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ORIGINAL PAPER

Carotenoid Content of Underutilized Tropical Fruits

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Abstract This study was conducted to evaluate the total carotene content (TCC) and beta carotene (BC) in the selected underutilized tropical fruits. TCC of underutilized fruits estimated by spectrophotometric method was in the range of 1.4–19.8 mg/100 g edible portion. The TCC of these fruits decreased in the order: Jentik-jentik>Durian Nyekak 2>Durian Nyekak 1>Cerapu 2>Cerapu 1>Tampoi Kuning>Bacang 1>Kuini>Jambu Mawar>Bacang 2>Durian Daun>Bacang 3>Tampoi Putih>Jambu Susu. BC contents estimated by HPLC method were highest in Jentik-jentik, followed by Cerapu 2, Durian Nyekak 2, Tampoi Kuning, Durian Nyekak 1, and Cerapu 1, which had a range of 68–92% of BC in TCC. These underutilized fruits have an acceptable amount of carotenoids that are potential antioxidant fruits.

Keywords Beta carotene · HPLC · Total carotene · Underutilized fruit

Introduction

Carotenoids are widely distributed in the plant kingdom. These components contribute to the red, orange, yellow, purple and green colours of fruits and vegetables [1, 2]. A number of carotenoids including alpha carotene, beta carotene and beta cryptoxanthin have pro-vitamin A activity [3], and they are converted to retinal by mammals [1]. However, other carotenoids such as lycopene and lutein have no provitamin A activity [4]. A study has reported that carotenoids have potential antioxidant capacity [5].

Carotene is present in cruciferous vegetables (broccoli, capsicum, carrot, chili, pumpkin, sweet potato) [6] and fruits (apricot, durian, mango, guava, papaya, plum, and passion-fruit) [7], while BC is found in yellow and orange coloured fruits. The BC rich vegetables are carrot, pumpkin and sweet potato, and fruits such as apricot, plum, durian, papaya and mango. Since most of our experimental samples have yellow to orange coloured pulp, the crude estimation of carotenoid content was expressed as TCC as carotene is the major soluble carotenoid in this fruit hexane fraction. As reported by Peng et al. [8], carotenoids in the tested samples also expressed as total carotenoid content were found to be rich in zeaxanthin dipalmitate.

Mangifera is a species of plant in Anacardiaceae family. Bacang or horse mango (*Mangifera feotida*) and kuini (*M. odorata*) are *Mangifera* fruits which are considered as underutilized tropical fruits. Cerapu (*Garcinia prainiana*) or button mangosteen is one of the attractive fruits in Malaysia. A ripe fruit has orange colour. Durian is the fruit of the genus *Durio* belonging to the *Malvaceae*. In Southeast Asia, durian is widely known and revered as the "King of Fruits". *Durio kutejensis* and *D. graveolens* are considered as underutilized durian. Tampoi and Jentik-jentik are also some of the rare species fruits and are not popular among Malaysians. Jambu Susu (*Syzygium malaccense*) or milk apple is considered a highly nutritious fruit, while Jambu Mawar (*S. jambos*) or rose apple is native to the Indo-Malaysian region.

Bioversity International recommended that underutilized fruits can be used as alternative sources to combat hidden hunger such as vitamin A deficiency [9]. Previous studies have showed that underutilized fruits (*Bouea macrophylla*

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and *Syzygium malaccense*) contained comparable amounts of carotenoids as in commercial fruits [10, 11]. However, there are still limited studies on the potential nutritional benefits of these fruits, especially their antioxidant components. The present study was conducted to evaluate the TCC and BC contents in fourteen types of Malaysian underutilized fruits.

Materials and Methods

Materials

Chemicals and Standards *n*-Hexane, methanol (HPLC grade) and dichloromethane (HPLC grade) were obtained from Merck (Darmstadt, Germany); ethanol was obtained from GmbH Chemical (Hamburg, Germany). Acetonitrile (HPLC grade) was purchased from Mallinckrodt Chemicals (New Jersey, USA). BC standard was purchased from Sigma Chemical (Missouri, USA). Stock solutions of BC (analytical grade) were prepared in hexane at concentrations of 0.22–7.0 μg/ml, then kept in brown bottles and stored at −20 °C. The (all-*trans*)-BC standards (97% HPLC purity) from Sigma (Missouri, USA) were used as calibration for HPLC analysis. The preparation of stock solutions and samples were carried out in a dark room with dim light.

