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Analysis of α-cryptoxanthin, β-cryptoxanthin, α -carotene, and β-carotene of *Pandanus conoideus* oil by high-performance liquid chromatography (HPLC)

Zita L. Sarungallo[a,b], Purwiyatno Hariyadi[a,c]*, Nuri Andarwulan[a,c], Eko H. Purnomo[a,b], Mitsuhiro Wada[d]

[a]*Departement of Food Science and Technology, Faculty of Agricultural Engineering and Technology, Bogor Agricultural University. Kampus IPB Darmaga, Bogor-16680, West Java. Indonesia.*
[b]*Agriculture Technology Department, The Papua State University. Gunung Salju Street, Amban, Manokwari-98314, West Papua, Indonesia.*
[c]*Southeast Asian Food and Agricultural Science and Technology (SEAFAST) Center, Bogor Agricultural University. Kampus IPB Darmaga, PO Box 220. Bogor-16680, West Java. Indonesia.*
[d]*Graduate School of Biomedical Sciences, Nagasaki University. 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan.*

Abstract

Pandanus conoideus is an *endemic* plant of Papua, Indonesia, reported to be very rich in carotenoids. The purpose of this study was to develop method for the determination of carotenoids (α-cryptoxanthin, β-cryptoxanthin, α-carotene and β-carotene) in *P. conoideus* oil (PO) by high-performance liquid chromatography (HPLC). sing the proposed method in this research, carotenoids content of nine clones of PO were analyzed which ranged from 5.4-138.5 ng/mg for α-cryptoxanthin, 3.9-29.4 ng/mg for β-cryptoxanthin, 3.5-80.0 ng/mg for α-carotene, and 10.8-118.0 ng/mg for β-carotene. Our results showed that four carotenoids content was very small as compared to total carotenoids content (3027-19959 ng/mg). This suggests that those four carotenoids were not a major component of the PO carotenoids. Using the principal component analysis, nine clones of *P. conoideus* can be grouped based on the proximity of its carotenoid content into group A (*Monsor, Mbarugum, Himbiak, Monsrus* and *Memeri*), group B (*Menjib Rumbai*), and group C (*Edewewits, Hibcau* and *Hityom*).
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Keywords: Pandanus conoideus oil, α-cryptoxanthin, β-cryptoxanthin, α-carotene, β-carotene, HPLC.

* Corresponding author.
E-mail address: phariyadi@ipb.ac.id

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INTRODUCTION

Pandanus conoideus is one species of the genus *Pandanus* that grows naturally in almost all of the land of Papua-Indonesia and Papua-New Guinea. The plant produces a fruit that have pericarp with dark red in color, which is used as a food source by inhabitants of the island. They have been utilizing the *P. conoideus* fruit as food and source of oil and also for ritual and medicine [1]. In addition, the oil of fruit is released upon cooking and mashing to form an oleaginous pulp which is used as 'butter sauce' on starchy foods or cooked with vegetable and meat [2]. In Indonesia, the *P. conoideus* fruit is known by the name of *pandan seran* while the Papuan in general recognize as *buah merah* (red fruit) and *buah tawi* (*tawi* fruit). The people of Papua New Guinea also use the fruit as a food and it is better known as *Marita* [2].

Studies on the composition and potential health benefits of *P. conoideus* oil have been reported [3-7]. Extract oil of *P. conoideus* has been reported as safe for human consumption and inhibit tumor growth and kill cancer cells [4, 5], provide anti-inflammatory activity and increase immune system [6], and reduce blood sugar of diabetic rats (*Rattus norvegicus*) [7]. The potential health benefits of *P. conoideus* oil was believed to be associated with its high antioxidant activity [8], owing to high content of carotenoids (pro vitamin A) and tocopherol (vitamin E), as well as its unsaturated fatty acid [3, 5, 9, 10].

Dark red colors of *P. conoideus* fruit is closely associated with carotenoids compound having at least seven conjugated double bonds. The higher the number of double bonds results in a shift in the maximum absorbance to the longer wavelengths, making the hue of carotenoids becomes more red. Carotenoids can be divided into two major groups: carotenes and xanthophyls. Carotenes consist of only carbon and hydrogen atoms (e.g., α-, β- and γ-carotenes and lycopene), while xanthophyls are oxygenated derivatives of carotenes containing hydroxyl-, keto-, epoxy- and methoxy-groups.

