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Research Article

Some Physico-chemical Properties of Red Fruit Oil (*Pandanus Conoideus* Lam) from Hexane and Chloroform Fractions

Novita Inar Arumsari[1], Sugeng Riyanto[1] and Abdul Rohman[1,2*]

[1]Faculty of Pharmacy, Gadjah Mada University, Yogyakarta, 55281 Indonesia.

[2]Research Center of Halal Products, Gadjah Mada University, Yogyakarta, 55281 Indonesia.

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ABSTRACT

As functional food oil, red fruit (*Pandanus conoideus* Lam) oil has been believed by local community to threat several degenerative diseases like cancer. Red fruit oil (RFO) has commanded high price value in Indonesian market. Therefore, the objective of the present study was to investigate several physico-chemical properties of RFO. Some parameters of RFO obtained from hexane and chloroform fractions have been evaluated. Such parameters include acid value, saponification value, anisidine value, conjugated dienes and trienes as well as *p*-anisidine value. Besides, absorptivity coefficient, fatty acid composition, and volatile compounds were also determined. Acid value and saponification value of RFO from chloroform fraction were higher than those in RFO from hexane fraction. In addition, RFO in hexane fraction has the higher iodine and anisidine values than that in chloroform fraction. Hexane is the best solvent to be used for analysis of RFO, as indicated by the highest absorptivity coefficient of RFO in hexane. The main fatty acid composed of RFO was oleic acid followed with palmitic acid. The main volatile compound present in RFO of hexane and chloroform fractions was 9-octadecenoic acid accounting of 41.57 % and 65.06 %, respectively.

Keywords: red fruit oil, physicochemical properties, hexane fraction, chloroform fraction

1. Introduction

Red fruit (*Pandanus conoideus* Lam) (Figure 1) is one of the uncommon fruits which is indigenous plant in the Province of Papua, Indonesia and in Papua New Guinea (Rohman *et al.*, 2010). Red fruit appears unusual shape with length about 68- 110 cm and with diameter of approximately 10-15 cm. According to its name, red fruit is red in color and contains large amount of oil (Rohman *et al*., 2012). The extracts of red fruit has been reported to inhibit lung carcinogenesis in rat induced by of 7,12-dimethylbenz[a]anthracene (Mun'im *et al*., 2006). Red fruit extract also revealed antioxidant activities *in vitro* (Rohman *et al*., 2010).

Red fruit oil was commercially obtained from solvent extraction of red fruit using non-polar solvents such as petroleum ether, hexane, and chloroform. For local communities, besides as food diet, red fruit oil is believed to theat several degenerative diseases such as cancer, arteriosclerosis, rheumatoid arthritis, and stroke

Figure 1. Red fruit (*Pandanus conoideus* Lam)

(Budi and Paimin, 2004). Its oil, known as red fruit oil (RFO), can be considered as functional food oils due to

* Corresponding author email: abdulkimfar@gmail.com

its ability to give several biological activities (Rohman and Che Man, 2012). RFO with different concentrations has been reported to significantly decrease the rate of Reactive Oxygen Intermediate (ROI) production of endothelial cells. RFO can neutralize oxidative stress of cells by reducing ROI production of endothelial cells exposed to severe malaria patient serum and neutrophils from healthy donor *in vitro* (Armiyanti *et al*., 2007). The effect of RFO on ALT and TNF-α serum levels of injured liver Sprague Dawley rat induced by CCl_4 has been investigated by Pertiwi (2008). RFO does not lower the serum level of ALT and TNF-α in the CCl_4-induced hepatotoxicity, but inhibits the levels of ALT when it is given before CCl_4 induction. In addition, RFO can increase spleen lymphocyte proliferation in mice after Listeria monocytogenes infections (Wahyuniari *et al*., 2009). Syarkiah *et al.* (2008) reported that RFO may significantly obstruct the formation of foam cell in rat treated with atherogenic diet at doses of 0.24 mL/day and 0.36 mL/day, respectively.

