Characterization of Chemical Properties, Lipid Profile, Total Phenol and Tocopherol Content of Oils Extracted from Nine Clones of Red Fruit (*Pandanus conoideus*)

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ABSTRACT

Red fruit (*Pandanus conoideus*) is an endemic plant of Papua, Indonesia, traditionally known as a source of edible oil. The chemical properties of oil free fatty acid (FFA)—iodine value, peroxide value, phosphorus—the lipid profile—fatty acid (FA) and triacylglycerol (TAG) compositions—and the total phenol and tocopherol contents were determined for oil extracted from nine clones of red fruit cultivated in three area of Papua. Red fruit oil (RFO) was extracted using a chloroform-methanol solution (2:1). RFOs from nine clones had FFA of 4.3 to 9.2%, peroxide value of 0.36 to 0.84 meq per kg, iodine value of 79.3 to 85.5 g per 100 g and phosphorus content of 38 to 374 ppm. The main FAs of the RFOs were oleic (49.36–64.47 g per 100 g), linoleic (4.13–16.06 g per 100 g) and palmitic (14.11–19.21 g per 100 g), while the major TAG species were OOO/triolein (26.84–39.81%), POO/palmito-diolein (24.23–31.08%), OLO/linoleo-diolein (7.23–17.43%) and PLO/palmito-linoleo-olein (3.72–14.3%). The RFOs contained total phenol (90–742 ppm), total tocopherol (234–1,728 ppm), α-tocopherol (52–272 ppm) and γ-tocopherol (16–287 ppm). The results of principal component analysis confirmed that the nine clones of red fruit can be divided into three groups based on their FAs and TAGs.

Keywords: red fruit (*Pandanus conoideus*) oil, chemical properties, tocopherol, composition of fatty acid and triacylglycerol

INTRODUCTION

The diversity of red fruit (*Pandanus conoideus*) clones in Papua (Indonesia) is very high as they are scattered in the highlands and lowlands (Walujo *et al*., 2007). Murtiningrum *et al*. (2012) explored the red fruits in five areas of Papua (Manokwari, Bintuni, South Sorong, Jayawijaya and Nabire) and registered 85 cultivars with varying physical characteristics and chemical composition of the fruit. Traditionally, the red fruit is used as a source of edible oil by the Papuans, with the fat content ranging from 11.2 to 30.7% on a dry basis (Murtiningrum *et al*., 2012).

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Some studies have reported potential health benefits of red fruit oil (RFO) such as inhibiting tumor growth and killing cancer cells (Mun'im *et al*., 2006; Surono *et al*., 2008), increasing anti-inflammatory and immune cells (Khiong *et al.*, 2009) and lowering blood sugar in diabetic rats (*Rattus norvegicus*) (Winarto *et al*., 2009). The functionality of RFO was due to the content of active compounds such as phenols, carotenoids, tocopherols and unsaturated fatty acids (Surono *et al*., 2008; Rohman *et al*., 2010; Rohman *et al*., 2012). On this basis, RFO has potential to be developed as a functional food ingredient.

The reported characteristics of RFO are different from those of other vegetable oils abundant in Indonesia such as coconut oil and palm oil. Coconut oil contains more than 90% saturated fatty acid and is dominated by lauric acid (47.5%) with an iodine value (IV) of 7 to 12 g per 100 g (Canapi *et al*., 2005), whereas, palm oil mainly consists of oleic (35.99%), linoleic (14:53%) and palmitic (39.93%) acids, with an iodine value of 54.85 g per 100 g (Li *et al*., 2012). Moreover, palm oil contains β-carotene (542.09 ppm) and α-tocopherol (171 ppm), which are not present in coconut oil (Dauqan *et al*., 2011). RFO has been reported to contain β-carotene and α-tocopherol (Surono *et al*., 2008). Thus RFO has potential as an alternative source of carotenoids and tocopherols.

The physical, chemical and functional characteristics of oils from different sources can be determined by their lipid profile (fatty acid and triacylglyceols composition) and the presence of other non-triglyceride components (deMan, 1999), which could greatly affect their applications in the food and nonfood industries. The composition of fatty acids also affects the stability of the oil against oxidation (Besbesa *et al.*, 2004). Therefore, characterization of oils is very important for the identification and determination of oil stability, and furthermore, for its potential industrial applications.

This study characterized and evaluated the levels of free fatty acids (iodine value, peroxide value, phosphorus content), lipid profile (fatty acid and triacylglycerol composition), and the total phenol and tocopherol contents of oil extracted from nine clones of red fruit from three areas in Papua.

MATERIALS AND METHODS

Materials

Nine clones of red fruit originating from Papua (Indonesia) were used as samples—*Menjib Rumbai, Edewewits, Memeri, Monsrus, Monsor* (cultivated in the Experimental Garden of the State Papua University (UNIPA), Manokwari), *Mbarugum* (District Koya, Jayapura), and *Hityom, Himbiak, Hibcau* (District Minyambow, Manokwari). Chemical reagents for the analysis of chemical properties of RFO were of analytical grade.

