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Research Article

QUANTIFICATION OF PRO-VITAMIN A ACTIVITIES AND CONTENT IN 22 SELECTED '*ULAM*' SPECIES OR MALAYSIAN TRADITIONAL VEGETABLES

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ABSTRACT

Recently there is a considerable interest in combating vitamin A deficiency (VAD) by manipulating carotenoid content and composition in plants through genetic manipulation to meet the daily requirements for vitamin A. In Malaysia and most developing countries many people survive largely on plant-based diets or monotonous consumption such as cereals and legumes that are poor sources of vitamin A. *Ulam* or traditional vegetables consumed by Malaysian comprise more than 120 species representing various plant leaves, fruits, seeds, tubers and roots are valuable sources of nutrients and pro-vitamin A. These pro-vitamin A compounds can profoundly protect our well-being or risk of disease throughout our lives. Therefore, identifying nutritional quality of food crops and its ingredients for VAD is one of the urgent health issues and research worldwide. This study established that traditional vegetables or *ulam* differ greatly with respect to types and concentrations of pro-vitamin A. A total of 22 *ulam* species were evaluated for quantitative and qualitative pro-vitamin A composition through HPLC analysis. Three main pro-vitamin A carotenoids were identified namely β-cryptoxanthin, α-carotene and β-carotene. These pro-vitamin A groups content and ratio varies between *ulam* species but were detected higher in *cekur manis* (68.44±0.23 µg/g DW), *pegaga* (2142.71±11.70 µg/g DW) and *daun selom* (3085.02±5.87 µg/g DW). Total retinol equivalent activity presented by these pro-vitamin A groups in every *ulam* species demonstrated that *beluntas* has the highest activity (656.59 µg/g DW). The information gathered in this study embarked that almost all of the *ulam* species are good and potent sources of pro-vitamin A.

Keywords: pro-vitamin A, Carotenoid, β-cryptoxanthin, α-carotene, β-carotene and *ulam*.

INTRODUCTION

Vitamin A deficiency (VAD) has been pronounced as an urgent public health issue which is affecting almost all nations worldwide especially in low-income and developing countries[1]. This deficiency had caused major mortality among children aged below five years old and symptoms ranging from night blindness, xerophthalmia to those of keratomalacia which lead to the total blindness. Without adequate vitamin A supplementations, the children are not only having risks to be blind but also vulnerable to various kinds of diseases[2]. Therefore, improved dietary vitamin A status would be important for the population survival especially among the children thus eliminating VAD as a continuous public health concern[3]. Dietary vitamin A may present in two forms; pre-formed vitamin A from animal-based foods and pro-vitamin A that usually originating from plant-based foods. Pro-vitamin A that comes from photosynthetic plants and bacteria is collectively known as carotenoids[4,5]. They are lipid soluble pigments of yellow-orange-red colour which primarily act as antioxidant in photosynthetic tissues. Even though they are yellow-to-red coloured pigments, they are found concentrated in green leafy vegetables[6-10]. There are more than 700 carotenoids have been characterised where 40 of them were found in human diet such as α-carotene, β-carotene, lutein and lycopene[11].Consumption of carotenoids in human diet has been reported to be associated with better health status and reduced fatality due to chronic diseases[11-16].

There are more than 120 species of traditional vegetables or *ulam* in Malaysia. They are important to the local community for their own unique taste in various dishes as well as their health benefits[17].They are reported to be nutritious as they are rich in carbohydrates, proteins, minerals and vitamins. Some of the vegetables are claimed to have medicinal properties in such way they are beneficial in lowering the incidence of cancer and age-related diseases. They are also popular for their anti-ageing properties[18,19]. Despite of their popularity, scientific reports on their medicinal properties especially regarding the ones related to pro-vitamin A carotenoids' activities are still lacking. Therefore, this research is primarily aimed to explore alternative sources for pro-vitamin A carotenoids from various *ulam* species.

MATERIALS AND METHODS

Sample preparation

Edible parts of all samples were freeze-dried (EYELA FDU-1100, Japan) for 72 hours, after which the samples were ground into fine powder and kept at -20°C until further analysis.

Extraction of carotenoids

0.1 g of each powdered sample was rehydrated with adequate distilled water and extracted with a mixture of acetone and methanol (7:3) until the solution became colourless. Equal amount of hexane and distilled water were added to the combined solution to extract out the carotenoids. After separation of layers was achieved, the upper hexanal layer was collected and dried down under a gentle stream of oxygen-free nitrogen[21].