P. conoideus fruit has been identified as a good source of carotenoids including α- and β-carotene and β-cryptoxanthin [5]. Some study have reported that β-carotene and β-cryptoxanthin content of *P. conoideus* oil ranging from, respectively, 123 to 2250 ng/mg and 5 to 90 ng/mg [3, 5, 10, 11]. Variation of reported value may be due to the difference of clone and origin of *P. conoideus* fruit, and/or methods of analysis used.

High-performance liquid chromatography (HPLC) combined with various detectors system have become the most common analytical method for determination of carotenoids, both qualitatively and quantitatively [12, 13]. The determination of carotenoids in *P. conoideus* oil by HPLC-UV/Vis using two columns (Handy OD5 column of 150 x 4.6 mm i.d. and Develosil Combi RP-5 column of 50 x 4.6 mm, i.d., 5 µm, Nomura Chemical), isocratically eluted with acetonitrile:methanol:ethyl acetate (68:23:9) for 60 minutes of running time was reported by Wada et al. [11]. Recently, Wardayani [14] succesfully developed an HPLC-UV/Vis method for separation and determination of *α*-carotene, *β*-carotene, *α*-cryptoxanthin and *β*-cryptoxanthin in astaxanthin supplement product by using a Develosil Combi RP-5 column (50 x 4.6 mm, i.d., 5 µm, Nomura Chemical) utilizing two pump and two mobile phase (gradient elution) for 35 minutes.

In this study, based on a method of Wardayani [14], an HPLC-UV/Vis method was developed and

proposed for the determination four carotenoids contents such as *α*-carotene, *β*-carotene, *α*-cryptoxanthin and *β*-cryptoxanthin in *P. conoideus* oil. The optimization was needed due to the differences in the matrix of the sample analyzed. Using the proposed method, determination of the carotenoids in nine (9) clones of *P. conoideus* oils from Papua (Indonesia) was performed.

MATERIALS AND METHODS

Chemicals and reagents

Standards used in this study were *α*-carotene and *α*-cryptoxanthin (Wako Pure Chemical Industries, Ltd. Osaka, Japan), *β*-cryptoxanthin (ChromaDex, Inc. CA, U.S.A.), and *β*-carotene (Kanto Chemical Co., Ltd. Tokyo, Japan). Other reagents such as potassium hydroxide (KOH), sodium chloride (NaCl), acetonitrile (HPLC grade), ethyl acetate (HPLC grade) and triethylamine (TEA) were obtained from Wako Pure Industries, Ltd. Tokyo, Japan. Ethanol (99.5%), hexane and methanol were purchased from Nacalai Tesque, Inc. Kyoto, Japan, and ascorbic acid from Sigma-Aldrich Corp., St. Louis, Mo., U.S.A. Water for solution and mobile phase was passed through a pure line WL21P (Yamato Scientific Co., Ltd., Tokyo, Japan) and other chemicals used were of extra pure grade.

Sample of *Pandanus conoideus*

Nine clones of the *P. conoideus* fruit originated from Papua (Indonesia) were used as samples for the study; namely *Menjib Rumbai, Edewewits, Memeri, Monsrus, Monsor* (cultivated in the orchard of The Papua State University, Manokwari, West Papua Province, Indonesia), *Mbarugum* (cultivated in District Koya, Jayapura, Papua Province, Indonesia), and *Hityom, Himbiak, Hibcau* (cultivated in District Minyambow, Manokwari, West Papua Province, Indonesia). The fresh fruit of *P. conoideus* was collected from the local farmer, which is harvested on period July-September 2012. Descriptions of ripe fruit are dark red fruit, grains already contains a full and hold tightly on the pith, fruit position in the tree with a slope of 180 °, and the open leaf sheaths and dried approximately ± 50% [15].

Oil extraction method

The *P. conoideus* oil was extracted by the method of Folch et al. [16]. Approximately 12 g of pericarp (pulp) of the fruit was macerated with 80 ml of solvent mixture of chloroform and methanol (2:1, v/v), stirred using a magnetic stirrer, at room temperature for 1 hour. The resulted solution was filtered using a vacuum pump, added 16 ml 0.88% NaCl, and then separated by the separating flask. The oil extract obtained was then evaporated with a rotary evaporator at 40 °C, packaged in dark bottles, dried with nitrogen gas, and stored at -20 °C until analyzed.

