Jurnal Teknologi

Full Paper

# DETERMINATION OF CHEMICAL PROPERTIES, COMPOSITION OF FATTY ACID, CAROTENOIDS AND TOCOPHEROLS OF DEGUMMED AND NEUTRALIZED RED FRUIT (*Pandanus conoideus*) OIL

Zita Letviany Sarungallo, Budi Santoso*, Risma Uli Situngkir, Mathelda Kurniaty Roreng, Meike Meilan Lisangan

Department of Agricultural Technology, Faculty of Agricultural Technology, Papua University. Jl. Gunung Salju, Amban, Manokwari, West Papua, Indonesia-98314

**Article history**
Received
*1 April 2020*
Received in revised form
*15 October 2020*
Accepted
*21 October 2020*
Published online
*27 October 2020*

*Corresponding author
bd.santoso@unipa.ac.id

**Graphical abstract**

## Abstract

Refining of crude red fruit oil (CRFO) through the degumming and neutralization steps intended to produce oil free of impurities (non triglycerides) such as phospholipids, proteins, residues and carbohydrates, and also reducing the amount of free fatty acids (FFA). This study aims to determine the effect of red fruit oil purification through degumming and neutralization stages on chemical properties, fatty acid composition, carotenoid content and tocopherol of red fruit oil (RFO). The results showed that degumming of CRFO did not affect the decrease in water content, FFA levels, peroxide numbers, iodine values, carotenoids and tocopherols content; but decrease in levels of phosphorus, β-carotene and α-tocopherol. Neutralization of degummed-RFO (DRFO) did not affect the decrease in water content, iodine value, carotenoid, tocopherol and α-tocopherol; but the FFA levels, peroxide number, phosphorus and β-carotene levels decreased significantly. The fatty acid composition of RFO was dominated by unsaturated fatty acids (± 75%), which increases through degumming and neutralization stages. β-carotene is more sensitive than α-tocopherol during refining process of crude oil, but in general, this process can improve the RFO quality.

*Keywords*: Red fruit (*Pandanus conoideus*), refining oil quality, carotenoid, tocopherol, fatty acid compotition



## 1.0 INTRODUCTION

Red fruit (*Pandanus conoideus*) oil is the yield of extraction from red fruit grains, which were known as a source of natural antioxidants [1] and proven safe for consumption [2]. The active component of red fruit oil consists of α-carotene, β-carotene, β-cryptoxanthin, and α-tocopherol, as well as unsaturated fatty acids, especially oleic, linoleic, linolenic and palmitoleic acids [3, 4]. However, the results of red fruit extraction were crude oil containing non-triglyceride components (phospholipids or protein-fat complexes and sticky carbohydrates such as gum and mucus, pigments, heavy metals, and free fatty acids) which affect the taste and oil storage period. Therefore, a purification process is needed to improve the quality of the oil. According to Greyt and Kellens (2005), refining was intended to remove unwanted components by minimizing the possible negative effects and loss of the desired component in oil [5].

Refining of crude oils in general includes degumming, neutralization, bleaching and deodorization. Degumming removes phospholipids, proteins, and also mucilaginous gums. The alkaline refining takes off free fatty acids (FFA), phospholipids, metal ions, and chlorophyll; while bleaching reduces the amount of colored compounds such as chlorophyll and carotenoids, metal ions and oxidation products. In the final deodorization of volatile aroma compounds and free fatty acids, but also tocopherols, phytosterols, phenolic compounds and carotenoids are removed from the oil [6]. Usually, oil refining process is done to eliminate the unwanted minor components, which make oils not suitable for sale and consumption. Furthermore, the refining process is trying to reduce possible damage to the neutral oil as well as minimize refining loss [7]. Refining of red fruit oil is intended to produce oil with low levels of phospholipids and FFA so that it's stability increases, but still retains the active component content of carotenoids and tocopherols. Therefore, in this study the purification of crude red fruit oil was only done with 2 stages of purification, namely the degumming process and followed by neutralization.

Degumming process can be done in some methods are described by various researchers, such as, water degumming, acid degumming such as phosphoric, citric, oxalic or tartaric [8], super degumming, total degumming process [9], and enzymatic degumming [10]. However, the use of acid proved to be efficient in cost and many variations have been developed to improve the consistency of the results [8]. Some researchers reported that degumming process of crude red fruit oil using 0.2-2% phosphoric acid [11] and 0.2% citric acid [12] can reduce phosphorus levels. While the neutralization process of degummed oil with alkali (NaOH and KOH) is the most widely used in industry because it is cheaper and more effective in reducing FFA of crude oil [13]. Through the neutralization process, red fruit oil is expected to be used as edible oil with high levels of carotenoids and tocopherols, and can also be used as ingredients for the development of edible oil-based products or carotene concentrates that can be used as raw material for functional food products. Murtiningrum (2004) reported that neutralization of alkaline degummed red fruit oil could reduce FFA from 16.17% to 1.4% [11]; as well as Santoso *et al*. (2018) reported that the FFA levels of degummed RFO (1.15%) could be lowered to 0.31% by neutralization with alkaline solution [14], but its effect on fatty acid composition and active components has not been reported.