In retail market, the price of RFO was 10 – 15 times higher than that of common plant oils such as palm, corn, and soybean oils (Rohman *et al*., 2011). As a consequence, RFO can be target of adulteration with other oils. Therefore, its quality control by assesing several phisico-chemical properties of RFO is highly needed in order to assure its authenticity.

2. Materials and Method

2.1. Preparation of red fruit oil

Red fruit oil (RFO) was obtained from the solvent extraction of red fruit using hexane and chloroform as extracting solvents during partition of ethanolic extract of red fruit. Red fruit was cut into small pieces using a commercial cutter and subsequently blended with ethanol using commercial blender used for making fruit juice. In this stage, one part of fruit was mixed with one part of ethanol. The ethanolic extract yielded was then subjected to maceration with ethanol (1: 3 volume/volume) for 4 days. The mixture was stirred 2 times/day, each with an approximately of 10 minutes. The extract was filtered using Buchner filter connected to vacuum pump, evaporated with vacuum rotary evaporator at 70° C. The concentrated extract was subjected to partition using liquid-liquid extraction with hexane three times (1: 1 volume extract/volume hexane). The residue of ethanolic extract was also partitioned with chloroform using the same condition as hexane partition. The hexane and chloroform fractions containing RFO were evaporated at 60 °C. The RFO yielded was subsequently used for determination of physico-chemical properties of RFO.

2.2. Determination of acid, saponification, and iodine values

Acid value (Ca 5a-40), iodine value (Cd 8-53), and saponification value (Cd 1d-92) of RFO obtained from hexane and chloroform fractions were determined according to the official methods of the Assosciation of Official Analytical Chemists (AOCS) methods (1996).

2.3. Analysis of anisidine value

Anisidine value (AV) was determined according to AOCS (1996). A-0.5 g of RFO was accurately weighed on an analytical balance (sensitivity of 0.1 mg) and dissolved with 25 mL of *n*-hexane. The absorbance of this solution was read using a UV-Vis spectrophotometer U-2810 (Hitachi, Tokyo, Japan) against *n*-hexane as reference and recorded as A_1. In another separate run, a 5 mL portion of the sample solutions was mixed with 1 mL of *p*-anisidine solution (2.5 g/L glacial acetic acid). Accordingly, a blank solution composed of 5 mL of *n*-hexane instead of sample solution was prepared. The sample and blank solutions were kept in the dark at ambient temperature for 10 min and the absorbance of the sample solution (A_2) was measured against a blank solution. *p*-AV was calculated as:

$$\text{p-AV} = 25\frac{1.2A_2 - A_1}{m}$$

A_1 and A_2 are the absorbancies measured as described above; *m* is mass of oil.

2.4. Determination of conjugated dienes and trienes

The conjugated dienes (CDs) and conjugated trienes (CTs) were determined by specific absorptivity values at 232 and 270 nm, as described by Besbes *et al.* (2004) using UV-Vis spectrophotometer U-2810 (Hitachi, Tokyo, Japan). Oil samples were diluted in isooctane, and theabsorbances obtained were used for calculating the specific absorptivity ($E_{1cm}^{1\%}$) as:

$$E_{1cm}^{1\%} = \frac{A_\lambda}{(c_L \text{ x } l)}$$

$E_{1cm}^{1\%}$ is the specific absorptivity, A_λ is the absorbance measured at either 232 nm (for CDs) or 270 nm (for CTs), c_L is the concentration of the oil solution in g/100 ml, and 1 represents the path length of the cuvette in cm (Pegg, 2005).

