DETERMINATION OF α- AND β-CRYPTOXANTHINS, AND α- AND β-CAROTENES IN BUAH MERAH OIL BY HPLC-UV DETECTION

Mitsuhiro Wada[a], Kaori Fujimoto[a], Toshiaki Nishigaki[b], Erna Febriyanti[c], Rie Ikeda[a], Kenichiro Nakashima*[d]

[a] Graduate School of Biomedical Sciences, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan
[b] M&K Laboratories Inc., 1286-18 Yamato, Azusagawa, Matsumoto, Nagano 390-1701, Japan
[c] Center For Agro-Based Industry, Jl. Ir. H. Juanda No. 11, Bogor, Indonesia
[d] Faculty of Pharmaceutical Science, Nagasaki International University, 2825-7 Huis Ten Bosch, Sasebo, Nagasaki, 859-3298, Japan
nakaken@niu.ac.jp

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PENENTUAN α- DAN β-CRYPTOXANTHINS, DAN α- SERTA β-CAROTENES PADA MINYAK BUAH MERAH DENGAN HPLC-UV

ABSTRACT

A high-performance liquid chromatography-UV detection method for determination of α- and β-cryptoxanthins and, α- and β-carotenes in Buah Merah oil was developed. The separation of the four carotenoids was achieved by a combination of a Handy ODS column (150×4.6 mm, i.d.) and a Develosil Combi-RP-5 (50×4.6 mm, i.d.) *via* a 3-port switching valve. The mobile phase used was a mixture of CH_3CN/CH_3OH/ethyl acetate (=68:23:9, v/v/v). The retention times of α- and β-cryptoxanthins and, α- and β- carotenes were 18, 20, 53 and 60 min, respectively. The clean-up of Buah Merah oil was performed by liquid-liquid extraction after saponification with 13.5 M KOH solution. The calibration curves of the carotenoids showed good linearity ($r \geqq 0.999$). The detection limits of four carotenoids at a signal-to-noise ratio of 3 were from 0.36 to 1.14 ng/mg. Furthermore, the proposed method could be successfully applied to determine the carotenoids in 10 Buah Merah oil samples.

***Keywords**: Buah Merah oil, α- and β-carotene, α- and β-cryptoxanthin, HPLC-UV detection*

ABSTRAK

Sebuah kinerja tinggi metode deteksi kromatografi cair-UV untuk penentuan α- dan β cryptoxanthins dan, α- dan β-karoten dalam minyak Buah Merah dikembangkan. Pemisahan empat karotenoid dicapai oleh kombinasi penanganan kolom ODS (150 × 4,6 mm, id) dan Develosil Combi-RP-5 (50 × 4,6 mm, id) melalui 3-port beralih katup. Fase gerak yang digunakan adalah campuran CH3CN/CH3OH/ethyl asetat (68:23:9 =, v / v / v). Waktu retensi α- dan β- cryptoxanthins serta α-dan β-karoten adalah 18, 20, 53 dan 60 menit, berturut-turut. Pembersihan minyak Buah Merah dilakukan dengan ekstraksi cairan-cairan setelah saponifikasi dengan 13,5 solusi M KOH. Kurva kalibrasi karotenoid menunjukkan linearitas yang baik ($r \geqq 0,999$). Batas deteksi empat karotenoid pada rasio signal-to-noise dari 3 adalah 0,36-1,14 ng / mg. Selain itu, metode yang diusulkan dapat berhasil diterapkan untuk menentukan karotenoid dalam 10 sampel minyak Buah Merah.

***Kata kunci**: Minyak Buah Merah, α- and β-carotene, α- and β-cryptoxanthin, HPLC-UV detection*

INTRODUCTION

Pandanus conoideus Lam (call as Buah Merah) oil is consumed as a diet by the inhabitants in the higher mountain areas of Papua island, Indonesia. The oil has been focused on as a healthy food owing to its several benefits for health such as antioxidant activity, inhibition of cancer and anti-inflammatory [1]. As active ingredients, carotenoids, phenolic compounds, flavonoids and unsaturated fatty acids have been known [1-3]. Especially, carotenoids involving carotenes (α- and β-carotenes) and xanthophyls (α- and β-cryptoxanthins) contribute

antioxidant to the activity of Buah Merah oil, and thus their determination might be useful for a quality control process.