Purification of red fruit oil has never been applied by producers in Papua. The oil separation process is only carried out by precipitation (± 24 hours) and filtration or by centrifugation [15]. In those methods are only removes insoluble impurities in oil. The purpose of this study was to determine the effect of refining process of crude red fruit oil, through degumming and neutralization stage on the chemical quality, composition of fatty acids, carotenoid content and tocopherol red fruit oil.

## 2.0 METHODOLOGY

### Materials

The main raw material used in this study was red fruit oil extracted by wet rendering. The chemicals used were various analytical grades for analysis of free fatty acids (FFA), peroxide numbers, iodine numbers, total carotenoids, β-carotene, total tocopherol, and α-tocopherol, and fatty acid composition.

### Refining Process of Crude Red Fruit Oil

The refining process of crude red fruit oil was done with degumming stage and followed by neutralization. The degumming stage begans by mixing the crude red fruit oil with 2% citric acid for 10 minutes. Then it was washed with water (50°C) and precipitation (centrifuged). Furthermore, washing of the oil was repeated until the pH is neutral [16]. Degummed red fruit oil (DRFO) was stored in a dark glass bottle until to the neutralization stage.

The DRFO was neutralization using a wet method. The neutralization process begans with mixing red fruit oil as a result of degumming process with NaOH solution and stirred for a few minutes, then added with distilled water. Washing of oil was then carried out using distilled water with a ratio of oil and water 1:1, and repeated 7 times [14]. The neutralized red fruit oil (NRFO) was packaged in dark glass bottles and stored until analyzed.

### Analysis of Oil Chemical Quality

Analysis of the chemical quality of oil includes water content [17], free fatty acids (FFA) were determined by titration method [17], iodine value using Wijs method [18], and peroxide numbers using acetic acid-chloroform method [17].

### Analysis of Total Carotenoids Content

Total carotenoids content (TCC) of oil was determined according to the method of Knockaert *et al*. (2012) with slight modifications [19]. 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 470 nm, using hexane and 0.1% BHT as a blank. The analysis was conducted in triplicate. Total carotenoids were calculated using Equation 1.

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

where: A is the absorbance value at λ max (470 nm), volume is the total volume of sample solutions, $E^{1\%}_{1cm}$ is

the extinction coefficient = 2560 for β-carotene in hexane [20].

### Analysis of β-carotene

Sample preparation method was a modification of AOAC (2005) [18]. One gram sample was placed inside the test tube then homogenized with 10 ml KOH 5% in methanol. Gas nitrogen was blown into the tube for 30 seconds then the tube was immediately closed to prevent β-carotene oxidation. Saponification was done at 65°C for 30 minutes inside a waterbath. The extract were cooled and then 5 ml deionized water and 10 ml hexane were added. After centrifugation, the top layer were withdrawn. The extraction process was done three times and combine with the first extract, evaporated under nitrogen, and dissolve in 1 ml HPLC mobile phase (acetonitrile:isopropanol= 65:35). Twenty microliters of extract was injected into the HPLC column with 1 ml/min flow rate and wavelenght 450 nm. The β-carotene concentration was calculated using Equation 2.

$$\beta\ \text{carotene content}\left(\frac{\text{mg}}{\text{kg}}\right) = \beta\ \text{carotene standard (mg) x } \frac{\text{sample (ml)}}{\text{100 ml)}} \times \text{Dilution factor} \quad \text{....(2)}$$

### Analysis of Total Tocopherol

Total tocopherols were determined using the method of Wong *et al*. (1988) [21]. Approximately 0.01 g of oil was placed in a volumetric flask (10 mL), to which was added: 5 mL of toluene; 3.5 mL of 2,2 bipyridine (0.07% weight per volume (w/v) in 95% ethanol); and 0.5 mL FeCl3. 6H2O (0.2% w/v in 95% ethanol). The solution was added with 95% ethanol to 10 mL, and the absorbance was measured at a wavelength of 520 nm. A blank was made the same way without the sample. The total tocopherol concentration was calculated based on a standard curve of a-tocopherol (100-1,500 µg/mL in toluene). Total tocopherols were calculated using Equation 3.

$$\text{Total tocopherol}\left(\frac{\text{mg}}{\text{kg}}\right) = \frac{\text{A}}{\text{M x W}} \quad \text{..... (3)}$$

where: A is the absorbance value at λ max (520 nm); M is a gradient on the standard curve; and W is sample weight (g).

### Analysis of a-tocopherol

The a-tocopherol was analyzed by the method of AOAC (2005) [18]. A total of 0.20 g of oil was dissolved in 5 mL of methanol, sonicated and filtered through Millipore (0.45 µm) and 20 µL was injected into an HPLC LC-2040 (Shimadzu Corp.; Kyoto, Japan), equipped with a pump (Shimadzu LC-20 AD) an UV-Vis detector (Shimadzu SPD -20A), and a Combi Develosil column RP-5 (50 × 4.6 mm, internal diameter 5 µm; Nomura Chemicals; Tokyo, Japan). The mobile phase was methanol:water (95:5) with a flow rate of 1.0 mL.min-1. The absorbance was measured at a wavelength of 292 nm. The calculation was expressed with a standard calibration curve as a-tocopherol (58.0; 25.0; 15.0; 8.0; 1.6 and 0.1 µg/mL ).

