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Characterizaton of red fruit (*Pandanus conoideus* Lam) oil

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Abstract: The present study was carried out to characterize red fruit (*Pandanus conoideus* Lam) oil (RFO) in term of FTIR spectra, fatty acid composition, and volatile compounds. FTIR spectrum of RFO was slightly different from other common vegetable oils and animal fats, in which in the frequency range of 1750 – 1700 cm^{-1}, RFO appear two bands. The main fatty acid composition of RFO is oleic acid accounting for 68.80% followed by linoleic acid with the concentration of 8.49%. The main volatile compounds of RFO as determined using gas chromatography coupled with mass spectrometry (GC-MS) and headspace analyser are 1,3-dimethyl-benzene (27.46%), N-glycyl- L-alanine (17.36%), trichloromethane (15.22%), and ethane (11.43%).

Keywords: Red fruit oil, fatty acid, FTIR spectra, volatile compounds

Introduction

The fruits and vegetables are the component of human diet. As suggested by The World Health Organization (WHO), the consumption of at least 400 g of fruits and vegetables are needed for the healthy life style. Inversely, the low consumption of fruits and vegetables is considered a risk factor for the incidence of several diseases like heart attack, cancer, diabetes and obesity (Satphaty *et al*., 2011).

Red fruit (*Pandanus conoideus* Lam) (Figure 1) is an indigenous plant from Papua Province, Indonesia and Papua New Guinea. This fruit has uncommon shape with 68- 110 cm length and diameter of 10-15 cm, red in color, and contains large amount of oil. According to Sant'Ana (2011), this fruit can be taken into account as exotic fruit due to unusual shape. For local communities, it is believed that fruit of *P. conoideus* can treat several degenerative diseases such as cancer, arteriosclerosis, rheumatoid arthritis, and stroke (Budi and Paimin, 2004).

Figure 1. Red fruit (*Pandanus conoideus* L) (taken from Wijaya and Pohan, 2009)

Mun'im *et al.* (2006) have reported that red fruit extract inhibited lung carcinogenesis in rat female Sprague-Dawley induced by carcinogen agent of 7,12-dimethylbenz[a]anthracene. In addition, Rohman *et al*. (2010[a]) have studied the antioxidant activities of red fruit extracts and its fractions *in vitro*. These beneficial effects may be attributed from the several active compounds such as β-carotene and α-tocopherol contained in red fruit oil. Today, red fruit oil (RFO) is widely distributed in Indonesia and marketed as daily oil diet. In the market, the price of RFO is approximately 10 – 15 of commom vegetable oils like corn, palm and canola oils and can be potential to be used as functional food oils. Functional food can be defined as food or part of food which have the beneficial effect to human health (Rohman and Che man, 2011). Some of edible fats and oils can be considered as functional food oils such as olive oil due to its ability to prevent several disease (García-González *et al*., 2008), virgin coconut oil (Marina *et al.*, 2009), and cod liver oil, especially due to the high content of high levels of long-chain *n*-3 fatty acids of *cis*-5,8,11,14,17-eicosapentaenoic acid (EPA) and *cis*-4,7,10,13,16,19-docosahexaenoic acid (DHA) believed to play an important role for the prevention of cardiovascular disease and for the alleviation of other health problems (Moghadasian, 2008).

We have investigated the possibility of RFO to be subjected of adulteration with the lower price oils (Rohman *et al*., 2011); however, using literature searching, there is no reports related to the properties of RFO. Therefore, in this study, we characterize the physico-chemical properties of RFO, including fatty acid composition, triglyceride composition, volatile compounds, and FTIR spectral data.

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Materials and Methods

Materials

Red fruit was taken from Papua, Indonesia. Botanical identification was carried out in Department of Biological Pharmacy, Faculty of Pharmacy, Gadjah Mada University, Yogyakarta, Indonesia. Palm, corn, and extra virgin olive oils were purchased from local market in Yogyakarta, Indonesia. The profiles of fatty acid composition of palm, corn, and virgin olive oils were in accordance with those listed in Codex Allimentarius (2003). All chemical and reagents used were of analytical grade.