Red fruit oil extraction method

The RFOs were extracted by the method of Folch *et al.* (1957). Approximately 12 g of pulp of the red fruit was macerated with 80 mL of solvent mixture of chloroform and methanol (2:1, volume per volume, v/v) and stirred using a magnetic stirrer, at room temperature for 1 hr. The resultant solution was filtered vacuumed, 16 mL of 0.88% NaCl added and then the solution was separated using a separating flask. The oil extract obtained was then evaporated with a rotary evaporator at 40 °C, packaged in dark bottles, dried with nitrogen gas and stored at -20 °C until analyzed.

Analysis of chemical properties

The chemical properties of RFO that were analyzed were: water content using oven methods (Association of Analytical Chemists, 2005), free fatty acid using titration metods (Association of Analytical Chemists, 2003), iodine value using Wijs methods (Association of

Analytical Chemists, 2005), peroxide value using acetic acid-chloroform methods (Association of Analytical Chemists, 2003) and phosphorous content was analyzed using the methods of Association of Analytical Chemists (2005), using vanadate-molybdate reagent and nitric acid as a reducing agent, followed by measurement of the absorbance with an ultravioletvisible spectrum (UV-Vis) spectrophotometer at a wavelength of 400 nm. A standard curve, established by plotting absorbance against the concentration of P_2O_5, was used to determine the phosphorus content of each oil analyzed.

Analysis of fatty acid and triglyceride 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 Association of Analytical Chemists (2003). 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% BF3-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 g oil) of the total peak areas of all

the FAs in the oil sample.

The composition of triacylglycerols (TAG) oil was determined by Association of Analytical Chemists (2005) using high-performance liquid chromatography (HPLC), where 0.05 g of sample was dissolved in acetone (2:1, v/v) at a concentration of 5%, filtered and injected into a 20 mL Millipore sample loop of HPLC, eluted using an isocratic pump type, with a mobile phase of acetone/acetonitrile (85:15, v/v) and a flow rate of 0.8 mL.min^{-1}. The two columns used were Zorbax Eclipse XDB C-18 (250 × 4.6 mm, Agilent Technologies Inc.; Santa Clara, CA, USA) installed in series with a Microsorb MV column (250 × 4.6 mm, Rainin Instrument Co. Inc.; Columbus, OH, USA). The processing time of analysis was 50 min. The retention time of each standard TAG was referenced to the identification of the sample. The percentage of TAG species was calculated based on the TAG area per total area identified.

Analysis of total tocopherols and total phenols

Total tocopherols were determined using the method of Wong *et al*. (1988). 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 FeCl$_3$. 6H$_2$O (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 α-tocopherol (100–1,500 ppm in toluene).

Total phenols was determined using Folin-Ciocalteu reagent. The phenol component was extracted by dissolving 0.1 g of sample in 1 mL of 80% methanol, centrifuging at 1,100 revolutions per minute for 10 min and then collecting the filtrate. The extraction process was repeated four times (Seneviratne *et al.*, 2009) and the filtrate collected was added with 80% methanol to 5 mL.

Measurement of total phenols was done by adding 0.5 mL of 50% Folin-Ciocalteu reagent to the 1 mL sample, allowing to stand for 5 min, adding 1 mL of 5% Na_2CO_3, adding deionized water to 5 mL then incubating for 60 min. Absorbance was measured by spectrophotometry at a wavelength of 745 nm. A blank was made with the same procedure without a sample. Gallic acid was used as the standard for the calibration curve.

Analysis of the composition of tocopherol

The tocopherol composition was analyzed by the method of Association of Analytical Chemists (2003). 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 dimater 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. Tocopherol fractions were identified by the retention time standard. The calculation was expressed with a standard

calibration curve as α-tocopherol (58.0; 25.0; 15.0; 8.0; 1.6 and 0.1 ppm) and γ-tocopherol (45.0; 22.5; 11.3; 5.6; 2.8 and 0.1 ppm).

Data analysis

The XLSTAT software (version 2014.2.07; Addinsoft, New York, NY, USA) was used for principal component analysis (PCA) of the lipid profile data.

RESULTS

Chemical properties of oils

Chemical characterizations of the RFO samples from the nine clones are shown in Table 1. The water content of the oils ranged from 0.07 to 0.18%, while free fatty acids (FFA, as oleic acid) ranged from 4.3 to 9.2%. The peroxide value (used to measure fat oxidation) of RFO from the nine clones ranged from 0.36 to 0.84 meq per kg. The iodine value (IV) of the oils varied between 79 and 85.5 g per 100 g, indicating a high degree of unsaturation of the RFOs, which was supported by the data on the fatty acid composition (Table 2). The phosphorus levels of the RFOs varied between clones, ranging from 37 to 374 ppm.

Table 1 Mean values (±SD, 3 measurements) of chemical properties of red fruit oil from nine red fruit clones.

