Component, Fatty Acid And Mineral Composition Of Rice Bran Oil Extracted By Multistage With Hexane And Ethanol

Fajriyati Mas'ud, Meta Mahendradatta, Amran Laga, Zainal Zainal

Abstract: Rice bran oil (RBO) has been extracted from Celebes rice bran by multistage extraction with hexane solvent followed by ethanol to see the component, profile of fatty acids and mineral contained in both of them. As a comparison, RBO directly extracted with ethanol was also presented. Extraction process was performed using reflux method at 55^{o}C, for 5 hours with bran and solvent ratio of 1:7. Analysis of components and fatty acids of RBO was conducted with GC-MS QP 2010 Shimadzu. Oleic, linoleic and palmitic were found dominant in first stage extraction by hexane with concentration of 3716.56, 1630.78 and 1021.89 mg/L, respectively. Palmitic (6.34 mg/L), lauric (4.78 mg/L), and linoleic (3.52 mg/L) were dominant in the second stage extraction by ethanol. Linoleic (28.85 mg/L), stearic (2.88 mg/L) and myristic (2.02 mg/L) were found in extracted directly by ethanol. RBO extracted with hexane had 18.6% of saturated fatty acid and 81.4% of unsaturated fatty acids, with ratio of saturated fatty acids : monounsaturated fatty acids: polyunsaturated fatty acids of approximately 1: 2.3 : 1.3. It contained about 56.7% of monounsaturated, 24.7% of polyunsaturated, and 18.6% of saturated fatty acids. In the present paper, we provide also an analysis of mineral composition of RBO by X-ray Spectrometer and melting point of RBO by Differential Scanning Calorimeter (DSC) instrument.

Keywords: Edible oil, fatty acid, mineral, multistage extraction, solvent extraction

1 INTRODUCTION

Rice bran, a by-product which is obtained from the rice milling processes, it is can be used as a major source of RBO [1[, [2]. The utilization of RBO has increased considerable due to its well-known for wealth of phytochemical [3] and its fatty acid profile. Fatty acids played an important role in metabolism, which was the main fuel for the metabolism, storage and transportation of energy, as an essential component of all membranes, and as a gene regulator [4]. The nutritional recommendations indicated that 25% of energy should be covered by lipid [5] and total fat daily intake of 20%-35% of energy was recommended by Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO) [6].

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Lipid consisted of fatty acids which were classified based on the presence or absence of double bonds as saturated fatty acids (SFA), without double bonds; monounsaturated fatty acids (MUFA), with one double bond; and polyunsaturated fatty acids (PUFA), with two up to six double bonds [7]. The human body cannot synthesize PUFA with the first double bond on C3 and C6 of the methyl-end due to the absence of appropriate enzymes. Thus, these fatty acids must be obtained from diet [8], [9], [10]. Analysis of fatty acids in the food was very important because of the implications of nutrition and its effect on health [11]. Oil fractionation to determine the fatty acid composition was quite urgent to produce a product with physical or nutritional properties of interest to the food industry [12]. The content of fatty acids and the ratio of saturated and unsaturated fatty acids were important parameter for determining the nutritional value of the edible oil, because of the latest trends in the food processing industry to inform the composition of fatty acids in the oil. Related with it, the physical and chemical characteristics of oils and fats are strongly influenced by the kind and proportion of fatty acids on triacylglycerols [13], [14]. In general, fatty acids in oils and fats extracted from plant consisted of unsaturated fatty acids in high percentage. Unsaturated fatty acids were generally more susceptible to oxidative damage [14], [15], [16]. Therefore, it was important to know the composition of fatty acids from oils or fats, to identify their characteristics and to determine the possibility of adulteration as well as to know the stability and physicochemical properties of oils [14], [17]. One method used to determine the composition of the fatty acids was based on chromatography analysis of their methyl ester [18]. The major components of lipids were fatty acids, and their physical, chemical, and physiological properties depend largely on its fatty acid composition. The quality and freshness of the vegetable oil were determined by the constituent fatty acid, because long chain organic acids were not highly volatile compounds [19], [20]. On the other hand, although the overall quality of the product was generally defined by the culinary benefits, the presence of inorganic compounds in the oils has very important role in terms of food safety and general product longevity. The mechanisms whereby inorganic constituents

