Food ©2009 Global Science Books

Stability of Carotenoids from Hexane Fractions of 12 Malaysian Underutilised Tropical Fruits during Low Temperature Storage

Hock Eng Khoo • Amin Ismail *

Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, UPM Serdang, 43400 Selangor Darul Ehsan, Malaysia

***Corresponding author*: * amin@medic.upm.edu.my**

ABSTRACT

A study on the stability of carotenoids in fruit extract is necessary due to rapid degradation of carotenoid compounds in the extract. Twelve selected underutilised tropical fruits were studied for their total carotene content (TCC) from hexane fractions under storage for 5 h at 0°C and 12 days at −20°C. Kinetic study revealed that the degradation rate of TCC was highly depended on the storage time. Storage for 12 days at −20°C had TCC degraded for more than 30% in hexane fractions of Bacang 1 (*Mangifera foetida*), Bacang 3 (*M. foetida*), Kuini (*M. odorata*) and Tampoi Putih (*Baccaurea macrocarpa*). Less than 5% of TCC was lost in hexane fractions of Cerapu 1 (*Garcinia prainiana*) and Cerapu 2 (*G. prainiana*). Most of the fruit's hexane fractions demonstrated a moderate loss of TCC if stored at –20°C for about 2 weeks. A major loss of TCC was found in low antioxidant fruits. However, storage for 5 h at 0°C had TCC degraded for less than 15% in hexane fractions of Cerapu 2, Durian Nyekak 2 (*Durio kutejensis*) and Jentik-jentik (*Baccaurea polyneura*). In this study, a rapid degradation of carotenoids occurred if the fruit's hexane fractions were stored at 0°C than at −20°C. Various factors may contribute to the degradation of carotenoid compounds in the fruits' hexane fractions.

Keywords: hexane fraction, stability, storage, total carotene content, underutilised fruit

INTRODUCTION

Carotenoids are colourful pigments widely distributed in nature. In plants, carotenoids contribute to the fruits' red, orange and yellow colours (Hughes 2001; Burns *et al.* 2003). Carotenoids consist of carotenes and xanthophylls. Typical carotenes are α- and β-carotenes, and lycopene, while cryptoxanthin, lutein and zeaxanthin are classified as xanthophylls. A study has shown that α- and β-carotenes, and β-cryptoxanthin have pro-vitamin A activity (Hulshof *et al.* 2007). These carotenoids are converted to retinal by mammals in the intestine (Burns *et al.* 2003). Other types of carotenoids, especially lycopene and lutein, have no pro-vitamin A activity (Lee *et al.* 1989).

In Malaysia, more than hundred types of indigenous fruits can be found in the tropical rain forest. Most of them are considered as "underutilised". These fruits are from the genera *Artocarpus*, *Baccaurea*, *Durio*, *Garcinia*, *Mangifera*, *Musa*, *Nephelium* and *Syzygium*. About 29 *Baccaurea* species available in Malaysia and few of the species are endemic in this country. Only eight species of *Mangifera* fruits were commonly consumed in Malaysia. Other types of underutilised fruits are less common. Most of the studies conducted in Malaysia on underutilised fruits are mainly on nutritional composition and antioxidant capacity. However, limited studies have focused on carotenoid composition and its stability during storage.

The stability of carotenoids in hexane solution had been reported by Cvetković and Marković (2008). Craft (1992) reported that less than 10% of lutein and β-carotenes degradation were found in organic solvents (Craft 1992). However, no study has been published on the stability of fruit's total carotenoid in hexane. Recently, scientists from all over the world have drawn more attention to study focusing on the stability of carotenoids, perhaps due to limited study on carotenoids degradation under different storage conditions. The stability of carotenoids mainly depends on heat, light, oxygen and different food matrices, which will also have an effect on autooxidation of carotenoids (Eder 1996). Carotenoid compounds are not stable under certain conditions, such as exposure to heat or light (Clark *et al.* 2004; Vásquez-Caicedo *et al.* 2007). When temperature or exposure to light during storage increases, carotenoids in an all-*trans* form will degrade (Tang and Chen 2000). Scita (1992) demonstrated that the loss of β-carotene under specific storage conditions (−20°C, in the dark and under nitrogen) was less significant in the presence of 0.025% butylated hydroxytoluene (BHT), which is a synthetic antioxidant and potential chemopreventive agent (Smith *et al.* 1998).