Oil preparation

Oil from red fruit was obtained using solvent extraction. Briefly, fruits were cut into small pieces using a commercial cutter and subsequently subjected to commercial blender containing ethanol (one part of fruit was added with one part of ethanol). The ethanolic extract obtained was further macerated with methanol (1: 3 volume/volume) for 4 days. The extract was evaporated at 70^{o}C and partitioned three times using hexane (1: 1 volume extract/volume hexane). The hexane extracts containing RFO were evaporated at 60^{o}C. The oil obtained was further used for analysis of FTIR spectra, fatty acid composition, and volatile compounds based on the oil weight.

Fatty acid (FA) analysis

FA composition of red fruit oil (RFO) was determined using gas chromatography using flame ionization detector (GC-FID) as fatty acid methyl ester (FAME) according to Rohman and Che Man (2009) with slight modification. Approximately of 50 mg samples of fats and oils was dissolved in 0.8 ml hexane and followed by the addition of 0.2 ml sodium methoxide 1 M. The mixture was vigorously shaken for 1 min with vortex mixer, added with 5 drops of saturated NaCl to precipitate sodium glycerolate, and mixed again using vortex for 15 sec. Subsequently, 1 μL of the clear supernatant was taken and injected into gas chromatograph (Shimadzu GC-2010, Shimadzu Corp., Tokyo, Japan), using the conditions as follow:

Column	RTX-5 capillary column (0.25 mm internal diameter, 30 m length, and 0.2 μm film thickness; Restex Corp., Bellefonte PA).
Oven	50^{o}C (hold for 1 min), then increased to 180^{o}C (8^{o}C/min), 180 to 240^{o}C (8^{o}C/min), and finally held at 240^{o}C for 5 min
Detector	flame ionization detector (240^{o}C)
Carrier gas	N_2, at 6.8 mL/min
Injector	240^{o}C; split ratio (1: 20)

The standard FAMEs (Sigma Chemicals, St. Louis, MO) comprising of 37 FAMEs were used as authentic samples to calculate the percentage of FAs based on its peak area. Quantification of FAME was performed using normalization internal technique.

Carotenoid and vitamin E analyses

The β-carotene content of RFO and other oils were determined spectrophotometrically according to Schierle *et al.* (2003). In addition, the level of tocopherol was determined according to Chen *et al*. (2011). Briefly, the oil was diluted 20 times with tetrahidrofuran (THF): acetonitrile (ACN) in ratio of 40: 60 v/v. The separation of tocopherol was carried out with C18 column using mobile phase of THF: methanol (10: 90 v/v) and was detected using fluoresence at excitaion and emission wavelengths of 296 and 330 nm, respectively.

Analysis of volatile compounds using Headspace Gas Chromatography-Mass Spectrometer analysis

Analysis of volatile compounds was conducted according to Juliana *et al*. (2011). Briefly, approximately 10 gram of RFO was accurately weighted using analytical balance (sensitivity 0.1 mg) and transfered into a 20 ml headspace vial. The extraction of the volatile compounds of RFO was carried out using a headspace auto sampler G1888 (Agilent Technologies, Palo Alto, CA, USA). The transfer line from the headspace sampler was directly connected to the injector of the gas chromatograph. The oven temperature was set for 110°C. The extraction conditions in the headspace auto sampler were programmed as follows: 20.0 min for vial equilibration, 0.20 min for vial pressurization, 0.20 min for filling the injection loop, 0.05 min for loop equilibration, and 1.0 min for sample injection. The used carrier gas was helium with a purity of 99.999%. The volatile compounds were analyzed using gas-chromatography-mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). The column used was DB-5 (30 m, 0.25 mm. i.d, film thickness 0.25 μm). The column temperature was kept at 40°C for 10 min, increased to 240°C (6°C/min) and kept isothermal for 20 min. 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.

Measurement of FTIR spectra

FTIR spectra of red fruit oil were measured using FTIR spectrometer Nicolet 6700 (Thermo Nicolet Corp., Madison, WI) equipped with deuterated triglycine sulphate (DTGS) as a detector and potassium bromide (KBr)/Germanium as beam splitter, and connected to software of the OMNIC operating system (Version 7.0 Thermo Nicolet). The sampling compartment was Smart Attenuated Total Reflectance kit (Smart ARK, Thermo Electron Corp.) with dimension of 10 x 60 mm. The rest procedure was as described by Rohman and Che Man (2010).