### Analysis of Fatty Acid Composition

Analysis of the fatty acid (FA) composition of the oils was done by trans-esterification of triglycerides into fatty acid methyl esters (FAME) according to AOAC (2005) [18]. Aproximately 0.025 g of RFO was added to 1 mg internal standard solution (C17:0, Sigma Co.; St Louis, MI, USA) in 10 ml hexane, and then 1.5 ml 0.5 N NaOH in methanol was added, exhaled with N2 gas and heated for 5 min at 85 °C. After cooling at room temperature, 2 mL of 14% $BF_3$-methanol was added and heated at 85 °C for 30 min. After cooling, 1.5 mL hexane and 3 mL of saturated NaCl were added, mixed gently and the upper layer (FAME) was collected. The FAME (10 µL) was injected into a gas chromatograph (GC-2100 Series; Shimadzu Corp.; Kyoto, Japan) equipped with a flame ionization detector and a column (DB-23; 30 m × 0.25 mm and 0.25 µm thickness). The conditions for the analysis were: (a) injector at 250 °C; (b) oven at 120 °C to 230 °C for 6 to 25 min, at a rate of 3 °C.min-1; and (c) detector at 260 °C.

Individual peaks of FAME were identified by comparing their retention times with those of standards (FAME Mix C8– C22; Bellefonte, PA USA). Each individual FA composition was calculated using the peak areas of the FA species that appeared in the chromatogram (grams per 100 grams oil) of the total peak areas of all the FAs in the oil sample.

### Statistical Analysis

The discussion was based on one-way analysis of variance (ANOVA) followed by Duncan Multiple Region Test (DMRT) with the level of significance at $P<0.05$ for every treatment of refining oil. All statistical analyses were performed using the Statistical Analysis Software (SAS) Program 9.1.3.

## 3.0 RESULTS AND DISCUSSION

### Characteristics of the Chemical Quality of Refined Red Fruit Oil

Water content is very important in determining of oil quality because the excess water will trigger a hydrolysis reaction which can increase the levels of free fatty acids [22]. The range of moisture content of CRFO, DRFO and NDRFO in this study was 1.55-2.15%, while the maximum moisture content of the quality of cooking oil is 0.15% [23].

The data in Table 1 shows that the refining gives a significant difference (P<0.05) to the water content of red fruit oil. The water content of CRFO tends to be higher than the moisture content of DRFO and NDRFO. The high water content of CRFO can be caused by an oil extraction process that uses wet method, which the grains of red fruit were extracted using boiled water for a long time. In addition, washing process of the oil was repeated during the purification stage, so that it was suspected that there was still water left behind. Therefore, it was recommended that at the end of neutralization a vacuum drying is needed to reduce the water content of the oil. Pal *et al*. (2015) reported that oil vacuum heating after washing process (during neutralization step) at 77 °C below 75 mm Hg for 30 min was effective for removing moisture traces [24].

**Table 1** Chemical quality of crude red fruit oil (CRFO), degumming red fruit oil (DRFO) and neutralized red fruit oil (NDRFO)

| Chemical quality of oil | Red fruit oil | | |
|---|---|---|---|
| | CRFO | DRFO | NDRFO |
| Water content (%, wet basis) | 2.15±0.14[a] | 1.63±0.25[a] | 1.55±0.22[a] |
| Free fatty acid (%) | 1.08±0.01[a] | 1.15±0.01[a] | 0.28±0.03[b] |
| Peroxide value (meq/kg) | 0.48±0.07[a] | 0.45±0.04[a] | 0.26±0.01[b] |
| Iodine Value (g/100 g) | 80.9±0.1[a] | 82.4±0.9[a] | 81.0±0.1[a] |
| Phosphorus (µg/mL ) | 51.68±7.74[a] | 0.70±0.07[b] | 0.20±0.04[b] |

*Value followed by different letter within a row of each treatment indicate a significantly differences (P<0.05)

Free fatty acids (FFA) were important parameters for determining oil quality standards; the presence of FFA in oils may promote oxidation [25]. FFA levels in CRFO and DRFO were 1.08-1.15% (Table 1). The high levels of FFA in CRFO and DRFO can be caused by the hydrolysis reaction during the extraction process and the degumming process takes place which was influenced by temperature, air, long heating time and the container used in extracting red fruit (wet extraction method) [15]. In addition, the level of FFA was also influenced by the type of clones and the maturity of the red fruit [26]. Meanwhile, the main purpose of the degumming process is to remove impurity components without reducing the level of FFA in the oil [5]. Thus the degumming process does not significantly decrease (P<0.05) of FFA levels.

Data in Table 1 also shows that FFA levels of CRFO decreased significantly (0.28%). Thus, during the process of refining red fruit oil, the most of the FFA content was removed during neutralization formed soap due to alkaline treatment that improves oil quality, which meets the quality standards of CPO oil or for cooking oil. The maximum FFA level in CPO was 0.5% [27], while for cooking oil [28] was a maximum of 0.3%. Pal *et al*. (2015) also reported that FFA content of crude sunflower oil was found to be reduced from 1 to 0.24 (% oleic acid) after neutralization [24]. The decrease in FFA levels in the neutralization stage was caused by the reaction between FFA in oil and alkali which was used to neutralize oil. It was also added that the low FFA content of refined oil makes the oil edible and of higher quality. The odor of the refined oil was more agreeable than crude oil, which might be due to the low of its FFA content [24].