Red fruit clone	Water content (%, wet basis), Mean±SD	Free fatty acid (%), Mean±SD	Peroxide value (meq per kg), Mean±SD	Iodine Value (g per 100 g), Mean±SD	Phosphorus (ppm), Mean±SD
Menjib Rumbai	0.07±0.01	5.3±0.6	0.61±0.02	83.3±0.1	141±10
Edewewits	0.10±0.01	4.3±0.6	0.36±0.02	79.0±2.7	119±18
Memeri	0.18±0.02	8.6±0.9	0.84±0.10	85.5±6.4	153±18
Monsrus	0.13±0.02	7.8±0.8	0.62±0.10	84.8±6.4	374±25
Monsor	0.02±0.01	7.9±0.2	0.37±0.01	80.1±0.2	37±4
Mbarugum	0.08±0.01	7.4±0.8	0.79±0.06	81.2±0.8	57±2
Himbiak	0.08±0.01	5.6±0.6	0.56±0.03	80.0±2.1	38±2
Hibcau	0.09±0.01	9.2±1.0	0.68±0.01	84.5±7.9	134±4
Hityom	0.18±0.02	5.5±0.2	0.37±0.03	83.2±2.5	62±4

Table 2 Fatty acid composition mean values (±SD grams per 100 g oil; 3 measurements) of red fruit oil from nine clones.

| | | Red fruit clone | | | | | | | | |
		Menjib Rumbai	Edewewits	Memeri	Monsrus	Monsor	Mbarugum	Himbiak	Hibcau	Hfityom
Saturated Fatty Acid	C8	0.004±0.0005	0.03±0.001	0.01±0.001	0.01±0.0005	0.02±0.002	0.01±0.0002	0.01±0.0003	0.01±0.0004	0.01±0.001
	C10	0.001±0.0001	0.01±0.0004	0.004±0.0004	0.06±0.001	0.005±0.00003	0.04±0.001	0.003±0.0004	0.03±0.0002	0.02±0.003
	C12	0.047±0.001	0.22±0.01	0.14±0.001	0.22±0.002	0.18±0.002	0.17±0.0002	0.13±0.004	0.13±0.002	0.11±0.002
	C14	0.07±0.0003	0.14±0.005	0.08±0.001	0.11±0.0003	0.12±0.003	0.09±0.001	0.10±0.002	0.11±0.001	0.08±0.002
	C16	15.47±0.053	19.21±0.19	14.39±0.011	14.11±0.02	16.85±0.4	18.64±0.005	16.2±0.059	17.52±0.06	17.34±0.03
	C18	0.98±0.0015	1.67±0.08	0.99±0.003	1.43±0.02	1.28±0.1	1.38±0.002	1.0±0.005	1.11±0.001	1.09±0.003
	C20	0.17±0.001	0.15±0.003	0.09±0.002	0.10±0.0003	0.16±0.02	0.16±0.0004	0.10±0.002	0.11±0.003	0.10±0.002
	Total	16.73±0.049	21.42±0.12	15.66±0.051	16.04±0.003	18.62±**0.2**	20.49±0.001	17.56±0.06	19.02±0.06	18.74±0.03
Un-Saturated Fatty Acid	MUFA C16:1	1.04±0.0015	0.97±0.01	1.74±0.000	0.56±0.0004	0.93±0.1	2.02±0.01	1.49±0.01	0.75±0.004	0.50±0.002
	C18:1	56.99±2.65	49.36±0.13	56.81±0.292	64.47±0.11	61.54±2.1	55.50±0.29	56.96±2.39	52.01±0.11	54.02±1.70
	C20:1	0.27±0.001	0.10±0.001	0.15±0.003	0.16±0.0003	0.23±0.03	0.16±0.001	0.20±0.004	0.17±0.003	0.15±0.001
	Total	58.29±2.6	50.43±0.12	58.70±0.295	65.18±0.11	62.70±**2.2**	57.68±0.29	58.65±2.39	52.93±0.11	54.67±1.7
	PUFA C18:2	5.24±0.31	8.64±0.03	4.13±0.009	5.51±0.01	5.61±0.5	9.26±0.01	5.66±0.049	16.06±0.02	13.23±0.03
	C18:3	1.07±0.00004	1.42±0.003	1.00±0.002	1.42±0.03	1.08±0.05	1.21±0.01	0.78±0.002	1.05±0.0001	0.94±0.01
	Total	6.32±0.3	10.06±0.03	5.14±0.011	6.93±0.04	6.69±0.5	10.47±0.005	6.43±0.05	17.11±0.02	14.17±0.02
	Total	64.61±2.9	60.48±0.15	63.84±0.306	72.12±0.07	69.43±1.6	68.15±0.28	65.08±2.44	70.04±0.12	68.84±1.72
	Unknown	4.424±0.03	0.13±0.01	5.11±0.226	0.44±0.005	0.39±0.1	0.31±0.003	4.84±0.16	3.28±0.04	2.66±0.07
	Total fatty acid	85.765±3.04	81.91±0.03	84.61±0.132	88.60±0.07	88.43±1.4	88.94±0.28	87.48±2.66	92.34±0.02	90.25±1.76

MUFA = Monounsaturated fatty acid; PUFA = Polyunsaturated fatty acid; C8:0 = Caprilic acid; C10:0 = Capric acid; C12:0 = Lauric acid; C14:0 = Miristic acid; C16:0 = Palmitic acid; C16:1 = Palmitoleic acid; C18:0 = Stearic acid; C18:1 = Oleic acid; C18:2 = Linoleic acid; C18:3 = Linolenic acid; C20:0 = Arachidic acid; C20:1 = Arachidonic acid.