Statistical analysis

All data analyses (FA composition, FTIR spectra and volatile compounds) were performed at least in three replications. The data were expressed as mean ± standard deviation.

Results and Discussion

Fatty acid composition

Fatty acids (FAs), one of the lipid classes, are the most vital components of edible fats and oils in which they are typically found in the ester form with glycerol backbone (triglycerides). Due to the nutritional value, quantitative analysis of FAs composition is a pivotal work in food research area, especially for fats and oils characterization (Carrosco-Pancorbo *et al*., 2009). In addition, FAs composition can vary from one source to another or even from one organ to others; Therefore, FAs profiles can be used for some purposes such as authentication studies. For this reason, some researches devote their attention to analyze FAs composition in their fields (Hauff and Vetter, 2010).

Table 1 showed fatty acid composition of RFO. The main fatty acids composed of RFO were oleic (C18: 1) and linoleic (C18: 2) acids with the concentration of 68.80% and 8.49%, respectively. It is believed that the high presence of monounsaturated fatty acid (oleic acid) in RFO can contribute to the beneficial effects to human health (Fomuso and Akoh, 2002). Such effects are preventing cardiovascular diseases, reducing plasma triacylglycerol (TAG), increasing high density lipoprotein (HDL)-cholesterol levels, improving the postprandial lipoprotein metabolism, reducing blood pressure and the risk of hypertension, having anti-cancerogenic effects in animal models and in human cell lines, not promoting obesity and increasing the lipolytic activity in adipose tissue, and preventing age-related cognitive decline and dementia (García-González *et al*., 2008).

Tocopherol and β-carotene contents of red fruit oil

Tocopherols are contained in plant oils, which are natural antioxidants capable for retarding the oxidative rancidity. The tocopherol content of RFO as determined using liquid chromatography with reversed phase mode was 145,12 ± 1,14 µg/g, expressed as α-tocopherol. In addition, the tocopherol content of corn oil was 116 – 172 µg/g, palm oil of 129 – 215 µg/g, and olive oil of 63 – 135 µg/g (O'brien, 2009). In addition, the level of β-carotene in RFO determined using visible spectrophotometry was 3.12 ± 0.17 mg/g of RFO weight.

Table 1. Fatty acid composition of red fruit oil

Fatty acid	Concentration (%)
C12:0	0.06 ±0.02
C14:0	0.07 ±0.00
C14:1	0.15 ±0.00
C16:0	20.05 ±0.09
C16:1	0.15 ±0.01
C18:0	0.18 ±0.01
C18:1	68.80 ±1.29
C18:2	8.49 ±0.02
C18:3n6	0.17 ±0.00
C20:0	0.13 ±0.00

FTIR spectral data

FTIR spectroscopy can be used as potential means for the characterization of edible oils, because FTIR spectra are considered as "fingerprint tool" meaning that there are no two oil samples with the same FTIR spectra, either in the number of peaks or in the intensities of maximum peak (Che Man *et al*., 2011). Figure 2 exhibited FTIR spectra of red fruit oil (RFO). As a comparison, FTIR spectra of common edible oils of palm oil, corn oil, and extra virgin olive oil were also presented.

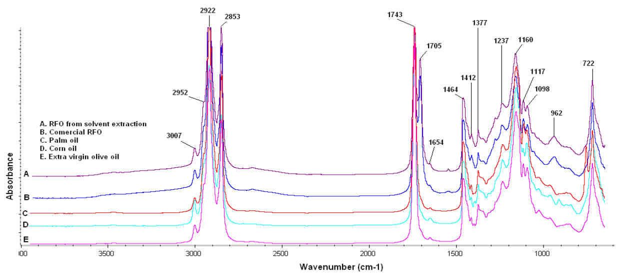

Figure 2. FTIR spectra of red fruit oil and other vegetable oils scanned at mid infrared region (4,000 – 600 cm^{-1})

FTIR spectra of RFO and selected vegetable oils appear quite similar. The Functional groups responsible to infrared absorption of common vegetable oils can be seen in our previous paper (Rohman and Che Man 2010[a;] Rohman and Che Man 2010[b]). Using detailed investigation, FTIR spectra of RFO exhibited some differences compared with other vegetable oils. There is a sharp peak at 1705 cm^{-1}, which is attributed from the vibration of carbonyl group. This peak was absent in palm, corn, extra virgin olive oil (EVOO) and other animal fats and vegetable oils (Che Man *et al*., 2011).