Determination of carotenoids composition

Sample preparation for carotenoids analysis

Extraction carotenoids from *P. conoideus* oil were done using procedure of Wada et al. [11]. Twenty-mg of oil were weighted on dark vial, 10 mg of ascorbic acid was added and then homogenized with 750 µl of ethanol. To the homogenized solution 200 µl of 76% KOH was added, vortexed and was saturated with nitrogen gas. Samples were then let to stands at room temperature for 30 min, to allow for the saponification. Saponification was stopped

by adding 250 µl of NaCl (25 mg/ml). The carotenoid was extracted with 750 µl of hexane:ethyl acetate (9:1, v/v) for 4 times, then combine the organic layer and evaporated to dryness at 40 °C. For analysis with HPLC, sample was dissolved in 400 µl of methanol, sonicated and passed through a membrane filter of 0.45 µm.

Chromatography

Chromatographic analysis were carried out using an HPLC system consisted of two Shimadzu LC-10AT*vp* chromatographic pumps (Kyoto), a Develosil Combi RP-5 C_{30}-column (50 x 4.6 mm, i.d., 5 µm, Nomura Chemical, Tokyo), and a Shimadzu SPD-10 AV UV-VIS detector (Shimadzu), and a 7125 injector with a 20 µl sample loop (Rheodyne, CA, U.S.A.).

Mobile system consisting of (A) a mixture of acetonitrile and water (80:20,v/v) containing 0.05% TEA and (B) a mixture of acetonitrile, methanol and ethyl acetate (68:5:27, v/v/v) containing 0.05% TEA. The gradient was programmed as follows: 0-4 min, 1-10% B; 4-25 min, 50-80% B; 25-35 min, 100% B; and 35-45 min, 1% B. The separated carotenoids were detected and measured at 450 nm. The total chromatographic run time was 45 min at flow rate of 1 ml/min.

Preparation of stock solution and calibration standards

Stock standard solutions of *α*-carotene, *β*-carotene, *α*-cryptoxanthin and *β*-cryptoxanthin (10 µg/ml) were diluted in ethanol. Standard curves were prepared by pipetting each standard 1.25, 2.5, 5, 10, 20, 40, 80 and 160 ng/mg and spiking them into oil. Calibration curves were calculated by linear regression analysis of the peak area versus the concentration of the nominal standard for each compound using Excel® 2007 (Microsoft Corp., Redmon, Wash, U.S.A). All calibration curves were required to have a correlation coefficient of at least 0.992.

Validation procedures

A chromatographic validation run included a set of calibration samples assayed in triplicate. Method of validation was performed in accordance with the procedures of Wada et al. [11]. The intra-day assay precision and accuracy were assessed using oil spiked with 5 and 40 ng/mg oil for the each carotenoid. Analyses of five replicate measurements for the each concentration were performed. The accuracies of the method were verified by comparing the concentration measured for *α*-cryptoxanthin, *β*-cryptoxanthin, *α*-carotene and *β*-carotene in oil with the actual added concentration.

Determination of total carotenoids content

Total carotenoids content (TCC) of *P. conoideus* oil was determined according to the method of PORIM [16] and Knockaert et al. [17] with slight modifications. Two milligrams of each sample was dissolved in hexane with 0.1% butylated hydroxytoluene (BHT) added. The absorbance of the sample solution was measured spectrophotometrically at wavelength of 446 nm and 470 nm, using hexane + 0.1% BHT as a blank. The analysis was conducted in duplicate. Total carotenoids were calculated as follows:

$$\text{Carotenoid concentration}\left(\frac{\text{ng}}{\text{mg}}\right)=\frac{\text{A x volume (ml) x }10^4}{\text{E}_{1\text{ cm}}^{1\%}\text{ x sample weight (g)}}$$

where: A is the absorbance value at λ max (446 nm and 470 nm), volume is the total volume of sample solutions,

$E^{1\%}_{1\ cm}$ is the extinction coefficient = 2560 for β-carotene in hexane [18].

Data analysis

The XLSTAT® Version 2013.6.01 was used for principle component analysis (PCA).

RESULTS AND DISCUSSION

Chromatography

The described method is suitable for the simultaneous determination of four carotenoids, namely *α*-cryptoxanthin, *β*-cryptoxanthin *α*-carotene, and *β*-carotene in *P. conoideus* oil. Separation between peaks of the individual carotenoids was satisfactory and no interferences from the *P. conoideus* oil matrix was observed (Fig. 1), and was confirmed by co-chromatography of sample spiked with carotenoids standards (Fig. 2).

These results showed that the analysis time of *α*-cryptoxanthin, *β*-cryptoxanthin, *α*-carotene and *β*-carotene analyzed by our methods (with average retention time of 21, 22, 32, and 33 minutes, respectively) were shorter as compared to that of reported by previous report of Wada et al. [11]. Using the HPLC-UV/Vis composing of two columns, Wada et al. [11] found that retention time of *α*-cryptoxanthin, *β*-cryptoxanthin, *α*-carotene and *β*-carotene in *P. conoideus* oil were 18, 20, 53 and 60 min, respectively. Consequently, our current method is more effective to analys *α*-cryptoxanthin, *β*-cryptoxanthin, *α*-carotene and *β*-carotene, with respect to the number of columns used and HPLC running time.