Samples Fourteen types of underutilized fruits were supplied from Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Selangor, Malaysia. A total of five genera of underutilized fruits were used in this study. The source of 14 types of underutilized fruit is shown in Table 1. The samples were collected conveniently due to the difficulty to obtain them. Each fruit was harvested at different time periods, as the fruits were seasonally grown except for Jambu Mawar. The fruits were randomly selected from plantation lot and transported to our laboratory at the Department of Nutrition and Dietetics, Universiti Putra Malaysia. The fruit was washed under running tap water and wiped with tissue paper. The peel was manually removed using a sharp knife. Then the fruit was cut into half and the kernel was removed. Edible portion (flesh/pulp) was put into a plastic bag and stored at −80 °C before lyophilization. The lyophilised samples were ground into fine particles. Laboratory sampling was carried out using diagonal sampling method. All samples were stored at −20 °C for less than a month before further analysis.

Extraction of Carotenoids

The extraction of carotenoids was carried out according to the method of Seo et al. [12] with slight modifications where the hexane layer was washed with the distilled water, and then the water was removed using anhydrous sodium sulphate. One gram of each sample was weighed and added with 10 ml of 95% ethanol. The mixture was stirred for 30 min in a Unimax 1010 DT shaking incubator (Heidolph, Schwabach, Germany). The mixture was filtered through a Whatman 1 filter paper and supernatant was added with equal volume of hexane to extract the lipophilic components. This step was repeated several times until lipid compounds were fully extracted. The pooled hexane layer was washed with distilled water. Aqueous layer was discarded, and the resulting hexane extract was added with anhydrous sodium sulphate. The hexane was evaporated from the extract using a Büchi rotary evaporator (Rotavor R-200, Flawil, Switzerland) at 39 °C. The remaining lipid layer was then dissolved in 10 ml hexane or in mobile phase for HPLC analysis, and then kept in amber bottle. It was stored at −20 °C for less than a week until further analysis.

Precision Measurements

The intra-batch precision was used to assess the repeatability of the method [13]. It was determined by performing the three times-analysis of a pooled sample hexane fractions and BC, while the inter-batch precision was determined for BC by performing the four times-analysis over a period of two weeks. More replicate analysis was not performed due to the limitation of resources.

Estimation of Total Carotene Content (TCC)

The method used for determining the TCC was performed on a UV–Vis spectrophotometer as described by Prache et al. [14]. Tee and Lim [15] reported that the total carotenoids in fruit extracts were measured at 450 nm. The absorbance for BC was scanned from 400 nm to 550 nm, and the λ_{max} was confirmed as 450 nm. Absorbance for both extracts and BC were read at 450 nm using Secomam UV–Vis spectrophotometer (RS232, Cedex, France). The TCC in the samples was determined by BC standard calibration curve: $A=mB+c$; where, A = absorbance value, m = slope of the BC standard calibration curve, B = concentration of BC (μg/ml), $c = y$ intercept of the curve. There is a limitation of the method as no prior separation process is carried out [15]. The absorbance taken at selected wavelengths is not specific for the compounds of interest. Therefore the results obtained would be referred as TCC. All data were subjected to Analysis of Variance (ANOVA) and LSD's multiple range tests using SPSS window version 15.

HPLC Determination of Beta Carotene (BC)

(All-*trans*)-BC contents were determined using the chromatographic method described by Hart and Scott [16] with

Table 1 Source of 14 types of underutilized fruits

Common name	Scientific name	Location	Fruiting season
Bacang 1	*Mangifera foetida*	Jerantut, Pahang	May–June
Bacang 2	*Mangifera foetida*	Gurun, Kedah	May–June
Bacang 3	*Mangifera foetida*	Gurun, Kedah	May–June, November–December
Cerapu 1	*Garcinia prainiana*	Jerantut, Pahang	July–September
Cerapu 2	*Garcinia prainiana*	Temerloh, Pahang	July–September
Durian Daun	*Durio lowianus*	Cheka, Pahang	July–August
Durian Nyekak 1	*Durio kutejensis*	Miri, Sarawak	November–December
Durian Nyekak 2	*Durio kutejensis*	Miri, Sarawak	November–December
Jambu Mawar	*Syzygium jambos*	Serdang, Selangor	Non-seasonal
Jambu Susu	*Syzygium malaccense*	Serdang, Selangor	June–September, November–February
Jentik-jentik	*Baccaurea polyneura*	Yan, Kedah	July–September
Kuini	*Mangifera odorata*	Arau, Perlis	May–June
Tampoi Kuning	*Baccaurea reticulata*	Jerantut, Pahang	July–August
Tampoi Putih	*Baccaurea macrocarpa*	Jerantut, Pahang	July–August