The volatile compounds of RFO

The volatile compounds in RFO were evaluated using gas chromatography-mass spectrometry using headspace analyzer (GC-MS-HS). Figure 3 showed the peak of volatile compounds. The volatile compounds corresponding to each peak was tentatively identified by mass spectra dataset supplied by instrument. Table 2 listed the main volatile compounds of RFO as determined using GC-MS-HS. There are 35 volatile compounds identified with the main components are 1,3-dimethyl-benzene (27.46%), N-glycyl- L-alanine (17.36%), trichloromethane (15.2%), and ethane (11.43%).

Table 2. The volatile compounds of red fruit oil as determined using GC-MS

Time retention (min)	Volatile compounds	Concentration (%)
0.610	Ethane	11.43
0.699	L-Alanine, N-glycyl-	17.36
0.948	Trichloromethane	15.22
1.476	Acetic acid	0.05
2.159	Acetamide, 2,2,2-trichloro-	0.27
2.438	Hexanal	9.98
7.940	Benzene, 1,3-dimethyl-	27.46
14.849	1H-4-Azacycloprop[cd]indene, octahydro-4-methyl-	0.17
15.531	Benzaldehyde	0.68
16.872	5-Hepten-2-one, 6-methyl-	0.45
17.056	Furan, 2-pentyl-	1.65
17.680	Octanal	1.57
17.959	2H-1,2-Oxaborin, 2,3,3-triethyl-3,6-dihydro-	0.14
18.653	5-Ethylcyclopent-1-enecarboxaldehyde	1.45
18.819	Cyclohexanone, 2,2,6-trimethyl-	0.28
20.570	Phenol, 2-methoxy-	0.21
20.819	4-Ethylcyclohexanol	0.16
21.128	3-Carene	0.49
21.187	6-Methyl-3,5-heptadiene-2-one	0.71
21.288	Nonanal	3.45
21.401	2,4-Hexadiene, 2,5-dimethyl-	0.73
22.220	Cyclopentasiloxane, decamethyl-	0.17
23.318	Benzaldehyde, 3,4-dimethyl-	0.11
23.591	Naphthalene	0.15
23.781	2-n-Heptylfuran	0.20
24.019	Dodecane	0.24
24.179	8-Azabicyclo[4.3.1]decan-10-one, -methyl-	0.15
24.547	Adrenalone	0.21
26.529	Cyclohexasiloxane, dodecamethyl-	0.25
28.660	Cyclododecane	0.12
28.838	Tetradecane	0.17
29.616	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	0.99
30.209	N-Benzyl-N-ethyl-p-isopropylbenzamide	0.18
30.577	3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	0.37
31.070	alpha-Farnesene	0.35

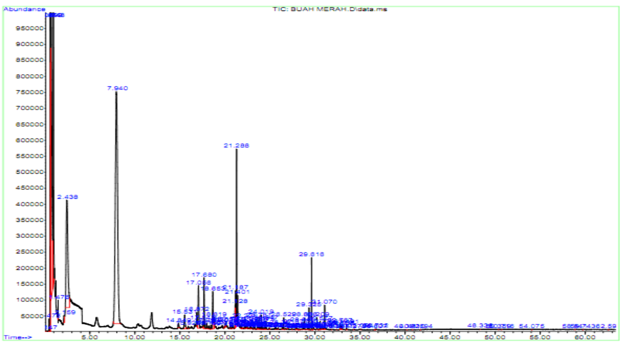

Figure 3. The GC-MS chromatogram of red fruit oil

Conclusion

Red fruit oil is one of interesting oils to be developed as functional food oils due to the high level of monounsaturated fatty acid (oleic acid) present in it. FTIR spectrum of RFO is unique which is different from other edible fats and oils, in which an additional peak in 1705 cm^{-1} was present. Furthermore, the investigation of the main volatile compounds present in RFO are 1,3-dimethyl-benzene, N-glycyl-L-alanine, trichloromethane, and ethane.

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