were incorporated into the oil include the natural uptake and accumulation of trace element by the plant and addition or adulteration at some point. The inorganic component could also reach the oil as contamination during processing [21]. Some elements might act as an accelerator auto oxidation, changing the taste and quality of products from time to time [22]. The quality of vegetable oil was directly related to the concentration of the mineral [23], the concentration of elements and substances that are significant in the end product can affect the taste, color and stability. One way of extracting neutral lipid fractions from plant sources of oil was the use of organic solvents [24]. Soxhlet extraction results in sesame revealed that polar solvent such as ethanol was good solvent compared to non-polar solvents. These results could be explained by the interaction between the unsaturated fatty acids with a polar solvent, compared with non-polar solvents [25]. On the other hand, hexane was the most commonly used, and preferred for the extraction of oil from seeds, due to its availability at a reasonable cost and its suitable functional characteristics for oil extraction. Among these characteristics are high-strength solvent to dissolve triglycerides at a fairly low temperature, non-reactivity with oil and miscella, and requires simple equipment. Related to solvents, some report studies had compared the effectiveness of hexane and ethanol in oil extraction [26]. But throughout our literature study, RBO extraction was generally carried out with one kind of solvent and repeated several times, or comparing the effectiveness of some kind of solvent with a certain ratio. Based on these, one method that was a major preoccupation in this study, for the first time, were multistage extraction of RBO by hexane followed by ethanol, so all the components of polar and non-polar of RBO and primarily long chain fatty acids and short chain contained in RBO can be extracted perfectly. As comparison, it was also presented fatty acid and mineral composition of RBO extracted directly with ethanol. In addition, analysis of mineral composition of RBO by X-ray Spectrometer and melting point of RBO by DSC was also conducted to assess its potential to be developed as raw material of oil-based food processing.

2 EXPERIMENTAL PROCEDURE

Celebes rice bran was obtained by milling rice grain in a local grinding mill Makassar-Indonesia during March to April in 2017. All chemicals and reagents used in the experiment were from Merck, Germany. Standards of Fatty Acid Methyl Ester (FAME) were from Supelco Inc., Bellefonte, PA (Supelco 37 Component FAME Mix) and other reagents from Merck, Germany. Freshly milled bran samples were directly collected from the milling system in polyethylene bags. The rice bran was screened through a 60 mesh sieve to have a uniform particle size and heated at autoclave (Hiclave HV-85 Hirayama) at 100^{o}C for 15 min to inactivate endogenous lipase. At experimental unit 75 grams of rice bran were weighed in the reactor 1.0 L four neck flasks, RBO is extracted with 525 mL solvent using a heating mantle connected with the thermometer setting at 55^{o}C for 5 hours, agitator on the top, speed of 200 rpm, RBO and bran residue are separated by centrifugation (refrigerated AX-521 centrifuge) at a speed of 3500 rpm, 20 min. The liquid part accommodated in the flask evaporator, solvents removed on a rotary evaporator Buchi R-215 incorporates vacuum Pomp V-700, speed of 60 rpm, the heating temperature of 35^{o}C, and the evaporation temperature 21°C. RBO obtained was packaged in a dark glass bottle and stored in a freezer for analysis. The bran residue from hexane extraction subsequently extracted with ethanol.

2.1 Analysis component of RBO

0.05 g of RBO dissolved in methanol-chloroform (1:1) and homogenized by vortex, inserted in the vial GC-MS, the sample was injected automatically. Component of RBO was analyzed using GC-MS ultra Shimadzu QP2010, instrument uses column Rxi SH-5Sil MS (length 30 m, diameter x thickness 0.25mm ID 0:25 um df). Helium was used as a carrier gas at a pressure of 76.9 kps with a flow of 14.0 mL/min, split ratio of 1:10. The initial temperature of the oven starting at 110^{o}C and hold for 2 min until the oven temperature to 200°C with an isothermal increase of 10°C/min, a final temperature of 280°C, hold for 9 min with an isothermal increase of 5°C/min, the total analysis time of 40 min. Scan interval 45-450 m/z. Ion temperature 200^{o}C and temperature interface 280^{o}C. Identification of compounds: the mass spectrum of the compound in the sample was obtained by electron ionization (EI) at 70 eV and the detector operator in scan mode from 45 to 450 m/z. Identification based on molecular weight, molecular formula, retention time and area (%).