some modifications. HPLC analysis was carried out on a Hewlett Packard (HP1100) system coupled with UV–Vis diode array detection. The column, a 150×4.6 mm, 3 µm Zorbax Eclipse RP C18 analytical column (Agilent, California, USA), was protected with a 15×2 mm, 5 µm Purospher Star RP-18e guard column (Merck, Darmstadt, Germany). The mobile phase consisted of acetonitrile: methanol: dichloromethane (75:20:5 *v*/*v*/*v*), and was filtered through a 0.22 pm PTFE membrane filter connected to a vacuum pump system and degassed in an ultrasonic cleaner 5510 E-MTH from Branson (Connecticut, USA). The separation was performed by isocratic elution at a flow rate of 1.5 ml/min. The injection volume was 20 µl and the column temperature at 30 °C. The peak of BC was identified at 450 nm. The calibration of the standard was carried out with the range of the concentrations (50–2,000 µg/ml).

Results and Discussion

Precision Measurements

The intra-batch relative standard deviation (RSD) of each fruit is shown in Table 2. Calculated RSD % were <5% for all tested fruits except for Jambu Susu (6.56%), Kuini (6.45%), and Tampoi Putih (7.3%); while Bacang 1, Cerapu 2, Durian SB, Jambu M, and Jentik-jentik had calculated RSD % <2%. The tested control (BC) demonstrated the very good precision in both intra-batch and inter-batch. Both precision % were lower than that recommended by AOAC [17], which reference RSD % were <1% for intra-batch precision and <2% for inter-batch precision. Inter-batch precision analysis was not carried out for all tested fruits due to the inestability of carotenoids in the food matrix.

All tested fruits had RSD % lesser than the RSD(r) %, which as recommended by AOAC [17] (Table 2). Besides, the reference RSD(r) % was determined based on the percentage of analyte in sample. Some of the fruit samples (TCC<5 mg/100 g) had slightly higher calculated RSD %. They include Bacang 2 (3.6%), Bacang 3 (4.2%), Durian Daun (4.0%), Jambu Susu (6.6%), Kuini (6.5%), and Tampoi Putih (7.3%). It may due to slight degradation of carotenoids in the sample matrix. More studies need to be carried out to determine the stability of carotenoids in fruits matrix under different storage conditions.

Table 2 Intra-batch variability of absorbance in hexane fractions of underutilized fruits

Underutilized fruits	Mean abs (n=3)	SD	RSD (%)	RSD(r) (%)
Bacang 1	0.082	0.0004	0.49	7.3
Bacang 2	0.071	0.0025	3.6	7.3
Bacang 3	0.041	0.0017	4.15	7.3
Cerapu 1	0.156	0.0077	4.94	7.3
Cerapu 2	0.379	0.0067	1.77	5.3
Durian Daun	0.065	0.0026	4.0	7.3
Durian Nyekak 1	0.284	0.0102	3.59	5.3
Durian Nyekak 2	0.372	0.0069	1.85	5.3
Jambu Mawar	0.025	0.0016	6.56	7.3
Jambu Susu	0.078	0.0015	1.95	7.3
Jentik-jentik	0.489	0.0046	0.95	5.3
Kuini	0.095	0.0061	6.45	7.3
Tampoi Kuning	0.126	0.0051	4.08	5.3
Tampoi Putih	0.032	0.0023	7.3	7.3
Beta carotene	1.734	0.0005	0.03	1.3

Beta carotene was used for comparison
Abs Absorbance, *SD* standard deviation, *RSD* relative standard deviation; *RSD(r)* intra-batch repeatability relative standard deviation [17]

Table 3 Total carotene content (TCC) and beta carotene (BC) content of underutilized fruits hexane fractions

Underutilized fruits	TCC (mg/100 g)	BC (mg/100 g)	% BC in TCC
Bacang 1	4.81 (0.1)	–	–
Bacang 2	3.25 (0.05)	–	–
Bacang 3	2.58 (0.05)	–	–
Cerapu 1	6.89 (0.11)	5.58 (0.10)	81
Cerapu 2	15.81 (0.1)	14.59 (0.14)	92
Durian Daun	3.04 (0.15)	–	–
Durian Nyekak 1	11.16 (0.14)	7.57 (0.01)	68
Durian Nyekak 2	14.97 (0.08)	10.99 (0.25)	73
Jambu Mawar	1.41 (0.07)	–	–
Jambu Susu	3.35 (0.07)	–	–
Jentik-jentik	19.83 (0.2)	17.46 (0.57)	88
Kuini	3.95 (0.04)	–	–
Tampoi Kuning	13.71 (0.06)	10.72 (0.35)	78
Tampoi Putih	1.47 (0.03)	–	–
Beta carotene	7.02 (0.15)	–	–