2.5. Determination of absorptivity coefficient

The absorptivity coefficient was determined in several solvents with different polarities. An approximately of 1.0 g of RFO was accurately weighed and dissolved in several solvents. The mixture was diluted in such a way that the absorbance values of RFO in the solvents fall into 0.2 – 0.8. The absorptivity coefficient was calculated based on Lambert-Beer law of $A = \varepsilon bc$. A is absorbance, ε is the absorptivity coefficient in mg RFO/100 mL.cm^{-1}, c is concentration of RFO in the solvents, while b is the thickness of solution (1 cm).

2.6. Analysis of fatty acid composition

The fatty acid composition of RFO coming from hexane and chloroform fractions was assesed using gas chromatography with flame ionization detector as described in El Kinawy (2010). The standard of 37 fatty

acid methyl esters (FAMEs) purchased from Sigma (Alldrich, USA) was used as authentic samples. The retention time of fatty acid in RFO was compared with that in standard of FAMEs. Quantification of fatty acid was calculated using internal normalization technique (Rohman and Che Man, 2009).

2.7. Analysis of volatile compounds

Volatile compouns present in RFO were determined using gas chromatographic-mass spectrometry (GC-MS) of Agilent GC 6890N 5975B MSD (Agilent Technologies, Palo Alto, CA, USA). The column used is HP-5MS (30 m x 0.25 mm. i.d, film thickness 0.25 µm). The oven was set at 80 °C, hold for 3 min, ramped to 300 °C and hold for 15 min. The carrier gas used was helium with the purity of 99,999 % and set at 1 ml/min. RFO was injected at 1µL with splitless mode. The mass selective detector was used in electron ionisation mode, and a mass range between 30 and 550 was scanned. The mass spectra were compared to the NIST Mass Spectral Search Program for the identification of volatile compounds.

3. Results and Discussion

3.1. Acid value, iodine value and saponification value

Table 1 exhibited acid value, saponification value and iodine value of red fruit oil (RFO) obtained from hexane and chloroform fractions. Acid value represents the free fatty acids present in fats and oils. The lower the acid value, the better the fats and oils. Compared with chloroform fractions, RFO in hexane fraction has the lower acid value. Thus, low levels of hydrolytic and lipolytic activities were observed in RFO from hexane fraction.

Table 1. Some parameter constants of red fruit oil in hexane and chloroform fractions

Physico-chemical properties	Mean ± SD		Unit
	Hexane fraction	Chloroform fraction	
Acid value	77.55 ± 3.36	100.63 ± 6.87	mg KOH/g MBM
Saponification value	127.30 ± 1.36	155.01 ± 0.52	mg KOH/g MBM
Iodine value	81.48 ± 4.59	70.73 ± 3.38	g I_2/100 gr MBM
Anisidine value	21.31 ± 1.70	19.46 ± 0.28	-
Conjugated dienes value	3.03 ± 0.07	3.02 ± 0.008	g/100 mLcm^{-1}
Conjugated trienes value	1.68 ± 0.04	2.56 ± 0.05	g/100 mLcm^{-1}

Saponification value can be used to estimate the molecular weight of fatty acids composed of RFO. This value is inversely proportional to the mean molecular weight of the glycerides in the oil. RFO in chloroform fraction has higher saponification value (155.01 mg KOH/ g RFO) than RFO in hexane fraction (127.30 mg KOH/g RFO). Thus, the average molecular weight of triglyceride composed of RFO in chloroform fraction was lower than that in RFO from hexane fraction. The iodine value indicates the unsaturation degree of fats and oils. The high level of iodine value is attributed to high unsaturation fatty acids composed of fats/oils. The slightly higher iodine value of RFO in hexane fraction than RFO in chloroform fraction gives the indication that RFO in hexane fraction contains more unsaturated fatty acid that than of RFO in chloroform fraction.