Method validation

Parameters of calibration curve for proposed method were summarized in Table 1. The calibration curves of *P. conoideus* oil that spiked with *α*-cryptoxanthin, *β*-cryptoxanthin, *α*-carotene, and *β*-carotene were obtained by plotting the peak area of each carotenoid versus the concentration in ng/mg oil. Calibration curve is obtained from five replications. The calibration curve was linear in range of 0.25-160 ng/mg for *α*-cryptoxanthin, 1.25-80 ng/mg for *β*-cryptoxanthin and *α*-carotene and 1.25-160 ng/mg for *β*-carotene with correlation coefficient of ($r \geq 0.992$) for all of the carotenoids.

Table 1. Calibration range of proposed method

Compound of standard	Concentration (ng/mg oil)	r^a	Equation[b]
α-cryptoxanthin	0.25-160	0.992	$y = 1.82\times10^5x + 2.45\times10^6$
β-cryptoxanthin	1.25-80	0.999	$y = 1.69\times10^5x + 1.88\times10^6$
α-carotene	1.25-80	0.995	$y = 1.85\times10^5x + 2.21\times10^6$
β-carotene	1.25-160	0.999	$y = 1.14\times0^5x + 6.93\times0^6$

[a] Correlation coefficient
[b] y= peak area of carotenoid, x = sample concentration, ng/mg oil

Intra-day precision of proposed method were evaluated by analyzing five replicates of *P. conoideus* oil spiked with known concentrations of *α*-cryptoxanthin, *β*-cryptoxanthin, *α*-carotene, and *β*-carotene (5 and 40 ng/mg oil). Intra-day precision indicated as relative standard deviation (RSDs), ranged from 6.3-10.9% for *α*-cryptoxanthin, 4.9-9.4% for *β*-cryptoxanthin, 6.1-10.1% for *α*-carotene, and 6.7-10.0% for *β*-carotene (Table 2). The accuracies of proposed method were 101%, 99-103%, 103-107%, and 101-103% for *α*-cryptoxanthin, *β*-cryptoxanthin, *α*-

carotene, and β-carotene, respectively (Table 2). The precision and the accuracy of the proposed method indicated were satisfactory.

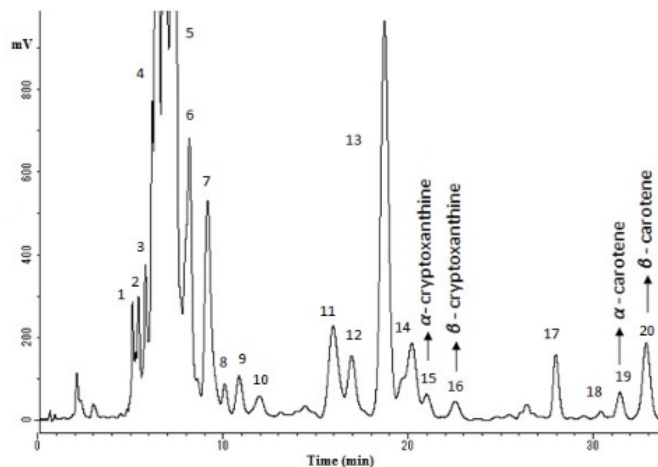

Figure 1. Chromatogram of four carotenoids of the *Pandanus conoideus* oil.

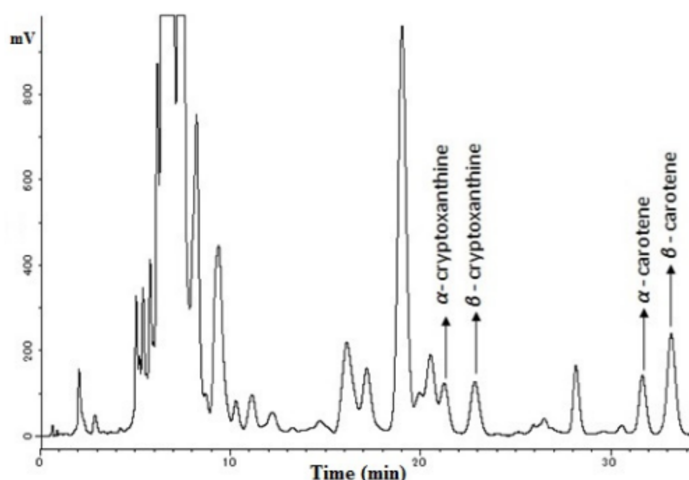

Figure 2. Chromatogram of four carotenoids of the *Pandanus conoideus* oil that spiked with standards (10 ng/mg oil of α-cryptoxanthin, β-cryptoxanthin, and α-carotene, and 40 ng/mg oil of β-carotene).