Fatty acid composition of red fruit oil

The fatty acid composition of the oils extracted from the nine clones of red fruit is presented in Table 2. The fatty acids of the RFO samples were dominated by unsaturated fatty acids (USFA) ranging from 60.48 to 72.12 g per 100 g, whereas the saturated fatty acids (SFA) ranged from 15.7 to 21.4 g per 100 g. The main USFAs of the red fruit oil were oleic acid/C18:1 (49.36–64.47 g per 100 g), linoleic acid/C18:2 (4.13–16.06 g per 100 g), linolenic acid/C18:3 (0.78–1.24 g per 100 g), palmitoleic acid/C16:1 (0.50–1.74 g per 100 g) and arachidonic acid/C20:1 (0.1–0.27 g per 100 g). The SFAs were dominated by palmitic acid/C16:0 (14.11–19.21 g per 100 g) and stearic acid/C18:0 (0.98–1.67 g per 100 g), whereas caprylic acid/C8:0, capric acid/C10:0, lauric acid/C12:0, myristic acid/C14:0 and arachic acid/C20:0 were detected in small amounts, ranging from 0.004 to 0.22 g per 100 g.

Triacylglycerol composition of red fruit oil

Triacylglycerols (TAG) of the RFO samples from the nine clones (Table 3) were identified as consisting of 10 to 14 species. The TAGs of the RFOs were dominated by OOO/triolein and POO/palmito-diolein (24.23–31.08%), followed by OLO/linoleo-diolein (7.23–17.43%), PLO/palmito-linoleo-olein (3.72–14.30%), POP/oleo-dipalmitin (7.0–10.21%), PLL/palmito-dilinolein (1.40–4.66%), PLP/linoleo-dipalmitin (1.2–2.2%), SOO/stearo-diolein (1.77–4.21%), PPP/tripalmitin (1.32–1.57%) and POS/palmito-oleo-stearin (1.21–1.54%).

TAG structures can be grouped into UUU (triunsaturates), SUU (diunsaturates), SUS (monounsaturates) and SSS (trisaturates) fatty acids and differences in the position and type of fatty acids on the glycerol molecule produce different types of TAG (Jahaniaval *et al*., 2000; Basiron, 2005). The unsaturated fatty acid in the TAG of vegetable oil is generally esterificated at the sn-2 position (Scrimgeour, 2005). Based on fatty acid saturation, the TAG of RFOs consisted of: UUU/triunsaturates (37.77–47.65%), which included OOO and OLO; SUU (38.45–46.839%) that is, POO, PLO, PLL and SOO; SUS/monounsaturates (9.21–12.42%) including POP, POS and PLP; while for SSS/trisaturates (1.32–1.57%), it was PPP (Table 3). These results are consistent with the fatty acid composition of RFOs (Table 2), which consist predominantly of unsaturated fatty acids (oleic, linoleic and palmitoleic acids) and saturated fatty acids (palmitic and stearic acids).

The principle component analysis (PCA) results in Figure 1 show that 91.37% of the variance was represented by nine variables comprising the composition of fatty acids (C16, C18:1, C18:2, total SFA, total MUFA and total PUFA) and TAG (OOO, OLO and PLO) of RFOs from the nine clones. The positions of the nine red fruit clones derived from four areas of cultivation were distributed in the four quadrant of the biplot.

Figure 1 shows that the components PLO, OLO, C18:2, total PUFA can be used as a primary identifier for red fruit clones in quadrant I (*Hibcau* and *Hityom* from Minyambou district). Clones of *Mbarugum* (Koya district, Jayapura) and *Edewewits* (UNIPA Experimental Farm, Manokwari) were located in quadrant II, with C16 and total USFA identified as primary identifier components. The five clones of red fruit from Minyambou district (*Himbiak*) and from the UNIPA Experimental Farm (*Memeri, Monsor, Menjib Rumbai* and *Monsrus*) were located across quadrants III and IV with primary identifier components of OOO, C18:1 and total MUFA.

Total phenol and tocopherol content of red fruit oil

The total phenol and tocopherol contents of the RFOs from the nine clones are shown in Table 4. Total phenols were in the range 90 to 742 ppm. RFO oil with a red color had a total phenol content of more that 100 ppm, while oil with a yellow color had a total phenol content of less than 100 ppm.

Table 3 Mean ± SD (three measurements) of triacylglycerol (TAG) composition of red fruit oil from nine clones.