3.2. Conjugated diene and triene values and anisidine value

The formation of peroxides is concurrent with conjugation of double bonds in polyunsaturated fatty acids, which can be measured using the specific absorptivity of conjugated dienes (CDs) and conjugated trienes (CTs) at 232 and 270 nm, respectively, in the UV spectrum (Wanasundara *et al.,* 1995). The detection of CDs and CTs in unsaturated lipids is a sensitive assay, but the magnitudes of changes in absorption are not easily related to the extent of oxidation. However, during the early stages of oxidation, the increase in UV absorption due to the formation of CDs and CTs is proportional to the uptake of oxygen and to the generation of peroxides. For this reason, the content of CDs and CTs can serve as a relative measurement of oxidation (Pegg, 2005). RFO from hexane fraction has CDs of 3.03 g/100 mL.cm^{-1} and is not statistically different from CDs of chloroform fraction. Futhermore, CTs value of RFO in chloroform fraction (2.56 g/100 mL.cm^{-1}) was higher than that in RFO from hexane fraction (1.68 g/100 mL.cm^{-1}).

Anisidine value (AV) is parameter used to measure the extent of secondary oxidation products in fats and oils. AV particularly measures the formation of 2-alkenals (Laguerre *et al*., 2007). This value is particularly appropriate for the heated oils, because most peroxides are destroyed during thermal oxidation using high temperature. AV can be successfully exploited for analysis of blended or deodorized oils heated above 200 °C and for analysis of some matters extruded at high temperature of 150 °C (Pokorny *et al*., 2008). AV of RFO in hexane fraction (21.37) was higher than that of RFO in chloroform fraction (19.46) meaning that the secondary products of RFO in hexane fraction was higher than that in chloroform fraction.

3.3. Analysis of absorptivity coefficient

In order to investigate the type of organic solvents having the best sensitivity for measurement of RFO, the absorptivity coefficient was evaluated. Table 2 and 3 exhibited the absorptivitiy coefficient of RFO coming from hexane and chloroform fractions expressed with gram RFO in 100 mL solvent measured with cuvette of 1 cm. The maximum wavelength of RFO in each solvent was also included in Table 2 and 3.

Four types of solvents namely hexane, chloroform, ethyl acetate and methanol were investigated. The results showed that hexane was the best solvents for RFO determination (either in hexane or in chloroform fractions). Hexane gives the highest absorptivitiy coefficient of RFO compared with other studied

solvents, i.e 80.44 mg/100 mL.cm^{-1} for RFO in hexane fraction and 58.23 mg/100 mL.cm^{-1} for RFO in chloroform fraction.

Table 2. The absorptivity coefficient of red fruit oil in hexane fractions at some organic solvents

Solvent	Maximum wavelength (nm)	Absorptivity coefficient (mg/100 mL.cm^{-1})
Hexane	467	80.44
Choloform	486	56.72
Acetid ethyl	473	61.13
Methanol	471	12.91

Tabel 3. The absorptivity coefficient value of red fruit oil in chloroform fractions at some organic solvents

Solvent	Maximum wavelength (nm)	Absorptivity coefficient (mg/100 mL.cm^{-1})
Hexane	467	58.23
Choloform	486	50.21
Acetid ethyl	473	49.68
Methanol	471	9.82

3.4. Fatty acid composition

The main fatty acid composed of RFO either from hexane or chloroform fractions is oleic acid (C18:1). The levels of oleic acid in RFO were 79.53% (hexane fraction) and 79.54% (chloroform fraction). RFO also contained high level of saturated fatty acid, i.e. palmitic acid (C16:0), in which RFO from hexane fraction accounted of 17.48%, while RFO of chloroform fraction contained 17.86 % of palmitic acid. Besides, some fatty acids with low levels were observed in RFO. The complete fatty acid composition of RFO coming from hexane and chloroform fractions was shown in Table 4. RFO was similar to olive oil in terms of the high level of oleic acid. Oleic acid in olive oil and in RFO was believed to take a role in several biological activities such as antioxidants (Fomuso and Akoh, 2002).