2.2 Preparation and analysis of FAME

Fatty acid composition of RBO was verified by gas chromatography (GC-MS ultra Shimadzu QP2010). Lipids were esterified by method adapted from Metcalfe [27] which consisted of lipid saponification with KOH 0.5 M in methanol solution, and catalyzed BF_3-MeOH reagent. The sample was solubilized by dichloromethane, from which 1 µL was injected for GC analyses. To separate and quantify the esterified fatty acid mixture, GC-MS QP 2010 by Shimadzu was used equipped with split/splitless injector, capillary column RTX®-1 (30 mx0.25 mmIDx0.25 µm) and flame ionization detector (FID). Helium was used as the carrier gas at flow of 1.25 mL/min. The injector and detector temperature were set to 260^{o}C. The chromatographic conditions for separation were column initial temperature of 50^{o}C, raising to 200^{o}C at a flow rate of 6^{o}C/min, holding during 4 min, the second step consisted in increased at a heating rate of 2^{o}C/min to 240^{o}C, and held for 10 min. FAME peaks were identified by comparing their retention time and equivalent chain length with respect to standard FAME.

2.3 Mineral and differential scanning calorimeter (DSC) analysis

Mineral analysis by S2 Ranger X-ray Spectrometer. Analysis according the user manual XRF (2012) by Bruker AXS GmbH, Ostiche Rheinbruckenstr, 49.76187 Karlsruhe, Germany. DSC analysis according to the user manual of Shimadzu DSC-60 Plus Series.

3 RESULTS AND DISCUSSION

Rice bran oil has been extracted from Celebes rice by multistage extraction with hexane (first stage) and ethanol (second stage). Analysis by GC-MS showed that some component obtained from first stage were: undecane; octane,2,3,7-trimethyl; tridecane; tetradecane; 9-Oxononanoic acid; hexadecane; tetradecanoic acid; 2-pentadecanone,6,10,14-trimethyl; pentadecanoic acid; hexadecanoic acid, methyl ester; n-hexadecanoic acid;

hexadecanoic acid,1-methylethyl ester; l-(+)-ascorbic acid 2,6-dihexadecanoate; n-hexadecanoic acid; heptadecanoic acid; pregn-5-ene-3,11-dione, 17,20:20,21-bis[methylenebis(oxy)]-, cyclic 3-(1,2-ethanediyl acetal); 9,12-octadecadienoic acid (Z,Z)-, methyl ester; 9-octadecenoic acid, methyl ester; 9-octadecenoic acid (Z); octadecanoic acid, methyl ester; l-(+)-ascorbic acid 2,6-dihexadecanoate; 9,12-Octadecadienoic acid, ethyl ester; ethyl oleate; cis-9-hexadecenal; 4,8,12,16-Tetramethylheptadecan-4-olide; hexadecanal; hexadecanal 2-methyl; tetratriacontane; hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester; 1,2-benzenedicarboxylic acid; 9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester; 9,12-octadecadienoic acid (Z,Z)-,trimethylsilyl ester; 2-[12-(2-oxiranyl)dodecyl]oxirane; oleoyl chloride; tetracosanoic acid, methyl ester; 9,12-octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester; cedrane, 8-propoxy; 2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E); 17-pentatriacontene; tetratetacontane; oryzanol, vitamin E; and 2H-1-benzopyran-6-Ool, 3,4-dihydro-2,7,8-trimethyl-2-(4,8,12,16,20,24,28,32-octamethyl. In the second stage obtained some component of RBO were: tetradecane; phenol, 2,4-bis(1,1-dimethylethyl); dodecanoic acid; hexadecane; tetradecanoic acid; 2-butanamine, n-(1-methylpropylidene); octadecane; 2-pentadecanone, 6,10,14-trimethyl; pentadecanoic acid; heneicosane; hexadecanoic acid, methyl ester; n-hexadecanoic acid; 4-ethenylhexahydro-4,7a-dimethyl-2(3H)-benzofuranone,[3ara-(3a .alpha.,4 .beta.,7a .beta)]; 9,12-octadecadienoic acid (Z,Z)-, methyl ester; 9-octadecenoic acid, methyl ester, (E); octadecanoic acid, methyl ester; 9-octadecenoic acid, (E); heneicosane; 9,12-octadecadienoic acid (Z,Z)-, methyl ester; hexatriacontane; pregn-20-YN-17-ol; 1,2-benzenedicarboxylic acid, oryzanol, ditridecyl ester; 9,12-octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester; hexadecanoic acid, tetradecyl ester; and hexatriacontane. Table 1 showed that the group of SFA was predominantly of Celebes RBO, although the composition was slightly. Oleic, linoleic, and palmitic acids were found in large numbers. In RBO extracted with hexane, all of fatty acids could be well extracted though the result of second stage extraction with ethanol was still found. RBO extracted with hexane had 18.6% of SFA and 81.4% of unsaturated fatty acids with ratio of SFA : MUFA : PUFA of approximately 1 : 2.3 : 1.3. It contained about 56.7% of MUFA, 24.7% of PUFA, and 18.6% of SFA. Oleic acid which found to be a predominant MUFA in the majority of samples was 56.39%. Concentration of palmitic acid as SFA and linoleic acid as PUFA were 15.51% and 24.74%, respectively.