TAG	ECN	Amount of double bond	Group of structure TAG	Rred fruit clone								
				Menjib Rumbai	Edewewits	Memeri	Monsrus	Monsor	Mbarugum	Himbiak	Hibcau	Hityom
PLL	44	4	SUU	2.63±0.04	1.40±0.10	1.77±0.03	2.26±0.14	1.97±0.04	1.62±0.05	2.76±0.28	4.66±0.02	3.58±0.08
OLO	46	4	UUU	7.38±0.39	9.70±0.01	7.23±0.04	8.32±0.13	7.59±0.01	11.3±0.004	8.85±0.92	17.43±0.09	13.43±1.53
PLO	44	3	SUU	7.01±0.28	10.15±0.06	3.72±0.05	6.05±0.12	6.96±0.08	11.63±0.26	8.00±0.14	14.30±0.13	12.11±0.65
PLP	46	2	SUS	1.36±0.16	2.21±0.11	1.81±0.01	1.10±0.17	1.21±0.20	2.03±0.02	1.84±0.23	3.69±0.20	3.51±0.41
OOO	48	3	UUU	34.77±0.6	28.07±0.12	39.81±0.13	39.34±0.49	35.43±0.36	29.58±0.34	33.34±1.16	26.0±0.20	26.84±0.26
POO	48	2	SUU	31.01±0.2	31.08±0.07	30.56±0.10	27.94±0.45	30.77±0.30	28.28±0.46	29.06±0.41	24.23±0.09	26.79±1.19
POP	48	1	SUS	8.62±0.06	10.21±0.12	7.41±0.03	7.84±0.08	8.49±0.33	8.25±0.30	8.56±0.62	7.0±0.07	7.98±0.76
PPP	48	0	SSS	1.57±0.10	-	-	1.36±0.05	-	-	1.32±0.17	-	-
SOS	50	1	SUS	1.77±0.23	4.21±0.02	2.40±0.07	2.84±0.12	2.34±0.09	3.38±0.21	2.64±0.19	-	2.22±0.01
POS	50	1	SOO	1.24±0.09	-	-	1.21±0.18	1.33±0.20	-	1.54±0.13	-	-
Unknown				2.76±0.17	2.97±0.04	5.26±5.35	2.06±0.26	3.91±0.09	3.98±0.10	2.09±0.13	2.7±0.003	3.53±0.15
TOTAL												
			UUU	42.15±0.9	37.77±0.13	47.04±0.17	47.65±0.62	43.02±0.37	40.83±0.34	42.19±2.08	43.43±0.29	40.28±1.79
			SUU	42.43±0.8	46.83±0.25	38.45±0.26	39.09±0.83	42.04±0.50	44.91±0.98	42.46±1.01	43.19±0.24	44.70±1.94
			SUS	11.21±0.3	12.42±0.23	9.21±0.04	10.16±0.43	11.03±0.74	10.28±0.32	11.94±0.98	10.69±0.27	11.49±1.17
			SSS	1.57±0.1	-	-	1.36±0.05	-	1.32±0.17	-	-	-

ECN = CN - 2DB (ECN = Equivalen Carbon Number. CN = Carbon Number. DB = Double Bonding); La = Lauric acid; M = myristic acid; P = palmitic acid; S = stearic acid; O = oleic acid; L = linoleic acid U = unsaturated; S : saturated; UUU (triunsaturates); SUU (diunsaturates); SUS (monounsaturate); SSS (trisaturates).

- = Not detected

The total tocopherol contents in the RFOs were in the range 234 to 1,728 ppm. Clones of red fruit with a yellow color (*Menjib Rumbai*) had a total tocopherol cotent of less than 250 ppm whereas those having a red color had a tocopherol content of more than 1,000 ppm. Overall, the RFOs of the nine clones analyzed contained α-tocopherol (52–229 ppm) and γ-tocopherol (16–287 ppm) as shown in Table 4. The clones of red fruit with α-tocopherol levels of more than 200 ppm were
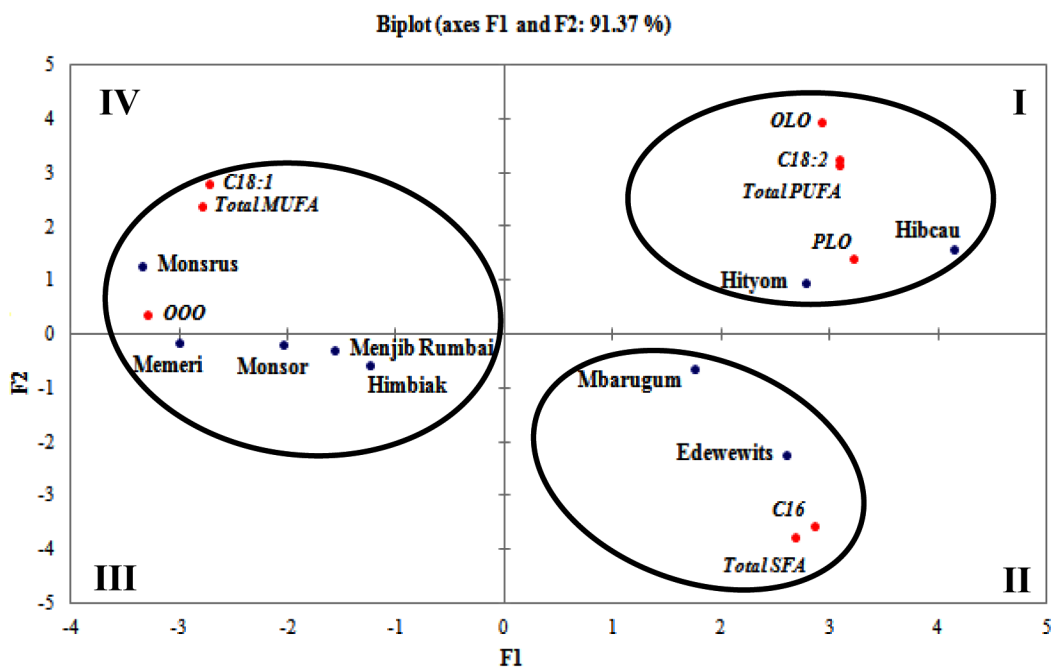

Figure 1 Distribution of nine red fruit clones based on principle component (PC) 1 (F1) and PC 2 (F2) with nine variables of the fatty acids (C16, C18:1, C18:2, total SFA, total MUFA and total PUFA) and triacylglycerols (OOO, OLO and PLO) composition of red fruit oils.