Values are expressed on dry weight basis. All data are the crude estimated mean TCC (SD) of three replicates

A wide range of RSD % from 0.5% to 7.3% for tested fruit samples may be due to the differences of analyte percentage in the food matrix. However, the carotenoid contents in most of the underutilized fruits were found to be degraded slightly. It was supported by Negi and Roy [18] that after storage of amaranth and fenugreek at cool chamber for 2 days, 10–30% of BC was degraded in the food matrix. Besides, it was noted that there was an increase in the amount of hexane loss during the analysis as the extract was exposed to air, thus contributing to the increase in the absorbance value of the hexane fraction. Moreover, the low solubility of semi-polar carotenoid compounds in hexane may contribute to high volatility of hexane [19]. In addition, carotenoid compounds could be lost due to oxidative degradation caused by the presence of oxygen during storage at low temperature [20]. At room temperature with exposure to dim or weak light, changes of individual carotenoid from one form to another may contribute to the increase or decrease in concentration [21]. Moreover, a study reported that antioxidants added to the sample could be able to slow down the degradation of

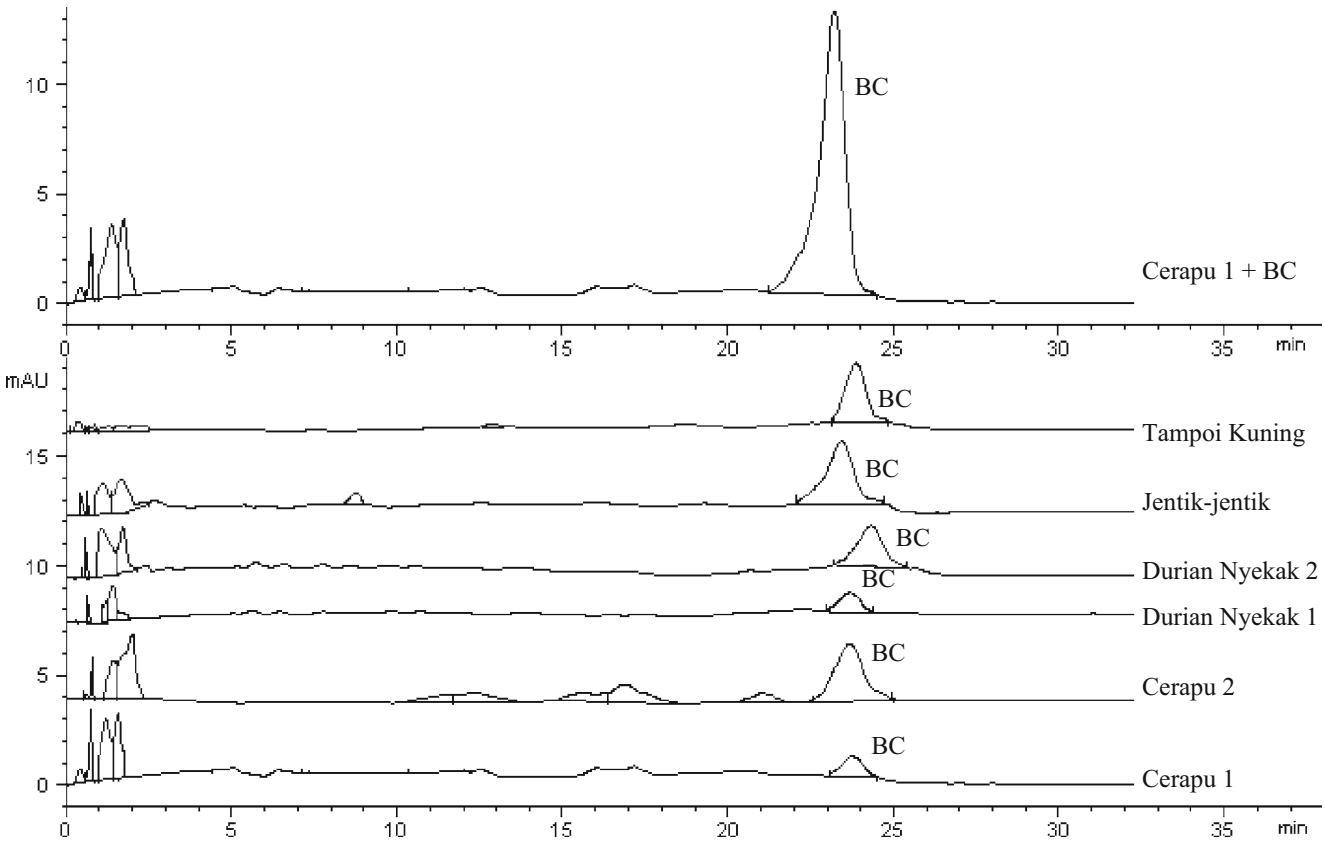


Fig. 1 HPLC chromatogram of the underutilized fruits hydrocarbon fraction extracted using hexane. The peak of BC was determined at 450 nm by spiking of the sample with BC standard. The peak of BC was detected at 24.0±0.8 min. Other peaks in the chromatogram were not determined

carotenoid [22]. Although precision measurement is important to determine the reliability of the analytical methods, the degradation of the carotenoid compounds in the fruit matrix during storage may affect the precision of the analytical methods. However, the stability of carotenoids in the fruit samples under different storage condition is questionable.

Total Carotene (TC) and Beta Carotene (BC) Contents

The estimation of TCC was performed without separation of the individual carotenoid. The fruit hexane fraction contained most of the non-polar carotenoid compounds, especially alpha and beta carotene, and the trace amount of lycopene remained soluble in the hexane fraction. The TCC of underutilized fruits is shown in Table 3. The TCC (mg/100 g edible portion) was found to be 19.83 in Jentik-jentik, 15.81 in Cerapu 2, 14.97 in Durian Nyekak 2, 13.71 in Tampoi Kuning, 11.16 in Durian Nyekak 1, 6.89 in