Saponification

Samples were saponified with a mixture ofacetonitrile and water (9:1) and methanolic potassium hydroxide solution (10% w/v). Base carotenoids were then extracted out with hexane and 0.1% butylated hydroxyl toluene (BHT), followed by addition of 10% NaCl until phase separation was achieved. After being washed, the extracts were dried under a gentle stream of oxygen-freenitrogen. All of the extraction and saponification procedures were detailed in our previous work[20]. Finally, the dried extracts were re-suspended in ethyl acetate prior to HPLC analysis.

HPLC analysis

The HPLC analysis of saponified extracts was performed on an Agilent model 1200 series as detailed by Othman[21]. The column used was a ZORBAX SB-C_{18} end capped 5µm, 4.6x250 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 at a flow rate of 1.0 mL min^{-1}. The column then was allowed to re-equilibrate in 100% A for 10 min prior to the next injection.

Throughout the analysis, temperature of the column was maintained at 20°C while the injection volume was 10μL each. Carotenoid standards for β-carotene and β-cryptoxanthin were obtained commercially from Sigma-Aldrich and α-carotene standard was isolated from orange-fleshed carrots as described by Rodriguez-Amaya and Kimura[22]. Each of the individual carotenoids was detected at the wavelengths of 454 nm for β-cryptoxanthin and β-carotene (maximum absorption for both carotenoids in the mobile phase) and 447 nm for α-carotene. Compounds from the extracts were identified by co-chromatography with standards and by elucidation of their spectral characteristics using a photo-diode array detector.

The total and individual carotenoid concentrations were expressed in terms of microgram per gram dry weight of freeze-dried matter (μg/g DW).

Calculation of vitamin A activity

The vitamin A activity of each of the detectable pro-vitamin A carotenoids was converted into retinol equivalent units as proposed by NRC[23], where 6 μg of β-carotene or 12 μg of α-carotene and β-cryptoxanthin is equal to 1 μg retinol equivalent (RE).

RESULTS AND DISCUSSION

A group of 22 species of commonly consumed Malaysian *ulam* (Table 1) had been selected for identification and quantification of their pro-vitamin A carotenoid content. As to date, there is no previous report on the carotenoid content for the following five *ulam* species (Table 1): *Daun Kesum*, *Hempedu Bumi*, *JarumTujuh Bilah*, *Kemangi* and *Mas Cotek*. Therefore, the results included in this report would provide new information for the said species with regard to their carotenoid accumulation.

Throughout this study, all of the experimental procedures were performed under dim light environment since the carotenoids are light-sensitive which otherwise would trigger to be photo-isomerised and photo-oxidised. In such case, analysis may come out with false positive results in the presence of carotenoid artifacts. Wherever needed; the extracts were completely dried under oxygen-free nitrogen gas prior to storage as to prevent individual carotenoid losses.

Table 1: Selected traditional vegetables (*ulam*) used in this study

Local name	Botanical name	Carotenoids detected in previous studies	References
Beka	*Oroxylum indicum*	β-carotene, lutein, violaxanthin	20
Beluntas	*Pluchea indica*	β-carotene, lutein, violaxanthin	20, 24
Cekur Manis	*Sauropus androgynus*	β-carotene, zeaxanthin, lutein, violaxanthin, neoxanthin	7, 9, 20, 24, 31
Daun Bawang	*Allium cepa*	β-carotene	10, 31
Daun Kari	*Murraya koenigii*	β-carotene, zeaxanthin, lutein, violaxanthin, neoxanthin	7, 10, 29, 31, 33
Daun Kesum	*Polygonum minus*	N/A	N/A
Daun Kucai	*Allium tuberosum*	β-carotene	7
Daun Selom	*Oenanthe javanica*	β-carotene, lutein	20
Daun Sup	*Apium graveolens*	β-carotene	25, 31
Gajus	*Anacardium occidentale*	β-carotene, lutein	7, 20, 24
Hempedu Bumi	*Andrographis paniculata*	N/A	N/A
JarumTujuh Bilah	*Pereskia sacharosa*	N/A	N/A
Kaduk	*Piper sarmentosum*	β-carotene, lutein	20, 25
Kemangi	*Ocimum americanum*	N/A	N/A
Mas Cotek	*Ficus deltoidea*	N/A	N/A
Salad	*Lactuca sativa*	β-carotene, zeaxanthin, lutein, violaxanthin, neoxanthin	10, 27, 32, 29, 30
Sawi	*Brassica chinensis*	β-carotene, zeaxanthin, lutein	7, 25, 26, 31
Selasih	*Ocimum basilicum*	β-carotene	25
Sirih	*Piper betle*	β-carotene, zeaxanthin, lutein	10, 29
Pegaga	*Centella asiatica*	β-carotene, lutein, violaxanthin, neoxanthin	20, 24, 25
TenggekBurung	*Euodia redlevi*	β-carotene, lutein	20
Ulam Raja	*Cosmos caudatus*	β-carotene, zeaxanthin, lutein	20, 24