Peroxide value (PV) or peroxide number was the most frequent measurement of lipid oxidation. It is used to assess in what extent rancidity reactions have been occurred during storage, it could be used as an indication of the quality and stability of fats and oils [29]. The degumming stage did not affect the peroxide number of DRFO with a range of 0.45-0.48 meq/kg (Table 1). Meanwhile, the neutralization stage can significantly reduce the peroxide number (P<0.05). Nevertheless, the PV of the red fruit oil was lower than the Indonesia Standard (SNI standard) that is the maximum for cooking oil is 2 mg $O_2$/100 g [28]. Wang and Johnson (2001) reported that the degumming and neutralization stages of wheat germ oil does not affect its PV [30].

The iodine value represents the degree of unsaturation of fatty acids in oil. The content of unsaturated fatty acids was very easily oxidized and degraded so that it can reduce iodine value [25]. This study shows that the iodine value of the red fruit oil produced at each stage of oil purification ranged from 80.9 to 82.4 g/100 g which did not show any significant differences between treatments (P<0.05) (Table 1). This result was agreed with Sarungallo *et al*. (2015b) that the iodine value oil from 9 red fruit clones was about 79-85.5 g/100g [4]. This iodine value of red fruit oil tends to be higher than palm oil which was 54.85 g/100g [31], but lower than sesame oil which was 112 g/100g [32], but equivalent to peanut oil around 82-107 g/100g [33].

The phosphorus content was an impure component in crude vegetable oil, whose presence was in the form of a phospholipid and was undesirable because it causes problems in purification. In crude oil, phospholipids containing nonhydratable phospholipids during the degumming stage are water insoluble, expanding, form a gel or settle from the oil and are not separated by centrifuges. Besides that, the presence of phosphorus affects the stability of oil because it can react with metals and free fatty acids, thus increasing the oxidative process [34]. The data in Table 1 shows that the process of degumming and neutralization were significantly effective in reducing the levels of phosphorus of crude red fruit oil (51.68 µg/mL), which was decreased to 0.70 and 0.20 µg/mL respectively. Murtiningrum (2004) also reported that crude fruit oil containing phosphorus 87.9 mg/kg and decreased

after going through the degumming process to 2.8 mg/kg [11]. It was also explained that the level of phosphorus of red fruit oil was strongly influenced by its clones, with a high variation of between 38-374 µg/mL [4]. Besides that Szydłowska-Czerniak and Szlyk (2003) also reported that the phosphorus content of crude oil rape seed was 251 µg/mL to 2 µg/mL after purification [34]. Wang and Johnson (2001) also added that the stage of degumming and neutralization of wheat germ oil can significantly reduce phosphorus levels [30].

## Active Component of Red Fruit Oil

Carotenoids and tocopherols in red fruit are very important roles as active components for human health [2], carotenoids and tocopherols were the primary fat-soluble vitamins that have epidemiological evidence of benefiting human health [35], associated with a lower incidence of agerelated macular degeneration, cardiovascular diseases, cancer and cataract formation [36]. According to Hart and Scott (1995), carotenoids exist as plant pigments, responsible for red, yellow and orange colour [20], and also have health-promoting effects. β-Carotene, β-crytoxanthin, α-carotene, lycopene, and lutein were the main compounds that contribute to the total carotenoids present in fruits and vegetables. However, during refining processes, a large amount of micronutrients and antioxidants such as polyphenols, tocopherols, sterols, carotenoids are lost, which substantially reduces the nutritional value and quality of vegetable oils.

The data in Table 2 shows that the stages of degumming and neutralization tend to reduce total carotenoid levels but not significantly different (P> 0.5) between treatments. The decrease in the total carotenoid of red fruit oil during the purification process was thought to be the result of degradation, especially in the oil washing stage after the degumming and neutralization stages. Washing using water could cause an oil hydrolysis reaction and oil oxidation reactions thus increasing the rate of damage of the active component [15]. Santoso *et al*. (2018) also pointed out that the neutralized RFO contains a total of carotenoids which are almost similar to crude oil or degummed oil [14]. Leong and Oey (2012) further stated that it is important to bear in mind that carotenoids are highly unstable in nature; they are both photo- and thermolabile and tend to oxidise if they are protected from light and atmosphere, because they have a structure with a double bonded conjugated system that contains many reactive electrons and was easily oxidized [37].

Data in Table 2 also shows that the decrease in β-carotene levels was significantly affected by the oil refining process, although the total carotenoids were not significantly different. This shows that the derivatives of carotenoids were more sensitive to the purification stages applied. Further, explained that the processing process by using heating, homogenization and high pressure processes can cause isomerization and degradation of β-carotene in some food products [19].

Tocopherols were also the key bioactive constituent in the human diet, well-known for its potent chain-breaking antioxidant, anticancer and anti-inflammatory activities [38]. Table 2 shows that like the total carotenoids, the refining process did not significantly affect the total tocopherols of oil. However, the degumming stage can reduce α-tocopherol levels significantly. Wang and Johnson (2001) also reported that the total tocopherol content of wheat germ oil did not significantly change during degumming, neutralizing, and bleaching, although α-tocopherol was reduced about 14% after neutralizing [30]. Same trend also noted by Santoso *et al*. (2018) for this result [14].