***Table 1**: Fatty acid composition of Celebes RBO obtained by multistage extraction*

Fatty acid	Lipid Numbers	Group	Multistage extraction	
			Stage 1 (Hexane) (mg/L)	Stage 2 (Ethanol) (mg/L)
Lauric acid	C12:0	SFA	0.002	4.78
Myristic acid	C14:0	SFA	0.002	2.22
Palmitic acid	C16:0	SFA	1021.89	6.34
Pentadecyclic acid	C15:0	SFA	1.73	0.03
Palmitoleic	C16:1	MUFA	17.03	0.03
Stearic acid	C18:0	SFA	125.81	1.81
Oleic acid	C18:1	MUFA	3716.56	0.55
Linoleic acid	C18:2	PUFA	1630.78	3.52
Arachidic acid	C20:0	SFA	47.74	0.03
Behenic acid	C22:0	SFA	17.5	0.05
Tricosylic acid	C23:0	SFA	1.43	0.02
Lignoceric acid	C24:0	SFA	9.97	0.01

***Table 2:** Fatty acid composition of Celebes RBO obtained by direcly extraction using ethanol*

Fatty acid	Lipid Numbers	Group	Single stage extraction (Ethanol) (mg/L)
Lauric acid	C12:0	SFA	4.78
Myristic acid	C14:0	SFA	2.02
Palmitic acid	C16:0	SFA	0.01
Pentadecyclic acid	C15:0	SFA	0.01
Palmitoleic	C16:1	MUFA	0.08
Stearic acid	C18:0	SFA	2.88
Oleic acid	C18:1	MUFA	0.08
Linoleic acid	C18:2	PUFA	28.85
Arachidic acid	C20:0	SFA	0.04
Behenic acid	C22:0	SFA	0.04
Tricosylic acid	C23:0	SFA	0.01
Lignoceric acid	C24:0	SFA	0.01

In the second stage extracted with ethanol produced the highest levels of linoleic acid (PUFA) amounted to 84.44%. As for the results of direct extraction with ethanol obtained linoleic (28.85 mg/L), stearic (2.88 mg/L) and myristic (2.02 mg/L) (Table 2). Extraction with ethanol could produce the highest levels of PUFA [28]. Ethanol resulted the food-grade extract which was rich in polar lipid and PUFA [29] and food grade hexane extract which rich in neutral lipids [30]. For absorption in the human body and their biological functions, differences in lipid structure were considered as very important [31]. Figure 1 shows the GC-MS chromatogram of fatty acid on RBO extracted with hexane. It can be seen that oleic acid (9-octadecenoic acid) is a fatty acid mostly in RBO (peak number: 7). Oleic peak appearing at retention time of 21.215 with 36.05 %area, area/high (A/H) of 4.99, the area of 157103345. The area indicates the amount of compound concentration, the larger the area, the greater the concentration of these compounds. Can be seen on the enlarged scale that concentration of oleic of 3716.56 mg/L. The next dominant fatty acids were linoleic and palmitic seen in peaks numbered 6 and 4 with retention times of 21.075 and 18.291, respectively.