The duration of storage and exposure to the environment were found to have an effect on carotenoid degradation in food products (Lin and Chen 2005). Food processing enhances carotenoid isomerisation, thus increases the carotenoid storage stability, while all-*trans* carotenoid in unprocessed food is prone to oxidative degeneration (Mayer-Miebach and Behsnilian 2006). Hydrocarbon carotenoids such as α- and β-carotenes that have pro-vitamin A activity are non-polar compounds and highly soluble in hexane. As the study was focused on stability of hydrocarbon carotenoids in hexane, total carotene content (TCC) of Malaysian underutilised tropical fruits in hexane fractions was determined, and their storage stability for up to 5 hours at 0°C and 12 days at −20°C was assessed.

MATERIALS AND METHODS

Chemicals and standard

Ethanol was obtained from GmbH Chemical Company (Hamburg, Germany), while *n*-hexane was obtained from Merck (Darmstadt, Germany). β-Carotene standard was purchased from Sigma (Missouri, USA).

Samples

A total of 12 underutilised fruits from five genera provided by the Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Selangor, Malaysia were selected for this

Received: 27 May, 2008. Accepted: 10 October, 2009.

Original Research Paper

Table 1 Description and fruiting period of Malaysian underutilised tropical fruits.

Local name	Scientific name	Location	Fruit description	Fruiting period
Bacang 1 Bacang 2 Bacang 3	*Mangifera foetida*	Gurun, Kedah	The fruit is oval in shape, measuring 8.5-13.5 cm long by 5-10 cm wide. The fruit skin is smooth, dull and greenish colour scattered with dark spots. The flesh is fibrous and orange-yellow in colour.	December-March, May-July
Cerapu 1 Cerapu 2	*Garcinia prainiana*	Temerloh, Pahang	The fruit is button shape-liked. The unripe fruit is bright green in colour, smooth and shiny. The ripe fruit is orange in colour and juicy; with pale orange-colour flesh consists of 5 to 7 segments. The skin is thin and can be easily peeled off.	July-September
Durian Daun Durian Nyekak 1 Durian Nyekak 2	*Durio* sp. *Durio kutejensis*	Cheka, Pahang Miri, Sarawak	Each species of durian has its own characteristics. Some species have strong stunning flavour and others are mild and light. Aril colour ranges from pale yellow to crimson red.	April-June October-January
Jentik-jentik	*Baccaurea polyneura*	Yan, Kedah	The fruit is oval in shape, measuring 2-2.5 cm long and with hard pericarp. The fruit has 2-3 seeds covered by orange colour flesh and have sweet taste.	April-May
Kuini	*Mangifera odorata*	Arau, Perlis	Most of the characters are similar as bacang and has a strong smell; juicy sweet flesh has a light orange colour.	July-September
Tampoi Kuning Tampoi Putih	*Baccaurea puberula* *Baccaurea macrocarpa*	Jerantut, Pahang	The fruit have almost round in shape, measuring 5-7 cm width. It has about 1 cm thick hard pericarp. The skin colour is dull brownish grey or brownish yellow and slightly rough with little brown spots.	May-June

Table 2 Average weight (g) of fruit sample whole fruit, flesh, peel and seeds.

Fruits	Whole	Edible portion		Peel	Seed
		Flesh	Flesh + Peel		
Bacang 1 [b]	199.6	129.9	-	26.6	43.1
Bacang 2 [b]	188.2	117.6	-	32.7	37.9
Bacang 3 [b]	387.8	241.6	-	102.3	43.9
Cerapu 1 [d]	4.6	2.2	-	0.93	1.5
Cerapu 2 [c]	44.7	32.9	-	8.9	2.9
Durian Daun [a]	1568.5	15.6	-	1536.4	16.5
Durian Nyekak 1 [a]	1493.2	14.8	-	1467.8	10.6
Durian Nyekak 2 [a]	1541.8	16.6	-	1514.7	10.5
Jentik-jentik [d]	4.4	1.4	-	3.0	-
Kuini [b]	173.5	87.4	-	41.7	44.4
Tampoi Kuning [c]	67.0	13.6	-	53.4	-
Tampoi Putih [c]	76.5	18.9	-	57.6	-

Sample size: [a] n=2, [b] n=3, [c] n=5, [d] n=7

study (**Table 1**). The fruits were collected conveniently at different periods, as the fruits were grown seasonally. Although more than one type of Bacang, Cerapu and Durian Nyekak were obtained from same district, these fruits differed in their variety.