The β-carotene in red fruit oil was the most sensitive to degumming and neutralization treatment compared to α-tocopherol (Table 2). Liu *et al.* (2015) also reported that α-tocopherol was the most effective to the antioxidation of β-carotene at the concentration of 0.10% under light exposure [39].

**Table 2** Total content of carotenoids, β-carotene, total tocopherol and α-tocopherol from crude red fruit oil (CRFO), degumming red fruit oil (DRFO), and neutralized red fruit oil (NDRFO)

| Active component | Red fruit oil | | |
|---|---|---|---|
| | CRFO | DRFO | NDRFO |
| β-carotene (µg/mL ) | 105±29$^{a}$ | 91.8±85$^{b}$ | 79.5±53$^{c}$ |
| Total Carotenoid (µg/mL) | 6,784±206$^{a}$ | 6,446±120$^{a}$ | 6,308±154$^{a}$ |
| α-tocopherol (µg/mL ) | 74.0±44$^{a}$ | 64.6±52$^{b}$ | 71.2±27$^{ab}$ |
| Total Tocopherol (µg/mL) | 1,765±57$^{a}$ | 1,711±28$^{a}$ | 1,700±111$^{a}$ |

*Value followed by different letter within a row of each treatment indicate a significantly differences (P<0.05)

## Fatty acids Composition of Red Fruit Oil

The composition of fatty acids of red fruit oil was determined by Gas Chromatography after the oil through the esterification reaction becomes the methyl ester form. Table 3 shows that the fatty acids identified in red fruit oil are lauric acid, myristic acid, palmitic acid, palmitoleic acid, oleic acid, and linoleic acid, but dominated by unsaturated fatty acids with a total of 70.3-75.07 g/100g consisting of oleic acid (C18: 1) 61.65-65.69 g/100g, palmitoleic acid (C16:1) 1.3-1.4 g/100g and linoleic acid (C18:2). Meanwhile, the saturated fatty acid content of red fruit oil ranges from 18.38-23.4 g/100g, which was

dominated by palmitic acid (C16:0) 14.9-20.8 g/100gr. Sarungallo *et al*. (2015b) also reported that the fatty acid composition of the 9 red fruit clones was dominated by unsaturated fatty acids (USFA) with a range of 60.48-72.12 g/100g oil and contained saturated fatty acids (SFA) ranging from 15 7-21.4 g/100g of oil [4]. It was also explained that differences in levels of fatty acids composition can be influenced by differences in red fruit clones [4], ripening stage of fruit [26] and also the extraction method used [15].

Refining of red fruit oil with degumming and neutralization steps does not affect the degree of saturation of oil (the amount of unsaturated fatty acids) which was indicated by not significantly of Iodin Number (Table 1). Aluyor *et al*. (2009) also reported that the refining process did not affect the composition of fatty acids in crude oil and the results of degumming of peanut oil [40]. However, the total saturated fatty acids of red fruit oil differed significantly between treatments (Table 3).

**Table 3** Fatty acids composition of crude red fruit oil (CRFO), degumming red fruit oil (DRFO), and neutralized red fruit oil (NDRFO)

| Fatty acids Composition (g/100g oil) | | | CRFO | DRFO | NDFRO |
|---|---|---|---|---|---|
| Saturated fatty acids | | C10 | 0,04±0,01 | 0,04±0,01 | 0,05±0,01 |
| | | C12 | 0,65±0,04$^{b}$ | 0,15±0,01$^{c}$ | 1,44±0,03$^{a}$ |
| | | C14 | 0,19±0,01 | 0,09±0,01 | 0,53±0,02 |
| | | C15 | 0,09±0,01 | 0,09±0,01 | 0,08±0,01 |
| | | C16 | 20,81±0,21$^{a}$ | 17,03±0,01$^{b}$ | 14,93±0,07$^{c}$ |
| | | C18 | 1,24±0,001$^{a}$ | 1,14±0,01$^{b}$ | 1,25±0,01$^{a}$ |
| | | C20 | 0,12±0,001 | 0,12±0,01 | 0,10±0,01 |
| | | **Total** | 23,4±0,17$^{a}$ | 18,65±0,22$^{b}$ | 18,38±0,08$^{b}$ |
| Unsaturated fatty acids | **MUFA** | C16:1 | 1,31±0,01$^{b}$ | 1,43±0,02$^{a}$ | 1,38±0,01$^{a}$ |
| | | C18:1*cis* | 61,65±0,53$^{c}$ | 65,69±0,49$^{a}$ | 62,81±0,20$^{b}$ |
| | | C20:1 | 0,35±0,001 | 0,37±0,01 | 0,35±0,01 |
| | | **Total** | 63,32±0,53$^{b}$ | 67,49±0,50$^{a}$ | 64,54±0,20$^{b}$ |
| | **PUFA** | C18:2 | 7,01±010$^{b}$ | 7,58±0,02$^{a}$ | 7,73±0,05$^{a}$ |
| | | C18:3 | - | - | - |
| | | **Total** | 7,01±010$^{b}$ | 7,58±0,02$^{a}$ | 7,73±0,05$^{a}$ |
| **Total** | | | 70.33±0.53$^{c}$ | 75.07±0.50$^{a}$ | 72.27±0.20$^{b}$ |
| Unknown | | | 0,13±0,01 | 0,14±0,01 | 0,14±0,01 |
| Total of fatty acids | | | 93,60±0,70$^{a}$ | 93,85±0,73$^{a}$ | 90,42±0,32$^{b}$ |