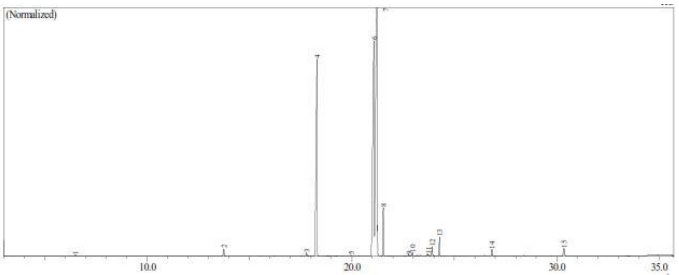

***Fig. 1**: GC-MS chromatogram of fatty acid on Celebes RBO*

Rice bran contained specific lipids in distinct ratios [32]. RBO has an optimal ratio of SFA, MUFA and PUFA of about 1: 2.2: 1.5, which was very close to the recommendation of WHO, RBO contained about 47% of MUFA, 37% PUFA and 20% SFA [33]. Major fatty acid of RBO was 40.2% linolelaidic, 33.6% oleic and 20.2% of palmitic acid [34]. Unsaturated fatty acids were about 75% (w/w) in oil; the major fatty acid compositions of RBO were oleic, linoleic and palmitic acids. It

mainly contained oleic (36-38%), linoleic (35-38%) and α-linolenic (from 1.8 to 2.4%) as unsaturated fatty acids. Palmitic (21- 25%) and stearic (2.7 to 3.0%) acids were SFA [35], [36], [37]. Recent studies had clearly shown a significant impact of PUFA in human health in disease prevention, particularly cardiovascular disease, coronary heart disease and cancer, inflammatory, thrombotic and autoimmune diseases, hypertension, type two diabetes, kidney disease, rheumatoid arthritis, ulcerative colitis, and Crohn's disease [38], [39]. RBO might assist reducing LDL cholesterol due to its fatty acid profiles and high quantity of unsaponifiable compounds [40]. Relative concentration and distribution of fatty acids in dietary fats had been reported to lower the risk of cardiovascular disease [41]. Diet with increasing the intake of linoleic and linolenic acids increased HDL-cholesterol and LDL-cholesterol lowering, while the intake of higher oleic acid could decrease LDL-cholesterol, but did not affect the HDL cholesterol level [42]. High content PUFA in oil indicate the high nutritional quality of oil. Owing to this high PUFA content, it exhibits properties prevention of thrombosis, inhabitation of cardiovascular diseases, reduction of cholesterol in serum, dilation of blood vessels, reduction cancer and regulation of autonomic nerves [43], [44]. Reports the results of the study show the potential of RBO as functional food. Short chain fatty acids generally had lower melting point and more water soluble. Long chain fatty acids had higher melting points. Unsaturated fatty acids had lower melting point compared to SFA of similar chain length. The rate of unsaturated fatty acid to SFA in edible oils and fats was very important for human nutrition. Therefore, high levels of SFA desirable on oil were beneficial to improve the stability. On the other hand, the presence of SFA in the oil was undesirable, because high levels of SFA might increase the concentration of low density lipoprotein (LDL), thus affecting the ratio of LDL to HDL (high density lipoprotein), promoting clothing and vascular smooth muscle proliferation [45]. Edible oils which rich in unsaturated fatty acids, especially MUFA had ability to lower the total and LDL cholesterol, triacylglycerol levels and increased HDL cholesterol that helps control blood pressure. In addition, they had anti thrombotic properties and also improved insulin sensitivity [46]. In rice bran lipids were concentrated approximately 19.4 to 25.5% [7], it was about 15-25%, which contained 95% of triacylglycerols and 4% of unsaponifiable lipids [47]. RBO main lipid fraction composed of neutral lipids, was the unsaponifiable fraction which included antioxidant, about 3 to 5% (w/w) [19], [48]. However, the component and composition of fatty acid in the oil varied depending on the source of oils, and technology process used for oil extraction, but each vegetable oils had a distribution of certain fatty acids depending on the plant source, so the impact on human health could assessed in accordance with the fatty acid content, because the fatty acids had different effects on human health and the risk of disease that could be caused [49]. In neem oil extraction, ethanol was good substitute for hexane, and able to provide the oil yield 41.11% compared to 42.29% at 50°C when hexane was used at the same temperature. A study proved the solubility of oil in higher ethanol at high temperatures, for example in the extraction of soybean oil. Hence, there was a possibility that more oil could still be extracted from the neem seed using ethanol at temperatures higher than 50^{o}C, and possibly more oil than that extracted using hexane at 50^{o}C, which was about the possible temperature limit of hexane due to its high risk of flammability [50]. Long chain fatty acid was generally well extracted by hexane, but it also depend on their solubility limit in hexane, so that the short chain fatty acid was few to be obtained from extraction with hexane. The long chain fatty acids were rather non-polar, the polarity would decrease with the increasing of chain. In contrast, short chain fatty acids were more extractable in ethanol, and solubility in ethanol depend on the length of the chain. This might explain why the long chain fatty acids were troublesome extractable by ethanol. The longer the carbon chain, the more difficult to dissolve oils and fats. Unsaturated oils and fats were more soluble in organic solvents than in SFA with the equal length of carbon chain. The fatty acids which had a high level of unsaturated were more soluble than fatty acids with low level of unsaturated [51]. The polarity of molecules depend on: (1) differences in solubility, the greater the difference, the more polar the molecule, and (2) the molecular structure, if the molecule was bound to organic substances, the molecule was more sensitive and the unpolarity increased. On the other hand, the determination of minerals in oils and fats has been the attention of the researchers, as mineral causes deleterious effects on the quality and stability of oils and fats. Minerals such as copper (Cu) and iron (Fe) had been known for fats and oils oxidative activity at low concentrations of 0.005 and 0.03 ppm, respectively. Cu was the strongest pro-oxidant in oil. To the best stability of the oil, Cu content should be below 0.02 ppm [52]. For the best stability of the oil, Fe levels recommended by the Codex STAN 19 [53] should be below 0.1 ppm and 0.10 - 0.40 ppm for Cu, this would increase stability and edibility of oil and extended the shelf life. Results were shown in Table 2 that the RBO which extracted with ethanol contained Cu over the recommended limit, whereas Fe was detected in the RBO extracted with hexane, the numbers also over the provisions recommended. Mineral generally a central mineral ions in organic molecules. The planes that symmetry which can be done by the molecule molecular symmetry. A molecule will be polar if the ability to separate the charge (ion positive and negative) is greater. Mineral solubility in the solvent depends on the nature of the compound, if these minerals are associated with the non-polar compounds such minerals will dissolve both the non-polar compounds, and vice versa. For example, potassium (K) in Table 3 was not detected in hexane solvent, but was detected in ethanol (Table 4), because K is generally a polar compound in salt form.