Content of carotenoid in *Pandanus conoideus* Oil

The proposed method was applied on *P. conoideus* oil for the evaluation of carotenoids content of 9 clones of *Pandanus conoideus* oils extracted using solvent (Folch method). The range of carotenoid content for α-cryptoxanthin, β-cryptoxanthin, α-carotene and β-carotene were 5.4-138.5 ng/mg, 3.9-29.4 ng/mg, 3.5-80.0 ng/mg and 10.8-118.0 ng/mg, respectively (Table 3). Data on carotenoid contents in the *P. conoideus* oil in literature is

limited, but this result corresponded well with data of Wada et al. [11], as well as Surono et al. [5]. The carotenoid content in some commercial *P. conoideus* oil from different brands and/or different production lots are about of 6-27 ng/mg α-cryptoxanthin, 14-90 ng/mg β-cryptoxanthin, 2-9 ng/mg α-carotene and 15-63 ng/mg β-carotene [11].While Surono et al. [5] reported *P. conoideus* oil extracted by dry rendering contain β-cryptoxanthin of 14.6 ng/mg, α-carotene of 0.13 ng/mg, and β-carotene of 19.8 ng/mg.

Table 2. Intra-day assay precision and accuracy for carotenoids spikes in *Pandanus conoideus* oil.

Compound	Added concentration (ng/mg oil)	Measured concentration, Mean ± S.D. (ng/mg oil)	Accuracy, Mean ± S.D. (%)[b]	Precision, RSDs (%)[a] Intra-day
α-cryptoxanthin	0	(10.6 ± 1.1)		
	5	15.5 ± 0.4	101 ± 11.0	10.9
	40	50.8 ± 2.5	101 ± 6.3	6.3
β-cryptoxanthin	0	(10.4 ± 0.8)		
	5	15.4 ± 0.4	103 ± 9.6	9.4
	40	50.1 ± 1.6	99 ± 4.8	4.9
α-carotene	0	(11.6 ± 0.6)		
	5	16.9 ± 0.5	107 ± 10.8	10.1
	40	52.9 ± 2.5	103 ± 6.3	6.1
β-carotene	0	(54.6 ± 2.5)		
	5	59.5 ± 0.4	101 ± 10.2	10.0
	40	95.6 ± 2.8	103 ± 6.9	6.7

[a]Relative standard deviation (n=5)
[b]Accuracy % = {(measured conc.- Original conc.)/Added conc.} x 100 (n=5)

Table 3. The results of the analysis carotenoid content of 9 clones *Pandanus conoideus* oil.

Clone of *Pandanus conoideus* oil	Content of carotenoid, Mean ± S.D. (ng/mg oil)[a]			
	α-cryptoxanthin	β-cryptoxanthin	α-carotene	β-carotene
Edewewits	11.4 ± 0.8	4.3 ±0.8	13.1 ± 1.7	24.6 ± 2.6
Memeri	10.38 ± 1.2	9.8 ±0.9	10.5 ± 1.6	55.8 ± 2.7
Monsrus	16.3 ± 0.4	16.4 ± 0.9	10.2 ± 1.0	50.3 ± 1.3
Monsor	36.0 ± 0.6	29.4 ± 0.1	19.8 ± 2.9	112.9 ± 0.6
Hibcau	1.6 ± 0.04	3.9 ±0.4	1.9 ± 0.2	10.8 ± 0.9
Himbiak	7.3 ± 0.6	19.8 ± 1.2	5.1 ± 0.6	50.7 ± 3.1
Hityom	5.4 ± 0.2	10.7 ± 0.3	3.5 ± 0.1	24.2 ± 0.4
Mbarugum	6.1 ± 0.4	15.1 ± 0.5	9.2 ± 1.0	118.0 ± 4.0
Menjib Rumbai	138.5 ± 2.5	15.7 ± 1.4	80.0 ± 1.3	66.9 ± 0.9

[a](n=3)

These differences of reported value could be influenced by cultivar/clone of fruit, the analytical method as well as the preparation method for oil extraction. Extraction of *P. conoideus* oil by dry extraction method used heat and high pressure treatment that can cause degradation of carotene. According to Knockaert et al. [17], with conjugated system of double bonds makes carotene also very susceptible to isomerisation and oxidation by heat as well as high pressure and mechanical processing like mixing or homogenizing during processing. The cis-isomers of β-carotene have a decreased provitamin A activity and an altered antioxidant activity.