*Value followed by different letter within a row of each treatment indicate a significantly differences (P<0.05)

The refining process tends to increase significantly the total levels of fatty acids not saturated, such as palmitic acid (C16: 1), oleic acid (C18: 1); linoleic acid (C18: 2). Thus the refining process tends to reduce significantly the total levels of saturated fatty acids in red fruit oil. Ayoade *et al*., (2015) also reported that the sum of saturated fatty acids in crude canerium seed oil was higher than that of refined oil monounsaturated and polyunsaturated fatty acid levels were higher in refined oil than the crude oil [41]. The total sum of fatty acids detected in crude oil is lower than that of refined oil, while total undetected fatty acid is higher in crude oil than refined one.

According to Garrow *et al.* (2000), plasma cholesterol was raised by saturated fatty acids (SFA) and lowered by polyunsaturated fatty acids (PUFA) [42]. Therefore, the refining oil by degumming and neutralisation step improved the quality of red fruit oils especially with increase in unsaturated fatty acids. In addition, the total fatty acid content was unsaturated in red fruit oil (70-72 g/100g), which was produced in this study was higher than palm oil 51.4-58.4 g/100g [43], but equivalent to olive oil 64.92-86.73 g/100g [44].

## 4.0 CONCLUSION

Degumming of CRFO did not affect the decrease in water content, free fatty acid (FFA) levels, peroxide numbers, iodine value, carotenoid and tocopherol; but decreased levels of phosphorus, β-carotene and α-tocopherol. Neutralization of degummed-RFO (DRFO) did not affect the decrease in water content, iodine value, carotenoid, tocopherol and α-tocopherol; but FFA levels, peroxide, phosphorus and

β-carotene levels decreased significantly. The fatty acid composition of RFO was dominated by unsaturated fatty acids (± 75%), which increases through degumming and neutralization stages. β-carotene is more sensitive than a-tocopherol during refining process of crude oil, but in general, this process can improve the RFO quality.

## Acknowledgement

The authors would like to thanks to Ministry of Research and Technology - Higher Education, Republic of Indonesia forfunding through the National Strategic Research Grant in 2016, with contract number 059/SP2H/DRPM/II/2016. Thanks also to the "Overseas Seminar Aid Program", Directorate General of Strengthening Research and Development, Ministry of Research and Technology - Higher Education which has funded the presentation of this paper at "Annual Conference and Exhibition Functional Foods, Nutraceuticals, Natural Health Products and Dietary Supplements, October 14-17 2018" in Vancouver, Canada.

## References

[1] Rohman, A., Riyanto, S., Yuniarti, N., Saputra, W. R., Utami, R. and Mulatsih, W. 2010. Antioxidant Activity, Total Phenolic, Total Flavanoid of Extracts and Fractions of Red Fruit (*Pandanus conoideus* Lam). *International Food Research Journal.* 17: 97-106.

[2] Surono, I., Endaryanto, T. A. and Nishigaki, T. 2008. Indonesian Biodiversities, from Microbes to Herbal Plants as Potential Functional Foods. *Journal of the Faculty of Agriculture Shinshu University.* 44(1.2): 23-27.

[3] Sarungallo, Z. L., Hariyadi, P., Andarwulan, N. and Purnomo, E., H. 2015a. Analysis of *α*-cryptoxanthin, *β*-cryptoxanthin, *α*-carotene, and *β*-carotene of *Pandanus conoideus* Oil by High-performance Liquid Chromatography (HPLC). *Procedia Food Science.* 3: 231-243.
DOI: 10.1016/j.profoo.2015.01.026.

[4] Sarungallo, Z. L., Hariyadi, P., Andarwulan, N. and Purnomo, E. H. 2015b. Characterization of Chemical Properties, Lipid Profile, Total Phenol and Tocopherol Content of Oils Extracted from Nine Clones of Red Fruit (*Pandanus conoideus*). *Kasetsart Journal (Nature Science)*. 49: 237-250.

[5] Greyt, W. D. and Kellens, M. 2005. Deodorization. In Shahidi, F. (Ed.). Bailey's Industrial Oil and Fat Products. Sixth Edition, Six Volume Set. New Jersey: John Wiley & Sons, Inc.

[6] Lin, L. and Koseoglu, S. S. 2005. Membrane Processing of Fats and Oils. In Shahidi, F. (Ed.). Edible Oil and Fat Products: Processing Technologies. Bailey's Industrial Oil and Fat Products. 6th ed. Volume 5. New Jersey: John Wiley and Sons, Inc.

[7] Sabah, E. and Majdan, M. 2009. Removal of Phosphorus from Vegetable Oil by Acid-activated Sepiolite. *Journal of Food Engineering.* 91 (3): 423-427.
DOI: 10.1016/j.jfoodeng.2008.09.020.