Table 4 Content (mean ± SD for three measurements) of total phenols, total tocopherol, α-tocopherol and γ-tocopherol of red fruit oil from nine clones.

Red fruit clone	Total Phenol (ppm), mean ± SD	Total Tocopherol (ppm), mean ± SD	α-Tocopherol (ppm), mean ± SD	γ-Tocopherol (ppm), mean ± SD
Menjib Rumbai	90 ± 4	234 ± 26	54 ± 2	19 ± 1.0
Edewewits	222 ± 15	1207 ± 124	229 ± 13	35 ± 1.5
Memeri	310 ± 4	1728 ± 97	272 ± 27	67 ± 3.2
Monsrus	292 ± 7	1538 ± 56	52 ± 4	26 ± 2.1
Monsor	429 ± 26	1614 ± 29	104 ± 1	25 ± 0.5
Mbarugum	742 ± 84	1538 ± 135	184 ± 4	287 ± 8.2
Himbiak	232 ± 31	1615 ± 124	153 ± 4	38 ± 1.7
Hibcau	705 ± 19	1060 ± 55	88 ± 2	16 ± 1.8
Hityom	127 ± 10	1381 ± 75	140 ± 9	39 ± 0.2

Memeri and *Edewewits* whereas the red fruit clones of *Mbarugum, Himbiak* and *Hityom* had α-tocopherol levels of 100 to 200 ppm. The levels of α-tocopherol content obtained in the current study were in agreement with that of Surono *et al.* (2008), who reported that the α-tocopherol content of RFO from Jayapura, Papua was 212 ppm. *Mbarugum* clone was reported to have the higest level (more than 200 ppm) of γ-tocopherol content, followed by *Memeri, Hityom, Himbiak* and *Edewewits*.

DISCUSSION

The water content is important in determining the quality of the oil, because the presence of water can cause hydrolysis reactions that lead to damaged oil, characterized by increasing levels of free fatty acids (Ngando *et al.*, 2006). The water content of the RFOs from the nine clones obtained in the current study (Table 1) was still relatively low. Using the Indonesian National Standard for crude palm oil (CPO) as a reference, the maximum water content allowed is 0.5% (SNI, 2006). This result indicated that the preparation and extraction method used in the current study was done properly.

However, the FFA levels of the RFOs from the nine clones were relatively high (Table 1), compared with those of the standard for CPO (FFA maximum of 0.5%; SNI, (2006)) and of cooking palm oil (FFA maximum of 0.3%; SNI, (2012)). High levels of FFA in the RFOs indicated the occurrence of oil hydrolysis, that is, breaking down of the ester bond between fatty acids and glycerol to produce FFA. Hydrolysis can be triggered by the presence of water and fruit internal lipase activity (Ngando *et al.*, 2006), which may occur during post harvest and during the oil extraction process. In this aspect, improvement of post-harvest handling of red fruit is needed. Furthermore, the difference in the percentage of FFA in the RFOs may also be influenced by the clone (that is, its genetic makeup) and fruit maturity (Sarungallo

et al., 2013). Furthermore. Salvador *et al.* (2001) reported that the olive oil at the end of maturation results in a higher acidity due to internal lipase activity and sensitivity to pathogen infection and mechanical damage becomes higher.

The IV of the RFOs of the nine clones varied. Oil from the *Memeri* clones had the highest IV, indicating the least saturated oil, whereas RFO from the *Edewewits* clones had the lowest IV, indicating the most saturated oil. RFOs with low IV (high saturation) were observed as solid at room temperature (such as the oils of the *Edewewit* and *Himbiak* red fruit clones) whereas those having high IVs (oils of *Memeri, Menjib Rumbai* and *Monsor* red fruit clones) remained liquid at room temperature.

Phosphorus (present as phospholipids) is undesirable because it can cause problems in purification, especially during the degumming process (Sambanthamurthi *et al.*, 2000; Szydlowska-Czerniak and Szlyk, 2003; Anderson, 2005). In addition, phosphorus can react with the metal and free fatty acids that can increase oxidative damage (Sambanthamurthi *et al.*, 2000; Szydlowska-Czerniak and Szlyk, 2003). Therefore, a red fruit clone with a low phosphorus content is more beneficial as a raw material because it can reduce the cost of purification.