Several studies on separation of carotenoid using HPLC have been reported. Inbaraj et al. [20] was developed a gradient solvent system of methanol-acetonitrile-water (84:14:2, v/v/v) and methylene chloride (100%) to resolve a range of carotenoids in chlorella tablet. They reported that the retention time of lutein and zeaxanthin were 33.49 and 33.02 min, while β-cryptoxanthin, α-carotene and β-carotene were 40.20, 45.19, and 47.29 min,

respectively. Furthermore, Liu et al. [19] reported that the carotenoids in human plasma could be separated by chromatographic method using isocratic elution of a mixture of acetonitrile and methanol (65:35, v/v) containing 0.065% of triethylamine at a flow rate of 1.5 ml/min, used C_{18} column and quantified at 450 nm. In this condition, the retention time of carotenoids lutein, zeaxanthin, and canthaxanthin were 4.2, 4.4, and 5.4 min, respectively; while β-cryptoxanthin, echinenone (IS3), lycopene, α-carotene, and β-carotene were 9.9, 11.2, 15.2, 22.4, and 24.4 min, respectively. Results of Inbaraj et al. [20] and Liu et al. [19] research on separation of carotenoids indicates that carotenoid components in *P. conoideus* oils (Fig. 1) were xanthophyls such as lutein and zeaxanthin that more polar and have structure similar to α- and β-carotenes, but differing in possessing hydroxyl groups on the rings.

Furthermore, there were more than 20 peaks appear on chromatogram of carotenoids of *P. conoideus* oil (Fig. 1). Total area of the 4 peaks identified as α-, β-cryptoxanthin and α-, β-carotene constitute only less than 10% of the total peak area (data not shown). Specifically, area of peak identified as β-carotene only contributes to about 5% of the total area of the peak recorded in the chromatogram. Consequently, our result suggests that β-carotene was not a major component of the carotenoids of *P. conoideus* oil.

In comparison with the others better known source of carotenoid, α- and β-carotene content of *P. conoideus* oil is lower than that of crude palm oil (CPO). CPO contains α-carotene of 181-253.4 ng/mg and β-carotene of 272-381 ng/mg [21]. However, *P. conoideus* oil has higher content of α- and β-carotene than that of carrot, sweet potato and corn. Carrot contains α-carotene of 25-49 ng/mg and β-carotene of 55-103 ng/mg [22]; while sweet potato-orange flesh (fresh weight) contain α-carotene of 3.8-9.0 ng/mg and β-carotene of 14.4-33.1 ng/mg [13]; and in white, yellow, red, blue and high carotenoid corn contain β-cryptoxanthin of 0.127-0.23 ng/mg and β-carotene 0.049-0.46 ng/mg [23].

Total carotenoids content (TCC)

The TCC of different clones of the *P. conoideus* oil were in the range of 3027-19959 ng/mg as showed in Table 4. These data indicated that the carotenoid was a pigment responsible for the yellow and red colour of *P. conoideus* fruit. Generally, clone with red fruit color have higher TCC than that with yellow color. Betwen red fruit color of the *P. conoideus* clones, clones having TCC more than 14000 ng/mg were *Monsor*, *Memeri*, *Himbiak*, *Monsrus*, and *Mbarugum*, while *Hityom*, *Hibcau* and *Edewewits* have TCC of 9000 to12000 ng/mg. The results are in agreement with those described by Andarwulan et al. [3] who reported that the TCC of *P. conoideus* oil from Jayapura (Papua) was in the range of 10022-21431 ng/mg.

The TCC of the *P. conoideus* oil in this study was much higher than that in the *P. conoideus* fruit from 16 accessions were ranged from 333 to 3309 ng/mg [24]. This is due to the fact that carotenoids are extremely lipophylic [25]. In addition, carotenoinds are located in cellular membranes or in lipophilic compartments, and in some plants hydroxylated carotenoids are esterified with various fatty acids, which make them even more lipophilic [24].