Tabel 4. Fatty acid composition of red fruits oil in hexane and chloroform fractions

Fatty acid composition	Concentration (%)	
	Hexane fraction	Chloroform fraction
C 12:0 (Lauric acid)	0.08	0.01
C 14:0 (Myristic acid)	0.08	0.09
C 14:1 (Myristoleic acid)	0.10	0.11
C 16:0 (Palmitic acid)	17.48	17.86
C 16:1 (Palmitoleic acid)	0.77	0.87
C 18:0 (Stearic acid)	0.14	0.13
C 18:1 (Oleic acid)	79.53	79.28
C 18:2 (Linoleic acid)	1.56	1.54
C 18:3n6 (Linolenic acid)	0.17	0.11
C 20:0 (Arachidic acid)	0.11	0.00

3.5. Analysis of volatile compounds

The presence of volatile compounds in RFO coming from hexane and chloroform fractions was evaluated using gas chromatography-mass spectrometry (GC-MS). Table 5 and 6 exhibited some of volatile compound present in RFO obtained from hexane fraction and chloroform fraction. RFO in hexane fraction has 21 compounds; meanwhile, there are 7 compounds in RFO of chloroform fraction. It is not surprising because hexane was more non-polar than chloroform. As a consequence, RFO in hexane fraction contained more volatile compounds than that in chloroform fraction.

Based on table 5, the highest levels of volatile compound present in RFO of hexane fraction was 9-decanoic acid and 11-octadecanoic acid accounting of 41.57% and 24.34, respectively %. Furthermore, the volatile compounds present in RFO of chloroform fraction (Table 6) were mainly 9-octadecanoic acid and 9-octadecanoic acid accounting of 65.06 % and 23.19 %, respectively.

Table 5. Volatile compounds of red fruit oil in hexane fractions

No	Compounds	Total compounds (%)
1	Limonene	0.09
2	Dodecanoic acid	0.04
3	Tetradecanoic acid	0.06
4	Pentadecanoic acid	0.09
5	9-Hexadecenoic acid	0.51
6	Hexadecanoic acid	9.11
7	Hexadecanoic acid (CAS)	13.85
8	11-Octadecenoic acid	24.34
9	Octadecanoic acid	2.93
10	9-Octadecenoic acid	41.57
11	9-Octadecenoic acid (CAS)	1.80
12	Oleic acid	3.03
13	9- Octadecenoic acid (CAS)	0.39
14	9- Octadecenoic acid (CAS)	0.05
15	9- Octadecenoic acid (CAS)	0.11
16	9- Octadecenoic acid	0.86
17	Oleic acid	0.13
	2,6,10,14,18,22	
18	Tetracosahexaene	0.14
19	Octadec-9-enoic acid	0.12
20	9- Octadecenoic acid	0.11
21	1.3-Dioxolane	0.46

Table 6. Volatile compounds in red fruit oil coming from chloroform fraction

No	Compounds	Total compounds (%)
1	9-Hexadecenoic acid	0.24
2	Hexadecanoic acid	6.02
3	Hexadecanoic acid (CAS)	0.43
4	Octadecanoic acid (CAS)	0.44
5	8- Octadecenoic acid	23.19
6	15- Octadecenoic acid	4.36
7	9- Octadecenoic acid (CAS)	65.06
8	9- Octadecenoic acid (CAS)	0.12
9	2,6,10,14,18,22 Tetracosahexaene	0.13

4. Conclusion

Some physico-chemical properties have been evaluated for the characterization of red fruit oil (RFO). The acid and saponification values of RFO from chloroform fraction were higher than those in RFO from hexane fraction. Meanwhile RFO in hexane fraction has the higher iodine and anisidine values compared to chloroform fraction. Hexane is the best solvent to be used for analysis of RFO as indicated by the highest absorptivity coefficient of RFO either in hexane or chloroform fractions. Oleic acid is the main fatty acid present in RFO, while. 9-Octadecenoic is the main volatile compounds present in RFO.

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