[8] Anderson, D. 2005. A Primer on Oils Processing Technology. In Shahidi, F. (Ed.). Edible Oil and Fat Products: Processing Technologies. Bailey's Industrial Oil and Fat Products. 6th ed. Volume 5. New Jersey: John Wiley and Sons, Inc.

[9] Dijkstra, A. J. and Opstal, M. V. 1989. The Total Degumming Process (TOP). In Erickson, D. R. (Ed.). Proceedings of World Conference on Edible Fats and Oils Processing. Champaign: AOCS Press. 176-177.

[10] Jahani, M., Alizadeh, M., Pirozifard, M. and Qudsevali, A. 2008. Optimization of Enzymatic *Degumming* Process for Rice Bran Oil Using Response Surface Methodology. *LWT Food Science and Technology.* 41: 1892-1898.
DOI: 10.1016/j.lwt.2007.12.007.

[11] Murtiningrum. 2004. Red Fruit (*Pandanus conoideus* L.) Oil Extraction Using Wet Rendering Method and Purification by Membrane Filtration. (in Indonesian). Thesis. Program Pascasarjana. Bogor: Institut Pertanian Bogor.

[12] Murtiningrum, Sarungallo, Z. L. and Roreng, M. K. 2011. The Active Component of Crude Oil and Degumming Red Fruit Oil (*Pandanus conoideus*), which was Extracted Traditionally. (in Indonesian). *Prosiding Seminar Nasional Perhimpunan Ahli Pangan Indonesia (PATPI), 15-17 September 2011*. Manado, Indonesia: PATPI. 157-160.

[13] Bhosle, B. M. and Subramanian, R. 2005. New Approaches in Deacidification of Edible Oil (A Review). *Journal of Food Engineering.* 69: 481-494.
http://dx.doi.org/10.1016/j.jfoodeng.2004.09.003.

[14] Santoso, B., Sarungallo, Z. L., Situngkir, R. U., Roreng, M. K., Lisangan, M. M. and Murni, V. 2018. Chemical Quality and Active Component of Red Fruit (*Pandanus conoideus* L.) Oil which Neutralized Using an Alkaline Solution. (in Indonesian). *J. Agritechnology.* 1(2): 66-76.

[15] Sarungallo, Z. L., Hariyadi, P., Andarwulan, N., Purnomo, E. H. 2014. The Effect of Extraction Method on the Chemical Quality and Fatty Acid Composition of Red Fruit (*Pandanus conoideus*) oil. (in Indonesian). *Jurnal Teknologi Industri Pertanian.* 24(3): 209-217.

[16] Sarungallo, Z. L., Santoso, B., Lisangan, M. M., Paiki, S. N. P., Situngkir, R. U., and Asokawati, E. 2018. Kinetics of Quality Changing of Degummed Red Fruit (*Pandanus conoideus*) Oil During Storage. (in Indonesian). *Jurnal Aplikasi Teknologi Pangan.* 7 (4): 156-162. https://doi.org/10.17728/jatp.2947.

[17] AOCS (American Oil Chemists' Society). 2003. *Official Methods and Recommended Practices of the AOCS*. 5th ed. Champaign, Illinois: American Oil Chemists' Society.

[18] AOAC (Association of Analytical Chemists). 2005. *Official Methods of Analysis.* 16th ed. Method 931.04; method 970.39. Wahington, DC, USA: Association of Analytical Chemists.

[19] Knockaert, G., Lemmens, L., Van-Buggenhout, S., Hendrickx, M. and Van-Loey, A. 2012. Changes in β-carotene Bioaccessibility and Concentration during Processing of Carrot Puree. *Food Chemistry.* 133: 60-67.
https://doi.org/10.1016/j.foodchem.2011.12.066.

[20] Hart, D. J. and Scott, K. J. 1995. Development and Evaluation of an HPLC Method for the Analysis of Carotenoids in Foods, and the Measurement of the Carotenoid Content of Vegetables and Fruits Commonly Consumed in the UK. *Food Chemistry.* 54: 101-111.

[21] Wong, M. L., Timms, R. E. and Goh, E. M. 1988. Colorimetric Determination of Total Tocopherols in Palm Olein and Stearin. *Journal of the American Oil Chemists' Society.* 65: 258-61.

[22] Sarungallo, Z. L., Santoso, B., Tethool, E. F., Situngkir, R. U., Tupamahu, J. 2018. Kinetics of Quality Changing of Red Fruit (*Pandanus conoideus*) Oil during Storage. (in Indonesian). *Agritech.* 38(1): 64-70.
DOI:10.22146/ agritech.25216.

[23] SNI (Indonesian National Standard). 2012. Cooking Palm Oil. SNI 7709-2012. (in Indonesian). Jakarta: Badan Standarisasi Nasional Indonesia.

[24] Pal, U. S., Patra, R. K., Sahoo, N. R., Bakhara, C. K. and Panda, M. K. 2015. Effect of Refining on Quality and Composition of Sunflower Oil. *Journal of Food Science and Technology.* 52(7): 4613-4618.
DOI: 10.1007/s13197-014-1461-0.

