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Procedia Food Science 3 (2015) 231 – 243

Procedia Food Science

International Symposium on Food and Agro-biodiversity

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

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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*).

Peer-review under responsibility of the organizing committee of Indonesian Food Technologist Community

Keywords: Pandanus conoideus oil, α-cryptoxanthin, β-cryptoxanthin, α-carotene, β-carotene, HPLC.

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Peer-review under responsibility of the organizing committee of Indonesian Food Technologist Community
doi:10.1016/j.profoo.2015.01.026

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