Carotenoid content of five accessions red fruit (*Pandanus conoideus* Lam.) oil

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Abstract. Red fruit (*Pandanus conoideus* Lam.) is a plant native to Papua, Indonesia. The extracted oil of red fruit has been known to possess potential medical properties, including anti-inflammatory, anti-tumor, and anti-diabetic. The natural antioxidant content such as carotenoids, α-tocopherol, and unsaturated fatty acids are considered to contribute to its bioactivity. In this study, five clones of red fruit, namely Maler, Bergum, Wesi, Uaghelu, and Keren, from different regions in Papua, were collected. Carotenoid content of the red fruit oil of the five clones, in particular β-carotene and β-cryptoxanthin, was determined by using HPLC analysis. The β-carotene content ranged from 193.9 to 1003.8 µg/ml, while β-cryptoxanthin content ranged from 3.3 to 48.9 µg/ml. Bergum oil has the highest content of both β-carotene and β-cryptoxanthin, thus it is suggested for cultivation.

1. Introduction

Red fruit (*Pandanus conoideus* Lam.) is a plant native to Papua, Indonesia. The fruit has a red color and has been used for food by the local communities. They squeeze the fruit pericarp to yield pulp and cook it as a sauce. Red fruit has become popular since the red fruit oil (RFO) is known to possess potential medical properties. Several studies showed that RFO could inhibit tumors and kill cancer cells [1, 2]. Besides, it could prevent preeclampsia symptoms in animal models [3], reduce LDL levels in white rat blood [4], have immunostimulant effect, and might be used as an adjuvant for HIV therapy [5], as well as as an anti-inflammatory.

Many health-related bioactivities are believed because of their high antioxidant content. RFO is rich in carotenoids, including α-carotene, β-carotene, and β-cryptoxanthin. β-carotene is known as an antioxidant, while β-cryptoxanthin can prevent lung cancer and emphysema [6, 7] as well as it has a beneficial effect against osteoporosis [8]. RFO is also rich in unsaturated fatty acids. The major constituent of the fatty acid is oleic acid, followed by linoleic acid [9]. The combination of oil and carotenoid present in RFO is beneficial because carotenoid is absorbed in the gastrointestinal tract in the form of mixed-micelles [10]. Therefore, the bioavailability of carotene increased by dietary fats and oils [11].

In Papua, there are many accessions of red fruit. According to Murtiningrum et al. [12], at least 85 accessions are distributed in Papua and West Papua. However, only 16 out of 85 accessions are potential to be developed because they are already cultivated, productive, resistant to disease, and rich in oil. In this study, five accessions of red fruit in Papua were collected. The fruits are known to have a different level of oil content. As mentioned previously, the oil content and carotenoid content are two important parameters for RFO quality. Therefore, we determined the level of β-carotene and β-
cryptoxanthin of the five accessions of RFO. The rich accession in the carotenoids can be suggested further for cultivation.

2. Materials and methods

2.1. Chemicals and reagents
Standard β-carotene and β-cryptoxanthin were purchased from Sigma. All solvent used in this study was pro analysis and HPLC grade.

2.2. Sample of Pandanus conoideus
Five accessions of red fruit were collected in Kurulu and Libarek District, Jayawijaya, Papua. Those accessions were Bergum, Wesi, Uaghelu, Maler, and Kenen.

2.3. Oil extraction
The pericarp of red fruit was hand-squeezed with the addition of a small amount of water to obtain red fruit pulp. The mixture of pulp and water was then centrifuged at 5000 rpm for 10 minutes, and the oil layer was pipetted into the dark vial. The oil was kept in freezer -20°C until analysis.

2.4. Sample preparation for carotenoids analysis extraction
Sample extraction was referred to Bhatnagar-Panwar et al. (2013) [13]. One milliliter of red fruit oil was diluted with 8 ml ethanol, which contained 0.5% butyl hydroxytoluene (BHT) and was warmed in water bath 37°C. After the addition of 2 ml hexane-acetone (2:1), the mixture was kept in the dark at room temperature for 10 minutes.

To allow saponification, an equal volume of methanol-KOH (15%), which contained 0.5% BHT, was added to the mixture. It was then incubated in a circulated water bath at 80°C for 15 minutes, followed by ice incubation for 5-10 minutes.

The carotenoid was extracted two times with the addition of 4 ml aquadest and 7.5 ml petroleum ether: diethyl ether (2:1), which contained 0.5% BHT. The organic layer was pooled together, and the solvent was removed by using a vacuum rotary evaporator. The sample was then dissolved in 10 ml of methanol: MTBE (6:4) and passed through a membrane filter of 0.45 μm for HPLC analysis.

2.5. HPLC analysis
The HPLC analysis was carried out using an HPLC system. It consisted of two Shimadzu LC-20 AB chromatographic pumps, a Discovery ® HS C18 column (150 x 4.6 mm i.d, 5 μm, Supelco), a CTO-20AC column oven (Shimadzu), a SIL-20AC autosampler (Shimadzu) and a Shimadzu SPD-20 AV UV-VIS detector (Shimadzu). The mobile system consisted of acetonitrile: water (9:1, v/v) with 0.25% of triethylamine (solvent A), and ethyl acetate with 0.25% of triethylamine (solvent B). The gradient was programmed as follows: 0-2 min, 20-50% B; 2-12 min, 50-75% B; 12-15 min, 75-20% B; and 15-25 min, 20% B. The carotenoids were detected and measured at 450 nm. The total run time was 25 minutes at the flow rate of 0.8 ml/min.