In laboratory, each fruit was washed, cleaned and weighed. The peel was manually removed and cut in half. The kernels of some fruits were also removed. Most of the fruits have non-edible seed and peel, and some have juicy pulp. The average weights of the whole fruit, flesh/pulp, peel and seed are shown in **Table 2**. The edible portion (flesh/pulp) was weighed, placed into a plastic bag and stored at −80°C before lyophilisation. All lyophilised samples were ground into fine particles. All samples were stored at −20°C for less than a month before further analysis.

Extraction of carotenoids

Carotenoids were extracted based on a method described by Seo *et al.* (2005) with slight modification, i.e. the hexane layer was washed with distilled water, and water was removed by using anhydrous sodium sulphate. In more detail, samples (1.0 g) were weighed and added to 10.0 ml of 95% ethanol. The mixture was stirred using a Unimax 1010 DT shaking incubator (Heidolph, Schwabach, Germany). After 30 min, the mixture was filtered through a Whatman No. 1 filter paper and the supernatant was mixed with an equal volume of hexane to separate the lipid layer from ethanol. The fruit residue was re-extracted three times. The hexane layer was removed and washed with distilled water to remove the protein residues and other water soluble antioxidant compounds. The water residue in the hexane fraction was removed and hexane was evaporated using a Rotavor R-200 rotary evaporator (Büchi, Flawil, Switzerland) at 39°C. The fruit extract was dried completely using nitrogen gas (Rodriguez-Amaya 2001). All the above steps were carried out in a dark room with dim light at room temperature (25°C). A known amount of hexane was used to dissolve the remaining lipid layer and stored in an amber bottle at –20°C in darkness for less than 2 weeks to study the total carotene degradation.

Estimation of total carotene content (TCC)

β-Carotene (analytical grade) stock solution were prepared in hexane at concentrations of 0.22−7.0 µg/ml, and stored in amber bottles at −20°C. The preparation of stock solution and samples were carried out in a dark room. Determination of TCC was carried out based on a method described by Prache *et al.* (2003). The TCC in the fruit's hexane fractions were measured using a RS232 UV-Vis spectrophotometer (Secomam, Cedex, France) at 450 nm. TCC in the samples was determined using β-carotene standard calibration: A = mB + c; where, A = absorbance value measured at 450 nm using UV−Vis spectrophotometer, m = gradient of the β-carotene standard calibration (straight line) graph, B = concentration of β-carotene (µg/ml), and c = y intercept of the straight line graph. As the absorbance taken at selected wavelengths is not specific for the compounds of interest (Tee and Lim 1991), the results obtained would best to be known as TCC (mg/100 g).

Stability of TCC in hexane fraction of underutilised fruits

The TCC in the studied fruit's hexane fractions was examined for kinetic degradation and storage stability. First, hexane fractions of three selected underutilised fruits (Jentik-jentik, Durian Nyekak 2 and Cerapu 2) were selected to study the kinetic of TCC degradation, which was stored at 0°C for up to 5 h. Briefly, 6 ml of hexane fraction was transferred into an amber bottle and stored at 0°C for up to 5 h. Within a day, TCC of the hexane fractions of the under-

Table 3 Underutilised fruits TCC (mg/100 g) in hexane fractions at day 0 and day 12.

Samples	Day		Kruskal Wallis
	0	12	Test sig-χ^2
Bacang 1	4.81 (0.1)	2.52 (0.03)	<0.05
Bacang 2	3.25 (0.05)	2.83 (0.05)	<0.05
Bacang 3	2.58 (0.05)	1.49 (0.04)	<0.05
Cerapu 1	6.89 (0.11)	6.61 (0.02)	0.086
Cerapu 2	15.81 (0.08)	15.17 (0.07)	<0.05
Durian Daun	3.04 (0.15)	2.3 (0.05)	<0.05
Durian Nyekak 1	11.16 (0.14)	8.29 (0.07)	<0.05
Durian Nyekak 2	14.97 (0.08)	11.28 (0.01)	<0.05
Jentik-jentik	19.83 (0.2)	15.02 (0.02)	<0.05
Kuini	3.95 (0.04)	2.76 (0.03)	<0.05
Tampoi Kuning	13.71 (0.05)	12.27 (0.07)	<0.05
Tampoi Putih	1.47 (0.03)	0.68 (0.02)	<0.05
β-Carotene	7.02 (0.01)	6.99 (0.02)	0.293

