et al.*, 1988; Mohd Salleh, 2005)
Pucuk gajus	lutein and β-carotene	β-carotene and lutein (Tee *et al.*, 1991)
Ulam raja	lutein and β-carotene	Lutein and zeaxanthin (Liu *et al.*, 2007)

and reported in this study. In fact, the saponification process was employed as to remove the major plant pigments and the unwanted ester-carotenoids, simultaneously.

Among the ten samples analysed, *cekur manis* has shown substantially higher total carotenoid (358.90 ± 21.43 µg/g DW) than other *ulam* species. It is followed by *daun selom* and *mengkudu* which contained 250.43 ± 16.35 and 235.36 ± 11.02 µg/g DW, respectively. Whilst, the least carotenoid content was found in *tenggek burung* valued at 98.09 ± 3.97 µg/g DW. It was found that the total carotenoid content reported in this study for *mengkudu*, *cekur manis* and *pucuk gajus* were found higher than those reported by Tee and Lim (1991) which are 70.47, 318.22 and 17.78 µg/g FW, respectively.

Carotenoid analysis performed by HPLC system detected at least four major carotenoid peaks: neoxanthin, violaxanthin, lutein and β-carotene. As shown in Table 2, neoxanthin was found highest in *mengkudu* (235.36 ± 11.02 µg/g DW); violaxanthin was highest in *pegaga* (83.98±6.86 µg/g DW), whereas lutein and β-carotene were detected in their highest levels in *daun selom* which are 70.44 ± 1.94 and 179.99 ± 1.20 µg/g DW, respectively. Zeaxanthin was not found in any of the 10 *ulam* species analysed. All the *ulam* species studied could be grouped into one of several classes depending on the accumulation of specific carotenoid pigments (Table 2). Three *ulam* species such as *pegaga*, *mengkudu* and *cekur manis* were found to have all four individual carotenoid pigments with a relatively high concentration of neoxanthin and violaxanthin. It was observed that all four individual carotenoid pigments were strongly correlated to the total amount of carotenoid accumulating. Two *ulam* species, such as *beluntas* and *beko*, were detected to have three of the four carotenoid pigments; violaxanthin, lutein and β-carotene. A group of five *ulam* species (*tenggek burung, ulam raja, pucuk gajus, kaduk* and *daun selom*) only accumulated lutein and β-carotene.

Previous studies have established that the carotenoid profiles in all *ulam* species except *beko*, *beluntas* and *tenggek burung* are dominated mostly by lutein, zeaxanthin and β-carotene (Tee *et al.*, 1991; Rodriguez-Amaya, 1997; Speek *et al.*, 1988; Mohd Salleh, 2005; Liu *et al.*, 2007).

Another interesting aspect of this study was the comparison of *pegaga*, *mengkudu* and *cekur manis* carotenoid profiles with previous studies (Table 3). The carotenoid profiles of these three *ulam* species were not similar. *Cekur manis* was found to have only 3 major carotenoids in previous studies compared to 4 major carotenoids in this studies, whereas 4 major

carotenoids were detected in *pegaga* and *mengkudu* in this studies compared to previous studies which detected merely 2 major carotenoids with an absence of neoxanthin and violaxanthin.

Conclusion

It can be concluded that the concentration of total carotenoid and the relative distributions of individual carotenoids within each grouping (neoxanthin, violaxanthin, lutein and β-carotene) did not necessary correlate to the levels of total carotenoids, indicating that high levels of carotenoids content may result from the accumulation of different levels of individual carotenoid pigments.

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