Many high-performance liquid chromatography (HPLC) methods have been developed to determine carotenoids in food. HPLC with UV/Vis detection is usually used because it's simple, economical and gives the value of sensitivity to determine carotenoids in foods [4-8]. An LC-MS/MS is also a powerful tool for the determination of carotenoids owing its high sensitivity and selectivity [9]. However, there are few methods to determine α- and β-cryptoxanthins and carotenes simultaneously.

The aim of this study was development of an HPLC-UV method for simultaneous determination of carotenoids (α- and β-cryptoxanthins and, α- and β-carotenes) in Buah Merah oil. The method validation was estimated to confirm the reliability of the method. Furthermore, the method was applied to determine the carotenoids in 10 kinds of Buah Merah oil from different brands and/or different production lots.

MATERIALS AND METHOD

Chemicals

α-Cryptoxanthin, α-carotene, NaCl, KOH, ascorbic acid, ethyl acetate and CH_3CN were obtained from Wako Pure Chemical Industries (Osaka, Japan). β-Cryptoxanthin used was from Chromadex (CA, USA). β-Carotene was purchased from Kanto Chemical Co. (Tokyo, Japan). Hexane, EtOH and CH_3OH were obtained from Nacalai Tesque (Kyoto, Japan). Water was passed through a pure line WL21P (Yamato Scientific Co., Tokyo). Other chemicals used were of analytical grade.

Pretreatment of Buah Merah Oil

All Buah Merah oil samples from Indonesia used were commercially available or raw materials for finished products. To 20 mg of oil sample in a brown flask, 10 mg of ascorbic acid and 750 μL of EtOH were added. Then 200 μL of 13.5 M KOH *aq*. solution were added for hydrolysis of esterified carotenoids and the air in a flask was replaced with N_2 gas. After standing for 30 min at room temperature, 250 μL of NaCl *aq*. solution (25 mg/mL) were put into the above mixture. The mixture was extracted with 750 μL of hexane/ethyl acetate (90:10, v/v) for 4 times. The collected organic layer was evaporated with a centrifugal evaporator (CE-1, Hitachi, Tokyo, Japan) at 40°C. The residue was reconstituted with 400 μL of CH_3CN and passed through a membrane filter (0.45 μm).

HPLC System and Conditions

The HPLC system for measurement of the carotenoids was consisted of a PU-1580 chromatographic pump (Jasco, Tokyo), a 7125 injector with a 20-μL of sample loop (Rheodyne, CA, USA), a Handy ODS (150×4.6 mm, i.d., column 1, Wako Pure Chemicals), a Develosil Combi-RP-5 (50×4.6 mm, i.d., column 2, Nomura Chemicals, Tokyo, Japan), a 3-port switching valve (GL Science, Tokyo, Japan), an 875-UV detector (Jasco) and an R-01 recorder (Rikadenki, Tokyo, Japan). A mixture of CH_3CN/CH_3OH/ethyl acetate (=68:23:9, v/v/v) was used as a mobile phase and flowed at 1.0 mL/min. The eluent was monitored at λ450 nm. The diagram of the HPLC system is shown in Figure 1. Carotenoids in the sample solution were retained on the column 1 and

interfering components were wasted. After 8.3 min of sample injection, valve position of the 3-port valve was changed to lead carotenoids to the column 2. for carotenoid separation process.

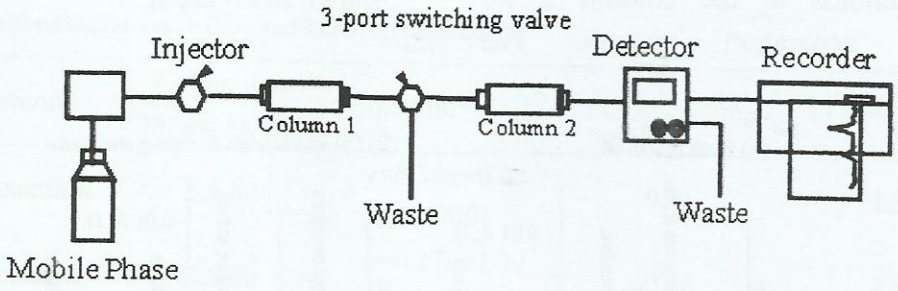

Figure 1. HPLC system and conditions for measurement of carotenoids in Buah Merah oil