utilised fruits was determined at every hour for up to 5 h. These three types of fruit's hexane fractions were selected due to limited samples of other fruits, as underutilised fruits are seasonal. To determine the effect of prolong storage time on TCC degradation; the hexane fractions of 12 underutilised fruits were stored at −20°C for up to 12 days. Every 4 days, the TCC of each hexane fraction was determined. All tests were run in triplicate.

Mean TCC of three replications of each fruit's hexane fraction were subjected to non-parametric Kruskal Wallis χ^2 test using SPSS window version 15. The significant level was set at $\alpha < 0.05$.

RESULTS AND DISCUSSION

Total carotene content (TCC)

TCC of the studied underutilised fruit's hexane fractions are shown in **Table 3**. The highest TCC was found in hexane fraction of Jentik-jentik and the lowest in Tampoi Putih. The TCC of the studied fruits was ranged from 1.47−19.83 mg/100 g. Although some of the underutilised fruits may have high tocopherol contents that are soluble in hexane, total carotenes have an optimum wavelength of 450 nm as compared to tocopherols at 292 nm (Gimeno *et al.* 2000).

Kinetic and stability study of TCC during 5 hours of storage at 0°C

In the present study, kinetic log absorbance of the three selected underutilised fruits' TCC in hexane fractions and control (β-carotene in hexane) was plotted against storage times shown in **Fig. 1**. The degradation of TCC in hexane fractions of Cerapu 2, Durian Nyekak 2 and Jentik-jentik was almost similar. The plots are linear with average R values of 0.99. The degradation kinetics seem to obey the first-order equation, $y = kx + n$ where y is the log absorbance of the hexane fraction (Cvetković and Marković 2008). The degradation rates of TCC (expressed as the slopes of the linear plots, k, in hour^{-1}) for the three selected underutilised fruit's hexane fractions are presented in **Table 4**. The kinetic trend suggests that the degradation of TCC was dependent on storage time. Other variables were not taken into consideration due to limited resources. The kinetic of TCC degradation in hexane fractions of Cerapu 2 and Durian Nyekak 2 exhibited a similar degradation rate (0.0056 k hour^{-1}), while Jentik-jentik had a higher degradation rate (0.012 k hour^{-1}) compared to Cerapu 2 and Durian Nyekak 2. Moreover, the control (β-carotene) was degraded at a lower rate (0.0017 k hour^{-1}).

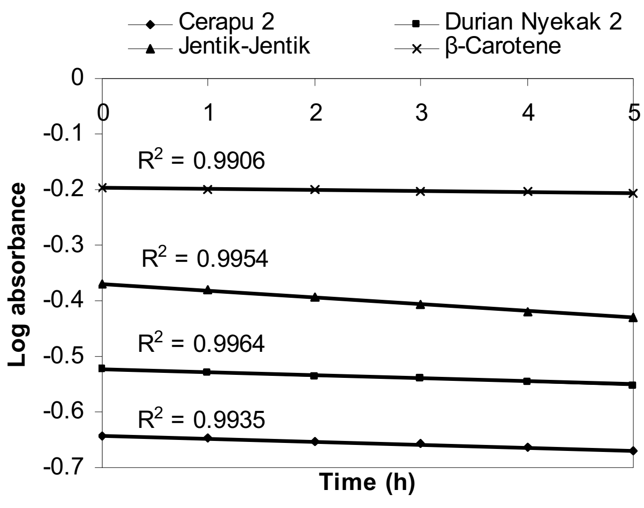

Fig. 1 Change in percentage of total carotenoids from hexane fraction of underutilised fruits during different days of storage at –20ºC.

Table 4 Kinetics and percentages of TCC degradation in hexane during storage for up to 5 hours at 0ºC for three selected underutilised fruits' hexane fractions.