In comparison with better known source of carotenoids, such as palm oil, carrot, sweet potato and some vegetables, the TCC of *P. conoideus* oil was the highest. Yap et al. [25] reported that CPO from different palm

species have total carotenoid content of about 700-800 ng/mg, and 1430 ng/mg in hybrids of *Elaeis Oleifera* and *E. guineensis*, and 2324 ng/mg in hybrids of *E. Oleifera* and *E. guineensis*. Meanwhile, TCC of sweet potato (*Ipomoea batatas* L.) of yellow-fleshed cultivars were ranged from 0.13 to 0.39 ng/mg (dry base) and of orange-fleshed cultivars were from 1.35 to 3.99 ng/mg (dry base) [26]. Furthermore, TCC of carrot was 1283-1474 ng/mg [17], yellow pumpkin (*Cucurbita maxima*) was 21.2 ng/mg, green chillies (*Capsicum annuum*) was 24.10 ng/mg, green beans (*Phaseolus coccineus*) was 16.5 ng/mg, tomato (*Lycopersicum esculentus*) was 31 ng/mg and red chilli (*Capsicum annum*) was 1130 ng/mg [27]. These results show that *P. conoideus* might be used as a food ingredient to develop value-added foods rich in carotenoids.

Table 4. Total carotenoids content (TCC) of 9 clones *Pandanus conoideus* oil.

Clones of *Pandanus conoideus* oil	Color of fruit	Total carotenoids content, Mean ± S.D. (ng/mg oil)[c]
Edewewits[a]	Red	9409 ± 83
Memeri[a]	Red	17179 ± 937
Monsrus[a]	Red	15485 ± 607
Monsor[a]	Red	19959 ± 1206
Hibcau[a]	Red	10338 ± 85
Himbiak[a]	Red	15518 ± 649
Hityom[a]	Red	11494 ± 292
Mbarugum[a]	Red	14666 ± 16
Menjib Rumbai[b]	Yellow	3027 ± 136

[a]Wave Length 446 nm
[b]Wave Length 470 nm
[c](n=3)

Principle Component Analysis (PCA) of *Pandanus conoideus* samples

In this study, every clones of the *P. conoideus* oil have some differents composition of the four carotenoids and also their TCC as showed in Table 3 and Table 4. The PCA was applied to investigate the relationship between 9 clones of *P. conoideus* by their components of carotenoid (α-cryptoxanthin, β-cryptoxanthin, α-carotene, β-carotene and total carotenoid).

Fig. 3 shows the results of PCA of nine clones of *P. conoideus* which can be classified into 3 groups based on the proximity of its carotenoid content. Group A (*Monsor*, *Mbarugum*, *Himbiak*, *Monsrus* and *Memeri*), which is characterized by a high amount of carotenoids, β-carotene and β-cryptoxanthin, located in quadrant III and IV, dominated by clones from the lowlands (from orchard of The Papua State University, Manokwari and Koya District, Jayapura), except *Himbiak* (from the highlands, District Minyambouw); and group B (*Menjib Rumbai*) that culitvated in orchard of The Papua State University, Manokwari, the yellow clone with low levels of total carotenoids and higher levels of α-carotene and α-cryptoxanthin is in quadrant I. Group C (*Edewewits*, *Hibcau* and *Hityom*) which characterized by low levels of total carotenoids, that dominated by clones of the highlands (District Minyambou), except *Edewewits* (derived from lowlands, orchard of The Papua State University, Manokwari), located in quadrant III.

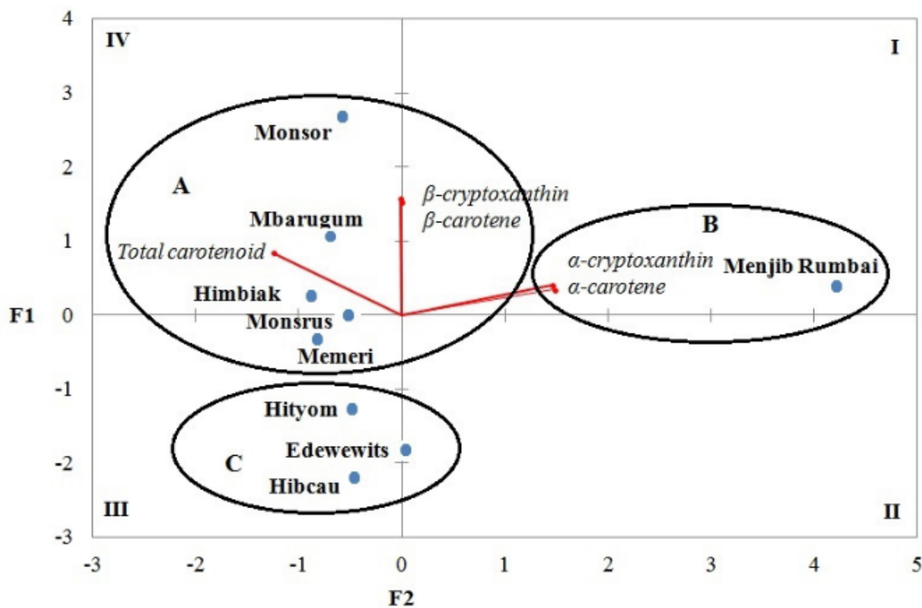

Figure 3. Distribution of 9 clones the *Pandanus conoideus* oil along principle component 1 (F1) and 2 (F2), using components of carotenoid.