N/A- Not available

Table 2: Pro-vitamin A activities and content in 22 selected traditional vegetables (*ulam*).

Local Name	BCR (μg per g DW)	AC	BC	Total (μg per g DW)
Salad	nd	nd	542.22±1.63	542.22±1.63
Kemangi	nd	nd	783.43±0.05	783.43±0.05
Mas Cotek	nd	379.35±0.06	874.62±0.04	1253.97±0.13
Tenggek Burung	nd	nd	1259.79±2.82	1259.79±2.82
Kaduk	nd	nd	1268.56±0.10	1268.56±0.10
Beka	nd	384.1 ±2.87	1123.96±3.42	1508.07±5.98
Daun Kari	nd	nd	1690.11±0.09	1690.11±0.09
Sawi	45.11±0.04	nd	1683.62±25.90	1728.73±25.94
Gajus	nd	nd	1738.12±0.09	1738.12±0.09
Hempedu Bumi	nd	nd	1766.17±2.45	1766.17±2.45
JarumTujuh Bilah	nd	nd	1863.58±15.55	1863.58±15.55
Daun Bawang	nd	nd	2066.85±2.45	2066.85±2.45
Selasih	nd	530.25±0.04	1950.47±24.11	2480.72±24.10
Daun Kucai	62.45±0.29	752.64±8.45	1856.99±0.09	2672.08±8.69
Sirih	67.64±0.34	nd	2739.45±9.88	2807.09±10.14
Daun Sup	58.99±0.03	nd	2757.76±8.90	2816.75±8.91
Ulam Raja	nd	1561.99±16.50	1430.11±5.17	2992.10±11.64
Daun Selom	nd	nd	3085.02±5.87	3085.02±5.87
Daun Kesum	nd	710.12±7.87	2532.96±25.38	3243.08±32.72
Pegaga	nd	2142.71±11.70	2387.64±6.20	4223.15±17.63
Cekur Manis	68.44±0.23	1357.80±0.14	2841.60±0.12	4267.84±0.45
Beluntas	nd	1915.74±15.58	2981.69±0.10	4897.43±15.51

nd- not detected, BCR- β-cryptoxanthin, AC- α-carotene, BC- β-carotene

Data represented as mean±standard deviation, n=5, significantly different at $p<0.0001$.

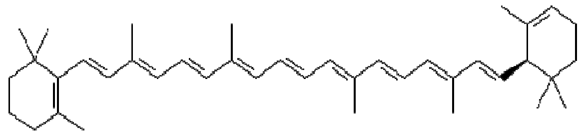
Total carotenoids are the sum of each pro-vitamin A carotenoid detected.

Under the experimental condition employed, it was observed that there was certain degree of variation in the level of total and individual carotenoid content among the selected species, ranging from one to nine fold (Table 2).

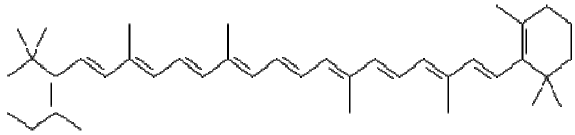
Among the species studied, HPLC analysis demonstrated that *Beluntas* accumulated substantially the highest total carotenoid (4897.43±15.51 µg/g DW) followed by *Cekur Manis* and *Pegaga* with 4267.84±0.45 µg/g DW and 4223.15±17.63 µg/g DW respectively. The total carotenoid content in all of the analysed samples was comparable with reports from previous studies. Interestingly, the total pro-vitamin A carotenoid concentrations for eight of the samples (*Tenggek Burung*, *Kaduk*, *Gajus*, *Ulam Raja*, *Daun Selom*, *Pegaga*, *CekurManis* and *Beluntas*) were found comparatively greater than the ones analysed in previous work elsewhere[20].On top of that, the total pro-vitamin A carotenoid content for *Salad* obtained here was quantified lower than the data reported by Burns *et al.*[27]yet higher than values obtained by Mamatha[32].