Samples	Day					k hour^{-1}
	1	2	3	4	5	
Cerapu 2	98.68	97.45	96.6	95.33	93.63	0.0056
Durian Nyekak 2	98.89	97.49	96.61	95.98	93.84	0.0056
Jentik-jentik	97.74	95.66	91.97	89.43	87.75	0.012
β-Carotene	99.69	99.43	98.96	98.49	98.18	0.0017

The kinetic obeys a linear first-order plot: $y = kx + n$, where y is log absorbance (TCC absorption in hexane at 450 nm), x the UV-irradiation time, and k is the first order rate constant for pigment bleaching.

Results showed that the studied fruits' hexane fractions and β-carotene were significantly degraded (sig-$\chi^2 < 0.05$) during 5 h of storage at 0°C. The percentages of TCC degradation during storage for 5 h at 0°C are shown in **Table 4**. The results also showed that during the storage period, less than 15% of TCC was lost in hexane fractions of Jentik-jentik, Durian Nyekak 2 and Cerapu 2, while less than 2% of β-carotene was degraded. However, low solubility of other carotenoid compounds (lycopene and β-cryptoxanthin) in hexane might affect the carotenoid degradation (Correa *et al.* 2001).

Stability study of TCC during 12 days of storage at −20°C

Results of the TCC degradation analysed by Kruskal Wallis χ^2 test are shown in **Table 3**. The TCC of all studied fruits' hexane fraction were significantly degraded (sig-$\chi^2 < 0.05$) during 12 days of storage at –20°C, except for the hexane fraction of Cerapu 1 and β-carotene (control). Among the studied fruits' hexane fractions, the TCC in Durian Nyekak 1, Durian Nyekak 2, Tampoi Putih, and Jentik-jentik was significantly degraded after every 4 days.

The percentages of TCC degradation during 12 days of storage at −20ºC are shown in **Table 5**. Results showed that on day 12th of storage, higher degradation of TCC was found in hexane fractions of Tampoi Putih (54%), Bacang 1 (48%), Bacang 3 (42%), and Kuini (30%); while less than 30% of TCC degradation was found in Durian Nyekak 1 (26%), Durian Nyekak 2 (25%), Durian Daun (24%), Jentik-jentik (24%), Bacang 2 (13%), Tampoi Kuning (11%), Cerapu 1 (4%), and Cerapu 2 (4%). The TCC in hexane fraction of Tampoi Putih had degraded more rapid than other *Baccaurea* fruits; similar as TCC degradation found in Durian Nyekak 1 if compared to other *Durio* fruits at day 12th of storage. The degradation of TCC in hexane fractions of Bacang 1 and Bacang 2 was more rapid than other *Mangifera* fruits. Moreover, rapid degradation of TCC was found in hexane fraction of Cerapu 2 than Cerapu 1. The control (β-carotene) had TCC degradation less than 0.5% during storage for 12 days at −20ºC.

The TCC was drastically decomposed in some of the fruits' hexane fractions when kept for 12 days. The loss of TCC in the hexane fraction of Cerapu was less than 5% at day 12th of storage. The kinetic study showed that hexane fraction of Cerapu 2 had lower degradation rate compared

Table 5 Percentages of TCC degradation in hexane fractions of under-utilised fruits during 12 days of storage at −20°C.

Samples	Day			
	0	4	8	12
Bacang 1	100 (0.10)	74.91 (0.63)	73.54 (2.64)	52.35 (0.48)
Bacang 2	100 (0.05)	95.89 (0.63)	93.82 (1.29)	86.82 (0.72)
Bacang 3	100 (0.05)	79.25 (0.93)	74.66 (7.18)	57.46 (0.68)
Cerapu 1	100 (0.11)	99.64 (3.31)	96.31 (1.32)	95.93 (1.48)
Cerapu 2	100 (0.08)	98.48 (1.10)	98.39 (0.14)	95.94 (1.09)
Durian Daun	100 (0.15)	81.04 (0.77)	80.36 (7.05)	75.88 (3.04)
Durian Nyekak 1	100 (0.14)	93.17 (1.13)	89.57 (1.56)	74.31 (0.43)
Durian Nyekak 2	100 (0.08)	90.41 (1.08)	85.13 (0.63)	75.37 (0.31)
Jentik-jentik	100 (0.20)	90.21 (0.84)	83.11 (0.48)	75.74 (0.53)
Kuini	100 (0.04)	95.25 (1.21)	72.17 (3.42)	69.81 (0.52)
Tampoi Kuning	100 (0.03)	99.51 (1.38)	96.48 (0.30)	89.54 (0.21)
Tampoi Putih	100 (0.05)	88.15 (3.18)	59.96 (2.24)	46.29 (1.99)
β-Carotene	100 (0.01)	99.81 (0.09)	99.71 (0.15)	99.68 (0.09)