Validation of Method

Calibration curves of each carotenoid was made by a spiked method where known concentration standards were put in Buah Merah samples. The concentration range was 2.5-80 ng/mg for α- and β-cryptoxanthins, and α-carotene, and 4.0-80 ng/mg for β-carotene, respectively. The limits of detection (LOD) and quantification (LOQ) were defined as the concentrations for which signal-to-noise (S/N) ratios were 3 and 10, respectively. Recovery of the compounds was evaluated by peak heights of the spiked standards (40 ng/mg) in Buah Merah oil to those of the corresponded standards. Accuracy, intra-day and inter-day precisions were examined by analyzing Buah Merah oil spiked with 2 concentrations of standards; the accuracy % was expressed as mean ± standard deviation (SD, n=5) and precision was expressed as the relative standard deviation (RSD) for five-replicate measurements. Accuracy % was calculated using the following equation:

$$\frac{\text{Found concentration} - \text{nominal concentration}}{\text{spiked concentration}} \times 100$$

Determination of Carotenoids in Buah Merah Oil

Ten kinds of Buah Merah oil samples were determined, and triplicate measurements for each sample were performed. The amounts of carotenoids in sample were indicated as mean ± SD (mg/100 g, n = 3).

RESULTS AND DISCUSSION

Optimization of Analysis Conditions

An octadecyl-silyl silica gel column (ODS) is ordinarily used [4-6] in separation of carotenoids. Recently, a C_{30} column which is more hydrophobic, is used to separate carotenoids in foods and achieved good separation of carotenoids and their esters [7,8]. Therefore, the Handy ODS (150 x 4.6 mm, i.d.) or the C30 column, Develosil Combi-RP-5 (50x4.6 mm, i.d.) were examined to separate α- and β-cryptoxanthins, and α- and β-carotenes, but their preferable separation could not be achieved. Next step, the combination of Handy ODS (column 1) and a Develosil Combi-RP-5 (column 2) through a 3-port switching valve was examined, and the good separation of α- and β-cryptoxanthin in Buah Merah oil was achieved. The

carotenoids were retained on the column 1 and interfering components were wasted. After 8.3 min of sample injection, valve position of the 3-port valve was changed to lead carotenoids to the column 2 for carotenoid separation process. The retention time of α-, β-cryptoxanthins, α- and β-carotenes were 18, 20, 53 and 60 min, respectively. Typical chromatograms of carotenoids in Buah Merah oil are shown in Figure 2.

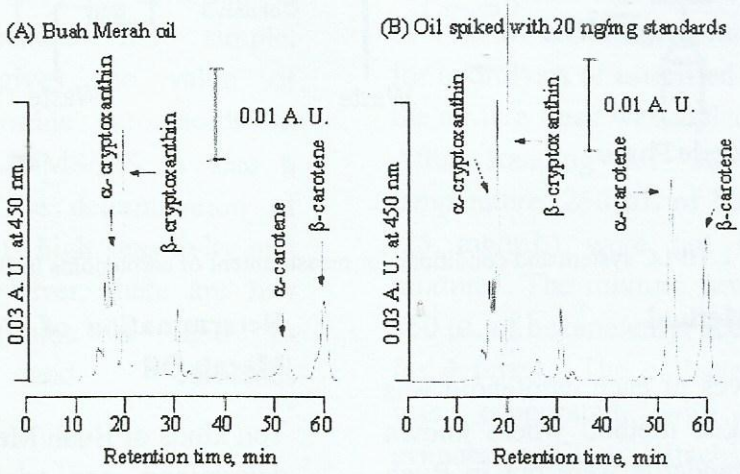

Figure. 2 Chromatograms of Buah Merah oil (A) and that spiked standards (B). Spiked concentration: 20 ng/mg of carotenoids.

Liquid-liquid extraction for clean-up of Buah Merah oil after saponification was used. Effect of organic solvent on the recovery of carotenoids from Buah Merah oil were examined. Hexane, ethyl acetate, EtOH, acetone and their mixtures were used as organic solvents. Among them, the maximum recovery was given by the mixture of hexane/ethyl acetate (=90:10, v/v) (data were not shown). Furthermore, 4 times extraction gave the maximum and constant recovery for all carotenoids.

Validation of Method

The analysis method of Buah Merah oil spiked with the standards of each carotenoid demonstrated good linearity ($r \geqq 0.999$) in the range of 2.5-80 ng/mg (α- and β-cryptoxanthins, and α-carotene) and 40-80 ng/mg (β-carotene), respectively (Table 1). The LOD and LOQ at a signal-to-noise ratio of 3 and 10 for α- and β-cryptoxanthins and, α- and β-carotenes were 0.41, 0.56, 0.36, and 1.14 ng/mg, and 1.38, 1.87, 1.20 and 3.81 ng/mg, respectively.