The FA composition of the RFOs was dominated by unsaturated fatty acids (USFA) (Table 2). *Monsrus* was the clone with the highest number of USFAs, while *Edewewits* had the highest level, which was also supported by its iodine number (Table 1). The results of the current study are similar with those of Rohman *et al.* (2012), which reported that the FA composition of RFO from Jayapura (Papua) consisted of oleic (68.8 g per 100 g), linoleic (8.5 g per 100 g), palmitoleic (0.15 g per 100 g) and palmitic (20.1 g per 100 g) acids.

The composition of FAs contributes both to the physical properties and chemical properties of the oil (Scrimgeour, 2005). The contents of oleic and linoleic acids in RFO are higher than

that of palm oil. For example, Li *et al*. (2012) reported that the contents of oleic and linoleic acids in palm oil were 31.5 g per 100 g and 12.7 g per 100 g, respectively, but palm oil contained a level of palmitic acid (39.9 g per 100 g) higher than red fruit. Chowdhury *et al.* (2007) reported that the linoleic acid content of soybean oil (52.18 g per 100 g) which was higher than in RFO in the current study, but their reported contents of oleic (23.27 g per 100 g) and palmitic (14.04 g per 100 g) acids were lower than in RFO. In comparison, the FA composition of RFO was more similar to that of olive oil, as Firestone (2005) reported that the respective acid contents in olive oil were oleic (57.3 to 71.6 g per 100 g), linoleic (7.62 to 15.13 g per 100 g) and palmitic (7.8 to 14.03 g per 100 g).

The composition of the TAGs of the RFOs (Table 3) was different from that of palm oil which is dominated by POO (20%) and POP (20.5%) according to Sundram *et al.* (2003), soybean oil dominated by LLL (20.5%), OLL (14.95%) and PLL (14.94%) according to Jahaniaval *et al*. (2000) or sesame oil dominated by OLL (17.9%), OOL (17.9%), POL (14.6%) and (OOP 13.1%) according to Jahaniaval *et al*. (2000), but resembled olive oil containing OOO (23.2–39.9%) and POO (22.0–26.0%) according to Firestone, (2005). Christopoulou *et al.*, (2004) reported that these quantitative differences in the TAG composition of vegetable oils can be used as markers for their detection of oil adulteration.

Generally, the melting behavior of oil can be estimated by the composition of FAs, with oils having a longer chain and and more saturated fatty acids having a higher melting point (Sundram *et al*., 2003). Data on the basis of UUU (triunsaturates), SUU (diunsaturates) and SUS (monounsaturates) fatty acids highlighted general similarities and differences among the RFOs of the nine clones (Table 3). For example, the total UUU of RFOs ranged from 37.77 to 47.65% and as a result, the melting point of the oil of the *Edewewits* clone was the highest and it was present in solid

form at room temperature, while the melting point of oil of the *Monsrus* clone was the lowest and it was present in liquid form at room temperature. The total UUU of RFOs was lower than for olive oil (51.4–56.1%) although their levels of the main species of TAG (OOO and PLO) were similar (Firestone 2005). Jahaniaval *et al*., (2000) showed that the total levels of UUU of corn oil (58.8%) and soybean oil (54.2%) were higher than in RFO but were similar with sesame oil (46.6%) although differing in the TAG species. Palm oil had a lower content of total UUU (9.53%) than RFO, as well as a lower content of SUU (37, 53% according to Sundram *et al.* (2003). Based on the TAG composition, it could be expected that RFOs have functional properties different from other vegetable oils.

Principle component analysis (PCA) based on the lipid profile of the RFOs from the nine clones, resulted in three groups in agreement with their content of FAs and TAG species (Figure 1). The distribution of red fruit clones of *Hibcau* and *Hityom* were located in quadrant I, having containing higher levels of C18:2, total PUFA (polyunsaturated fatty acid), PLO and OLO than in the other clones (Table 3). The red fruit clones of *Mbarugum* and *Edewewits* were located in quadrant II because they contained higher levels of C16:0 and total SFA than the other clones, while clones with a higher content of OOO, C18:1 and total MUFA (monounsaturated fatty acid) were located in quadrant III (*Memeri*, *Monsor*, *Menjib Rumbai* and *Himbiak*) and quadrant IV (*Monsrus*).

The location of the third red fruit cultivation area (Minyambou district) was in the highlands (above 1,000 m above sea level), while Koya district, Jayapura and the Experimental Garden of the State University of Papua and Manokwari are all located in the lowlands (below 200 m above sea level). According to the PCA results (Figure 1), it is common for red fruit clones from the highlands (Minyambou district) located in quadrant I (*Hibcau* and *Hityom*) to have a

characteristic component of USFA (linoleic acid). Turhan *et al.* (2010) also reported that the highest linoleic acid content of olive oil was found from the location having the highest average altitude and the lowest temperature. Harris *et al.* (1978) explained that increasing temperature resulted in an increase in the linoleic acid content of sunflower oil due to the effect of temperature on the activity of the desaturase enzymes that convert oleic to linoleic acid.