As shows on Table 3, clones of *P. conoideus* which are located in quadrants III and IV (group A) contain β-carotene (more than 50 ng/mg) and β-cryptoxanthin (more than 15 ng/mg) higher than other quadrants. While group C (quadrant III), tends to be far from the centre of the biplot, because they have *β*-carotene lower than 50 ng/mg. Group B is located in quadrant I has the highest concentration of *α*-carotene (more than 50 ng/mg) and *α*-cryptoxanthin (more than 100 ng/mg). On the other hand, group A have a lower content of *α*-carotene and *α*-cryptoxanthin (Fig. 3), and group C have the lowest level of *α*-carotene and *α*-cryptoxanthin (*Memeri, Edewewits, Hibcau* and *Hityom*). The concentration of *α*-carotene of less than 10 ng/mg are found in *Hibcau, Hityom, Himbiak dan Mbarugum*, and of more than 10 ng/mg are *Menjib Rumbai, Monsrus, Memeri, Edewewits* and *Monsor*.

Based on the results of PCA, generally clones which were grown in the lowlands tend to produce carotenoids higher than the ones grown in the highland. According Cazzonelli [28], the levels of carotenoids is influenced by several factors including the maturity stage of development, environment, stress, or a combination there of. It was also explained that the carotenoids found in the leaves and stems of plants that play an important role in the process of photosynthesis and protect against photo-oxidative damage [29], which levels in the chloroplasts of leaves is influenced by light intensity [30]. Thus the lowlands areas with temperatures relatively warmer environment with higher light intensity will trigger the plants produce carotenoids.

PCA results in Fig. 3 also shows that some clones of *P. conoideus* in Group A, that characterized with the high levels of total carotenoids (predominantly from lowland clones), as well as there are clones of lowland joined

in Group C, with the identifier levels of total carotenoids lower (dominated by clones of the highland). Therefore *P. conoideus* carotenoid levels are not only influenced by the location of growth, but can also be influenced by clones or genetics. Me'ndez et al. [31] also reported that five cultivars of red pepper have characteristic pattern biosynthesis different carotenoids. Matejkova and Petříková [32] also reported the levels of carotenoids of six carrot cultivars grown under conditions of environmental temperature, rainfall and soil composition showed the same significant differences between cultivars. Nicolle et al. [33] also reported that the carotenoid content of carrots varies among cultivars, where the influence of genotype, as well as the variety, location and year, not only effect by the levels of total carotenoids but also the proportion of β-carotene α- and in carrots. It was also explained that the levels of carotenoids from plants can be improved by genetic engineering. Further Me'ndez et al. [31] stated that the carotenoid biosynthesis of the plant can be affected by many factors such as the expression of genes that regulate carotenogenesis, physiological and morphological characteristics of the clones, as well as growth.

CONCLUSIONS

The HPLC-UV/Vis method for determination of carotenoids in *P. conoideus* oil sample has been developed. This method was considered precise as indicated by the RSDs value which is less than 11% and the accuracy is more than 90%. The method was successfully applied to determine the carotenoids content of nine different *P. conoideus* oil. The carotenoids content of 9 clones of *P. conoideus* oil were ranged from 5.4-138.5 ng/mg for α-cryptoxanthin, 3.9-29.4 ng/mg for β-cryptoxanthin, 3.5-80.0 ng/mg for α-carotene, and 10.8-118.0 ng/mg for β-carotene. Total carotenoids content of *P. conoideus* oil ranged from 3027-19959 ng/mg and β-carotene was not a major component of the carotenoids of *P. conoideus* oil. Clones of *P. conoideus* can be grouped based on the proximity of its carotenoid content using the PCA, which consist of group A with a higher total carotenoids (*Monsor, Mbarugum, Himbiak, Monsrus, and Memeri*); group B with a higher α-crytoxanthin and α-carotene (*Menjib Rumbai*); and group C with a lower total carotenoids (*Edewewits, Hibcau* and *Hityom*).

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