The sensitivity of the proposed method was comparable with the conventional HPLC-UV method [4,5] and was sufficiently sensitive to determine carotenoids in Buah Merah oil. To our knowledge, this is a first report to determine α- and β-cryptoxanthins in Buah Merah oil simultaneously.

The recoveries for α- and β-cryptoxanthins and, α- and β-carotenes were 93%, 91 %, 93% and 92%. Other validation parameters such as accuracy, precision for intra-and inter-day measurements were summarized in Table 2. The accuracy (ranging from 93.9

to 114.0%), intra-day precision (less than 11.4%) and inter-day assays (less than 11.8%) were acceptable for practical use (n = 5). As results, the developed method with acceptable validation parameters was reliable for the determination of α- and β-cryptoxanthins in Buah Merah oil.

Table 1. Calibration curves, LODs and LOQs of carotenoids

Compound	Calibration range, ng/mg	Equation*[1] (*r*)*[2]	LOD*[3], ng/mg	LOQ*[4], ng/mg
α-cryptoxanthin	2.5-80	y=0.36x+0.84 (1.000)	0.41	1.38
β-cryptoxanthin	2.5-80	y=0.27x+1.77 (0.999)	0.56	1.87
α-carotene	2.5-80	y=0.42x-0.01 (0.999)	0.36	1.20
β-carotene	4.0-80	y=0.13x-0.31 (0.999)	1.14	3.81

*[1]: x = concentration, ng/mg; y = peak height cm
*[2]: Correlation coefficient;
*[3]: Limit of detection at an S/N ratio of 3
*[4]: Limit of detection at an S/N ratio of 10.

Table 2. Accuracy, intra- and inter-precision for carotenoids

Compound	Spiked concentration, ng/mg	Found concentration (Mean±SD)*[1], ng/mg	Accuracy %	Precision RSD%	
				Intra-day	Inter-day
α-cryptoxanthin	0	19.7±0.4	-	-	-
	10.0	29.3±0.5	95.9	5.4	8.4
	20.0	42.3±0.7	114.0	6.9	7.5
β-cryptoxanthin	0	10.2±0.3	-	-	-
	5.2	15.1±0.4	93.9	8.8	11.8
	20.9	32.6±1.8	106.9	8.1	9.2
α-carotene	0	3.9±0.3	-	-	-
	4.6	8.8±0.5	108.3	9.2	9.6
	17.8	20.7±0.9	94.4	5.4	7.3
β-carotene	0	29.4±0.9	-	-	-
	10.8	40.2±1.2	99.4	11.4	11.4
	43.0	70.5±2.2	95.3	5.3	7.9

*[1]: n=5.

Determination of Carotenoids in Buah Merah Oil

Ten Buah Merah oil samples from Indonesia were determined (Table 3). Amounts for α-, β-cryptoxanthins, α- and β-carotenes were in the range of 0.6 - 3.1 mg/100 g, 1.4 - 9.0 mg/100 g, 0.2 - 0.9 mg/100 g and 1.5 - 6.7 mg/100 g, respectively. The four carotenoids in all samples examined could be determined, although their amounts varied widely and sample D from yellowish fruit was found no carotenoids contained.

Table 3. Amount of carotenoids in Buah Merah oil

	Sample	Amount (Mean±SD)*[1], mg/100g			
		α-cryptoxanthin	β-cryptoxanthin	α-carotene	β-carotene
A		2.7±0.2	7.0±0.6	0.9±0.2	6.3±0.5
B		2.2±0.2	5.5±0.3	0.8±0.1	5.3±0.1
C		2.6±0.1	6.5±0.6	0.8±0.1	6.0±0.1
D		ND*[2]	ND*[2]	ND*[2]	ND*[2]
E		0.6±0.0	1.4±0.3	0.2±0.0	1.5±0.2
F		1.5±0.2	4.8±0.5	0.7±0.0	4.3±0.3
G		3.1±0.2	9.0±0.5	0.9±0.2	6.7±0.4
H		2.0±0.3	6.6±0.2	0.6±0.1	4.8±0.3
I		2.0±0.2	4.5±0.2	0.4±0.1	3.3±0.2
J		1.8±0.3	4.4±0.1	0.4±0.1	3.2±0.2

*[1]: n=3; *[2]: ND, not detected

CONCLUSIONS

As the conclusion, the HPLC method combined two columns through the 3-port switching valve could determine the four carotenoids with acceptable validation. And the proposed method could be successfully applied to Buah Merah oils. The method is useful to evaluate quality of Buah Merah oil on the basis of carotenoid content.

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