to Jentik-jentik. Although hexane fraction of Durian Nyekak 2 had similar degradation rate as Cerapu 2, lower degradation of TCC was found in hexane fraction of Cerapu 2 than Durian Nyekak 2. It indicates that hexane fraction of Cerapu 2 might be protected by antioxidant compounds present in the extract. At day 12th of storage, hexane fractions of Bacang 1, Bacang 2, Kuini and Tampoi Putih had higher degradation of TCC might be due to the least protective effect by unknown antioxidants. However, the studied fruits' hexane fractions had been washed with distilled water to remove most of the polar antioxidant compounds. However, trace amounts of fat soluble ascorbic acid esters with long chain fatty acids such as ascorbyl palmitate or ascorbyl oleoyl that are soluble in hexane might have a protective effect.

A study done by MARDI-UPM reported that Bacang, Cerapu, Jentik-jentik and Kuini had 30.01, 27.31, 14.9 and 10.41 mg/100 g (edible portions) of ascorbic acid content, respectively (unpublished data); while Durian Nyekak had 15.9 mg/100 g ascorbic acid (Voon and Kueh 1999). Although Tampoi Kuning had low ascorbic acid content, the TCC degradation rate was low. It might be due to the protective effect of other hexane soluble antioxidants, such as tocopherol.

Carotenoids in freeze-dried form have been reported to deteriorate when the storage time and temperature increase (Tang and Chen 2000). The carotenoids may also have degraded due to autooxidation, which was in agreement with a study carried out by Rodriguez and Rodriguez-Amaya (2007). Although saponification process could further remove the interfering chlorophylls and carotenoid esters that caused carotenoid degradation and isomerisation (Eder 1996), it is not an ideal way as carotenoids decomposition may occurred. Besides, enzyme extracted carotenoids are much more stable than carotenoids extracted using a solvent (Çinar 2001). Moreover, the addition of antioxidants into the sample could be able to slow down the degradation rate of carotenoid (Zhang *et al.* 1998).

CONCLUSION

The solubility and stability of the study underutilised fruits' TCC in organic solvent are varied. The study indicated that degradation of TCC in the fruits' hexane fractions could not be due to a single factor. Analysis of carotenoid is recommended to be carried out immediately after extraction. If delayed for 12 days, there was a loss of more than 10% of TCC in hexane fractions of Bacang 3, Durian Nyekak 1, Durian Nyekak 2, Durian Daun, Jentik-jentik, and Tampoi Kuning; while more than 30% of TCC degradation occurred in hexane fractions of Bacang 1, Bacang 2, Kuini and Tampoi Putih. In addition, Cerapu extracts are a good source of antioxidant as TCC degradation occurred at a slower rate. However, the carotenoid-rich *Baccaurea* and *Durio* fruits are also potential sources of antioxidants.

ACKNOWLEDGEMENT

The authors would like to thank Dr. Salma Idris from the Division of Botany, Strategic Resource Research Center, Malaysian Agricultural Research and Development Institute (MARDI), Serdang, 43400 Selangor Darul Ehsan, Malaysia for providing the studied fruits.

REFERENCES

Burns J, Fraser PD, Bramley PM (2003) Identification and quantification of carotenoids, tocopherols and chlorophylls in commonly consumed fruits and vegetables. *Phytochemistry* **62**, 939-947

Çinar I (2001) Storage stability of enzyme extracted carotenoid pigments from carrots. *Electronic Journal of Environmental, Agricultural and Food Chemistry* **3**, 609-616

Clark S, Youngman LD, Chukwurah B, Palmer A, Parish S, Peto R, Collins R (2004) Effect of temperature and light on the stability of fat-soluble vitamins in whole blood over several days: implications for epidemiological studies. *International Journal of Epidemiology* **33**, 518-525