Cerapu J, 4.81 in Bacang 1, 3.95 in Kuini, 3.35 in Jambu Mawar, 3.25 in Bacang 2, 3.04 in Durian Daun (3.04), 2.58 in Bacang 3, 1.47 in Tampoi Putih and 1.41 in Jambu Susu. In this study, Cerapu 2, Durian Nyekak 2, Jentik-jentik and Tampoi Kuning had higher TCC (>10.0 mg/100 g) than other fruits. Tampoi Putih and Jambu Susu had low level of TCC, which was less than 1.5 mg/100 g. All the tested underutilized fruits had TCC in the range of 1.4–19.8 mg/100 g.

Determination of individual carotenoid by HPLC would be useful to quantify its content present in underutilized fruits. Based on spectrophotometric methods, BC-rich underutilized fruits were selected for HPLC analysis. The peaks of BC in the selected underutilized fruits are shown in Fig. 1. Results showed that Jentik-jentik had the highest BC content, followed by Cerapu 2, Durian Nyekak 2, Tampoi Kuning, Durian Nyekak 1 and Cerapu 1 (Table 3). The BC content of *Durio* fruit was about 70% in TCC, while *Baccaurea* and *Garcinia* fruits had more than 80% of BC in TCC, except Tampoi Kuning (78%). The BC content of other tested fruits was not determined due to limited resources.

High carotene content was found in the tested fruits that have yellow colour pigmentation. This is in agreement with some studies, a study by Ben-Amotz and Fishler [23] reported that the orange colour of carrot and sweet potato was attributed to their high level of BC. Ajila et al. [24] also reported that the yellow-orange flesh of ripened mango had high total carotenoid. Besides, Tee and Lim [11] found that commercial mango was rich in BC as compared to other local fruits. In this study, the underutilized *Mangifera* fruits had 3.6–4.8 mg/100 g of TCC, which were comparable to the commercial mangos grown in India (4.9 mg/100 g) [25] and Israel (4.93 mg/100 g) [23]. To best of our knowledge, the published works on carotenoids of other underutilized tropical fruits are limited for reference.

Conclusions

The estimation of TCC in underutilized fruits using a UV–Vis spectrophotometer is a rapid method compared to HPLC method. This method could under- or over-estimate the carotenoids content, if compared to other chromatographic methods. The intra-batch precision measurements showed that the analytical method is reliable. Among the tested underutilized fruits, Jentik-jentik was rich in TCC, while Jambu Susu and Tampoi Putih had low TCC. However, fruit with yellow-orange coloured flesh is a good source of carotene.

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