The HPLC analysis also established that in determination of three types of pro-vitamin A carotenoids; β-cryptoxanthin which is a rare carotenoid, had been successfully quantified in *Sawi*, *Daun Sup*, *Daun Kucai*, *Sirih* and *Cekur Manis.* In fact, up to present date there is none of the previous analysis on the selected vegetables and herbs species reported on their β-cryptoxanthin content. Therefore, the results from this study established new profiling for this particular carotenoid.

α-carotene and β-carotene are isomeric compounds which differ in the location of double bond in one of their rings (Figure 1). Among the 22 *ulam* species analysed, β-carotene had been detected as their major pro-vitamin A compound but α-carotene was present in only nine species.This phenomenon is probably due to their function and the regulation of their biosynthetic pathway. β-carotene is the most abundant carotenes in the leaves since they were the primary free radical scavenger in the photosynthesis whereas α-carotene was present as minor accessory pigment in a number of plant species only[34].



α-carotene



β-carotene

Fig. 1: Difference in the structures of α-carotene and β-carotene.

As this paperwork mainly discussed on pro-vitamin A content of the selected *ulam* species, hence the related retinol equivalent activity of respective samples was also included here for further evaluation on their relevancy as natural sources for vitamin A pre-cursors. The total retinol equivalent (RE) content for every sample was tabulated in Table 3. The total RE content was calculated in accordance to NRC[23,35]. Attentively, *Beluntas* ranked at the top to possess 656.59µg RE per g DW, followed by *Cekur Manis, Pegaga* and *Daun Selom* with 592.45µg, 576.50µg and 514.17µg RE/g DW respectively.

Table 3: Total retinol equivalent (RE) activity in selected 22 *ulam* species

Samples	Total RE	Samples	Total RE
Salad	90.37	*Daun Bawang*	344.48
Kemangi	130.57	*Selasih*	368.52
Mas Cotek	145.77	*Daun Kucai*	369.27
Tenggek Burung	209.97	*Sirih*	377.42
Kaduk	211.43	*Daun Sup*	462.21
Beka	219.34	*Ulam Raja*	464.54
Daun Kari	281.69	*Daun Selom*	481.34
Sawi	284.36	*Daun Kesum*	514.17
Gajus	289.69	*Pegaga*	576.50
Hempedu Bumi	294.36	*Cekur Manis*	592.45
Jarum Tujuh Bilah	310.60	*Beluntas*	656.59

Data represented in microgram per gram dry weight.

According to Ministry of Health (MOH), Malaysia, the recommended nutrient intakes (RNI) for vitamin A lies within range 375 to 850 µg RE per day (about 2 to 5 mg β-carotene per day). However, these values become specific based on gender, age and stage of life[5].From the analysis done in this study, RE values from eight of the 22 samples established to be within the range as suggested by both MOH and FAO/WHO[5,36]. There have been reviews on several plants to be used as interventional food in order to overcome VAD issue such as orange-fleshed carrots and orange-fleshed sweet potatoes[37,38]. As for comparison, analysis for orange-fleshed carrots was also carried out to check for their RE content which valued at 99.64 µg RE/g DW. Thereby, from the results obtained earlier, we have found that 21 out of the 22 selected *ulam* species are having better RE values than carrots ranged from 130.57 to 656.59 µg RE/g DW. The individual pro-vitamin A profiles are well-established here and thus providing an update on the nutritional values of our traditional vegetables (*ulam*). Further investigation need to be taken into account before the *ulam* species could be used for local food intervention program to eliminate VAD as a public health concern.

CONCLUSION

The retinol equivalent (RE) values in every sample are mainly correlated to their respective concentration of β-carotene. It was found that 21 of the analysed *ulam* species are having better RE activity in comparison to a well-known source of pro-vitamin A (orange fleshed carrots), hence could be further evaluated for local food intervention program to combat VAD problems.

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