Correa NM, Durantini EN, Silber JJ (2001) Substituent effects on binding constants of carotenoids to n-heptane/AOT reverse micelles. *Journal of Colloid and Interface Science* **240**, 573-580

Craft NE (1992) Relative solubility, stability, and absorptivity of lutein and β-carotene in organic solvents. *Journal of Agricultural and Food Chemistry* **40**, 431-434

Cvetković D, Marković D (2008) Stability of carotenoids toward UV-irradiation in hexane solution. *Journal of the Serbian Chemical Society* **73**, 15-27

Eder R (1996) Pigment. In: Nollet LML (Ed) *Handboook of Food Analysis: Revised and Expanded, Physical Characterization and Nutrient Analysis* (Vol 1, 2nd Edn), Marcel Dekker, NY, USA, pp 805-808

Gimeno E, Calero E, Castellote AI, Lamuela-Raventós RM, de la Torre MC, López-Sabater MC (2000) Simultaneous determination of α-tocopherol and β-carotene in olive oil by reversed-phase high-performance liquid chromatography. *Journal of Chromatography A* **881**, 255-259

Hughes DA (2001) Dietary carotenoids and human immune function. *Nutrition* **17**, 823-827

Hulshof PJM, Kosmeijer-Schuila T, Westa CE, Hollman PCH (2007) Quick screening of maize kernels for provitamin A content. *Journal of Food Composition and Analysis* **20**, 655-661

Lee CY, Simpson KL, Gerber L (1989) Vegetables as a major vitamin A source in our diet: New York's Food and Life Sciences Bulletin (126th Edn), New York State Agricultural Experiment Station, Geneva, Switzerland, pp 1-10

Lin CH, Chen BH (2005) Stability of carotenoids in tomato juice during storage. *Food Chemistry* **90**, 837-846

Mayer-Miebach E, Behsnilian D (2006) Stability and bioavailability of lycopene, lutein and zeaxanthin in fruits and vegetables as affected by thermal processing. *Stewart Postharvest Review* **5**, 1-10

Prache S, Priolo A, Grolier P (2003) Persistence of carotenoid pigments in the blood of concentrate-finished grazing sheep: Its significance for the traceability of grass-feeding. *Journal of Animal Science* **81**, 360-367

Rodriguez EB, Rodriguez-Amaya DB (2007) Formation of apocarotenals and epoxycarotenoids from β-carotene by chemical reactions and by autoxidation in model systems and processed foods. *Food Chemistry* **101**, 563-572

Rodriguez-Amaya DB (2001) *General Procedure and Sources of Errors in Carotenoid Analysis: A Guide to Carotenoid Analysis in Foods*, ILSI Press, International Life Sciences Institute, Washington DC, USA, pp 24

Scita G (1992) The stability of β-carotene under different laboratory conditions. *Journal of Nutritional Biochemistry* **3**, 124-128

Seo JS, Burri BJ, Quan Z, Neidlinger TR (2005) Extraction and chromatography of carotenoids from pumpkin. *Journal of Chromatography A* **1073**, 371-375

Smith WA, Arif JM, Gupta RC (1998) Effect of cancer chemopreventive agents on microsome-mediated DNA adduction of the breast carcinogen dibenzo[*a*,*l*]pyrene. *Mutation Research* **412**, 307-314

Tang YC, Chen BH (2000) Pigment change of freeze-dried carotenoid powder during storage. *Food Chemistry* **69**, 11-17

Tee E-S, Lim C-L (1991) Carotenoid composition and content of Malaysian vegetables and fruits by the AOAC and HPLC methods. *Food Chemistry* **41**, 309-339

Vásquez-Caicedo AL, Schilling S, Carle R, Neidhart S (2007) Effects of thermal processing and fruit matrix on β-carotene stability and enzyme inactivation during transformation of mangoes into purée and nectar. *Food Chemistry* **102**, 1172-1186

Voon BH, Kueh HS (1999) The nutritional value of indigenous fruits and vegetables in Sarawak. *Asia Pacific Journal of Clinical Nutrition* **8**, 24-31

Zhang YX, Simonne RDPE, Landen WO Jr., Eitenmiller RR (1998) Stabilization of all-*trans* beta-carotene in raw, minced sweet potato. IFT'S Annual Meeting: Fruits and Vegetables Poster Session II, Georgia World Congress Center, Atlanta, Georgia, June 22