3. Results and discussion
Five accessions of red fruit, namely Bergum, Maler, Wesi, Uaghelu, and Kenen, were collected from Papua. Bergum, Maler, and Wesi are known to contain a high amount of oil, while Kenen and Wesi are less. Therefore, they are less popular in the local community. The five accessions can be distinguished from their physical characters. Among those five accessions, Kenen tree is the smallest with 3 m in height and 30 cm in diameter (Table 1). The size of the fruit is also smaller than Maler and Bergum, but it is similar to Uaghelu (Table 2).

β-carotene and β-cryptoxanthin content of RFO from five accessions were determined by the HPLC method. Validation of β-carotene resulted in LOD and LOQ value, which was 3.19 µg/ml and 9.68 µg/ml, respectively. Precision from six times injection gave RSD value of 1.83 %, while accuracy was within the range of 93 – 105 %. Validation of β-cryptoxanthin resulted in an LOD value of 0.032
µg/ml and LOQ 0.098 µg/ml. Precision from six times injection gave RSD value of 0.7%, and the accuracy was 97 – 109 %.

### Table 1. Plant physical characters of five red fruit accessions.

| Accession | Height (cm) | Stem circumvent (cm) | Branches | Leave length (cm) | Leave width (cm) |
|-----------|-------------|----------------------|----------|-------------------|-----------------|
| Maler     | 703         | 39                   | 9.3      | 221.2             | 11.2            |
| Wesi      | 875         | 49                   | 13.5     | 242.4             | 11.9            |
| Bergum    | 550         | 40.7                 | 5        | 236.3             | 11.4            |
| Kenen     | 300         | 30                   | 7        | 255.3             | 9.7             |
| Uaghelu   | 550         | 41                   | 7        | 248.8             | 10.5            |

### Table 2. Fruit physical characters of five red fruit accessions.

| Accession | Length (cm) | Diameter (cm) | Weight (kg) |
|-----------|-------------|---------------|-------------|
| Maler     | 85.33       | 13.00         | 9.65        |
| Wesi      | 67.00       | 11.50         | 5.45        |
| Bergum    | 83.00       | 13.70         | 8.63        |
| Kenen     | 58.00       | 10.82         | 3.724       |
| Uaghelu   | 54.00       | 10.70         | 3.510       |

β-carotene and β-cryptoxanthin content of the five accessions of red fruit oil respectively ranged from 193.9 to 1003.8 µg/ml and 3.3 to 48.9 µg/ml (Table 3). Among the five accessions, Bergum had the highest content of β-carotene. A similar fashion was also observed in β-cryptoxanthin content, in which the highest level was found in Bergum.

### Table 3. β-carotene and β-cryptoxanthin content in five accessions of red fruit.

| Accession | β-carotene (µg/ml) | β-cryptoxanthin (µg/ml) |
|-----------|--------------------|-------------------------|
| Bergum    | 1003.8             | 48.9                    |
| Uaghelu   | 759.8              | 7.8                     |
| Wesi      | 715.2              | 6.1                     |
| Maler     | 505.3              | 7.4                     |
| Kenen     | 193.9              | 3.3                     |

The results showed that the β-carotene and β-cryptoxanthin content was higher than other previously reported [14]. Sarungallo et al. (2015) [14] have determined β-carotene and β-cryptoxanthin content of RFO from nine accessions, namely Monsor, Mbarugum, Himbiak, Monsrus, Memeri, Menjib Rumbai, Edewewits, Hibcau, and Hityam. The β-carotene content was 10.8 – 118.0 ng/mg, while β-cryptoxanthin content was 3.9 – 29.4 ng/mg. The different accessions and different oil extraction methods used in the study might have resulted in the different content of these carotenoids.

In comparison to other sources of carotenoids, RFO has a higher content of β-carotene compared to crude palm oil (CPO), carrot, and tomato. CPO contains β-carotene of 370 µg/g [15], while carrot and tomato contain β-carotene of 112.1 µg/g and 16.1 µg/g, respectively [16]. The RFO also has a higher content of β-cryptoxanthin compared to β-cryptoxanthin, which is known as rich sources such as tangerine and papaya. Tangerine contains β-cryptoxanthin of 4.28 µg/g, and papaya contains β-cryptoxanthin of 4.04 µg/g [17].

### 4. Conclusion

Among five accessions of red fruit, Bergum oil contained a high level of β-carotene as well as β-cryptoxanthin. The β-carotene content was 1003.8 µg/ml, while β-cryptoxanthin content was 48.9 µg/ml. Moreover, the oil content of Bergum fruit was relatively high. Therefore, among the five accessions of red fruit studied in this research, Bergum could be suggested for cultivation
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5. References
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