In the current study, most of the red fruit clones from the lowlands (Experimental Garden of the State University of Papua, Manokwari) were located in quadrant III (*Memeri*, *Monsor*, *Menjib Rumbai*) and quadrant IV (*Monsrus*) that had a high content of MUFA. Esmaeili *et al.* (2012) also reported that the differences observed between locations for FA composition may be explained by the different altitudes of the locations. They found that the lower temperatures reduced the amount of palmitic acid (SFA) and increased the level of oleic acid (USFA). In addition, the amount of USFA increased when the rainfall and humidity increased during fruit growth periods (Sadeghi and Talaii, 2002).

The different location in the lowlands in Koya distric (Jayapura)—namely, *Mbarugum*—was located in quadrant II with a higher SFA content. The temperature in Koya distric (Jayapura) was lower than in the Experimental Garden of the State University of Papua (Manokwari). This result was supported by Turhan *et al.* (2010) who reported that the level of palmitic acid in olive oil tended to increase in warmer locations. In addition, Flagella *et al.* (2002) showed the effect of sowing time on some FAs in sunflower and found that palmitic acid reduced early sowing compared to late sowing as a result of the lower temperature in the early sowing growth period.

Even though red fruits from the highlands were grouped in quadrant I, the *Himbiak* clone was separated in quadrant III because it had a higher content of oleic acid than Hityom and Hibcau. Consequently, as with the *Edewewits*

clone from the lowlands (Experimental Garden of the State University of Papua) it had a strong differing characteristic with regard to its palmitic acid content and grouping with the *Mbarugum* clone (from Koya distric) in quadrant II. This result indicated that the FA composition of red fruit also depended on genetic properties. Poiana and Mincione (2004) reported that the FA trends of olive oil from nine cultivars showed characteristics typical of the cultivar considered and that the FA and TAG compositions of red fruit oil varied widely depending on the clone (genetic material) and location of their cultivation. However some researchers reported a great influence of environmental factors such as year of harvest, location, season or climatic conditions (in particular the temperature and rainfall during fruit growth and ripening) on the variation of some FAs of vegetable oil (Sadeghi and Talaii, 2000; Flagella *et al.* 2002; Turhan *et al.* 2010; Esmaeili *et al.* 2012).

The phenolic content of oil is one of the important parameters associated with its functionality and quality. Phenolic compounds may contribute to oil flavor and protect the fatty acids from oxidation (Servili and Montedoro, 2002). The total phenol content of RFO (Table 4) was higher than that of grape seed oil extract (59.0–115.5 ppm) according to Bail *et al.* (2008), but similar with that of virgin olive oil and olive pasta (343–350 ppm and 207–210 ppm, respectively), according to Cioffi *et al*. (2010). Thus, the content of phenols in RFO can also play a role in increasing the activity of natural antioxidants.

The total tocopherol content of RFO in the current study was relatively higher than for crude palm oil. Sundram *et al.* (2003) reported that the total tocopherol content of crude palm oil ranged from 600 to 1,000 ppm and decreased during refining of the oil to 350–630 ppm. In terms of the α-tocopherol and γ-tocopherol contents, the RFOs from the nine clones were similar to that of palm oil, which was reported to contain 35–66.5 ppm

and 22–35 ppm, respectively, (Sambanthamurthi *et al.,* 2000; Li *et al.,* 2012). Similarly, olive oil was reprted to contain α-tocopherol with a range of 2 to 190 ppm (Firestone, 2005) and grape pomace to contain α-tocopherol from 18.4 to 50.2 ppm and γ-tocopherol from 1.3 to 47.5 ppm (Tangolar *et al.*, 2011). It was also reported by Tan *et al.* (2009) that CPO-low free fatty acid contained γ-tocotrienol at 570 ppm (± 44%) as a major part of the total tocopherol (1,293 ppm) followed by α-tocotrienol (309.7 ppm), α-tocopherol (235.3 ppm) and δ-tocotrienol (194.7 ppm). Therefore, it can be presumed that the RFOs had quite high levels of total tocopherol (above 1,000 ppm), including other tocopherol derivatives (tocotrienol), α-tocopherol and γ-tocopherol.

The results of this study also showed that each red fruit clone had unique chemical characteristics, including a unique lipid profile, with different phenol and tocopherol contents, generally higher than those of other vegetable oils (palm oil and olive oil). Based on this identified characteristic, some red fruit clones (*Mbarugum, Memeri, Monsor, Edewewits, Hityom* and *Himbiak*) can be selected to be developed for cultivation, due to their high contents of α-tocopherol and γ-tocopherol.

CONCLUSION

The chemical characteristics, lipid profile and the active component of RFO varied among clones. The main FAs of the RFOs were identified as oleic (49.36–64.47 g per 100g), linoleic (4.13–16.06 g per 100g) and palmitic (14.11–19.21 g per 100g) acids, while the major TAG species were OOO (26.84–39.81%), POO (24.23–31.08%), OLO (7.23–17.43%) and PLO (3.72–14.3%). Oil of red fruit also contained active compounds such as phenol and tocopherol as natural antioxidants. The existence of phenols and tocopherols contributed to the stability of the oils. The results of PCA showed that nine clones of red fruit can be clustered into three groups in

accordance with their composition of FA and TAG species. Overall, RFO has good chemical properties, as well as containing sufficiently high active components so that it can potentially be developed as an edible oil, as well as a functional ingredient for industrial applications.

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