[25] Scrimgeour, C. 2005. Chemistry of Fatty Acids. In: Shahidi F. (Ed.). Edible Oil and Fat Products: Chemistry, Properties, and Health Effects. Bailey's Industrial Oil and Fat Products. 6th ed. Volume 1. New Jersey: John Wiley and Sons, Inc.
[26] Murtiningrum, Sarungallo, Z. L., Santoso, B., Roreng, M. K. and Armiati. 2019. Chemical Properties, Carotenoid, Tocopherol and Fatty Acid Composition of Three Clones of Red Fruit (*Pandanus conoideus* Lam.) Oil of Different Ripening Stages. *International Food Research Journal.* 26(2): 649-655.
[27] SNI (Indonesian National Standard). 2006. Palm oil. SNI 01-2901-2006. (in Indonesian). Jakarta: Badan Standarisasi Nasional Indonesia.
[28] SNI (Standar Nasional Indonesia) 01-3741-2002. 2002. Cooking Oil. (in Indonesian). Jakarta: Badan Standarisasi Nasional Indonesia.
[29] Ekwu, F. C. and Nwagu, A. 2004. Effect of Processing On The Quality of Cashew Nut Oils. *Journal of Science and Agriculture, Food Technology and Environment*. 4: 105-110.
[30] Wang, T. and Johnson, L. A. 2001. Refining High-free Fatty Acid Wheat Germ Oil. *Journal of the American Oil Chemists' Society*. 78(1): 71-76.
[31] Li, R., Xia, Q., Tang, M., Zhao, S., Chen, W., Lei, X. and Bai, X. 2012. Chemical Composition of Chinese Palm Fruit and Chemical Properties of the Oil Extracts. *African Journal of Biotechnology*. 11(39): 9377-9382.
DOI: 10.5897/AJB11.3604.
[32] Chakraborty, D., Das, J., Das, P. K., Bhattacha, S. C. and Das, S. 2017. Evaluation of the Parameters Affecting the Extraction of Sesame Oil from Sesame (*Sesamumindicum*l.) Seed Using Soxhlet Apparatus. *International Food Research Journal.* 24(2): 691-695.
[33] Sanders, T. H. 2002. Groundnut (Peanut) Oil. In Gunstone, F. D. (Ed.). Vegetable Oils in Food Technology. Composition, Properties and Uses. Oxford, UK: Blackwell Publishing Ltd. 231-243.
[34] Szydłowska-Czerniak, A. and Szlyk, E. 2003. Spectrophotometric Determination of Total Phosphorus in Rape Seeds and Oils at Various Stages of Technological Process: Calculation of Phospholipids and Non-hydratable Phospholipids Contents in Rapeseed Oil. *Food Chemistry*. 81(4): 613-619.
DOI: 10.1016/S0308-8146(02)00562-9.
[35] Ju, J., Picinich, S. C., Yang, Z., Zhao, Y., Suh, N., Kong, A. N. and Yang, C. S. 2010. Cancer-preventive Activities of Tocopherols and Tocotrienols. *Carcinogenesis.* 31: 533-542.
Doi: 10.1093/carcin/bgp205.
[36] Saini, R. K., Nile, S. H. and Park, S. W. 2015. Carotenoids from Fruits and Vegetables: Chemistry, Analysis, Occurrence, Bioavailability and Biological Activities. *Food Research International.* 76: 735-750.
Doi: 10.1016/j.foodres.2015.07.047.
[37] Leong, S. Y. and Oey, I. 2012. Effects of Processing on Anthocyanins, Carotenoids and Vitamin C in Summer Fruits and Vegetables. *Food Chemistry*. 133(4): 1577-158.
DOI:10.1016/j.foodchem.2012.02.052.
[38] Saini, R. K. and Keum, Y. S. 2016. Tocopherols and Tocotrienols in Plants and Their Products: A Review on Methods Of Extraction, Chromatographic Separation, And Detection. *Food Research International.* 82: 59-70.
https://doi.org/10.1016/j.foodres.2016.01.025.
[39] Liu, Y., Hou, H., Yang, J. and Gao, Y. 2105. Effects of Antioxidants on the Stability of β-Carotene in O/W Emulsions Stabilized by Gum Arabic. *Journal of Food Science and Technology*. 52(6): 3300-3311.
DOI: 10.1007/s13197-014-1380-0.
[40] Aluyor, E. O., Aluyor, P. and Ozigagu, C, E. 2009. Effect of Refining on the Quality and Composition of Groundnut Oil. *African Journal of Food Science.* 3(8): 201-205.
[41] Ayoade, G. W., Amoo, I. A. and Akpambang, V. O. E. 2015. Physicochemical and Fatty Acid Composition of Crude and Refined Oils of African Canarium. *International Journal of Science and Technology.* 4(5): 230-234.
[42] Garrow, J. S., James, W. P. T. and Ralph, A. 2000. *Human Nutrition and Dietetics.* 10th Edition. London: Churchill Livingstone Press. 145-152.
[43] Sundram, K., Sambanthamurthi, R. and Tan, Y. A. 2003. Palm Fruit Chemistry and Nutrition. *Asia Pacific Journal of Clinical Nutrition.* 12: 355-362.
[44] Firestone, D. 2005. Olive oil. In Shahidi, F. (Ed.). Edible Oil and Fat Products: Edible Oils. 6th ed. New Jersey: John Wiley & Sons, Inc. 6: 317-321.