***Table 3**: Mineral composition of the Celebes RBO*

Mineral	Multistage extraction	
	Stage 1 (Hexane) (mg/L)	Stage 2 (Ethanol) (mg/L)
K	Nd	96
Cl	Nd	23
P	955	18
Si	13	907
S	Nd	566
Ba	26	35
Fe	17	Nd
Sb	Nd	13
Al	Nd	Nd
Sn	Nd	10
Cu	Nd	20
Zn	Nd	11

Nd, not detected

Table 4: Mineral composition of the Celebes RBO extracted by ethanol

Mineral	Single stage extraction (Ethanol) (mg/L)
K	245
Cl	101
P	25
Si	14
S	826
Ba	35
Sb	14
Al	353
Sn	11
Cu	26
Zn	15

Nd, not detected

In relation to the potential of RBO to be further processed as an oil-based processed feedstock, it is helpful to find data on the melting point and heat capacity of RBO analyzed using DSC. The data shown in Figure 2 RBO melts first at the temperature of 31.08^{o}C, peak melting point of 31.90^{o}C, at this point RBO requires the greatest energy to be able to melt at the perfect temperature of 32.89^{o}C. The data were in accordance with the results shown in Table 1 that the dominant fatty acids constituting RBO were oleic, linoleic and palmitic. Oleic, linoleic and palmitic have a melting point of 13^{o}C, -5^{o}C and 63^{o}C, respectively, so the mixture of them has a melting point around 31-32^{o}C. The data also informs that heat capacity RBO of 0.03 J/g.

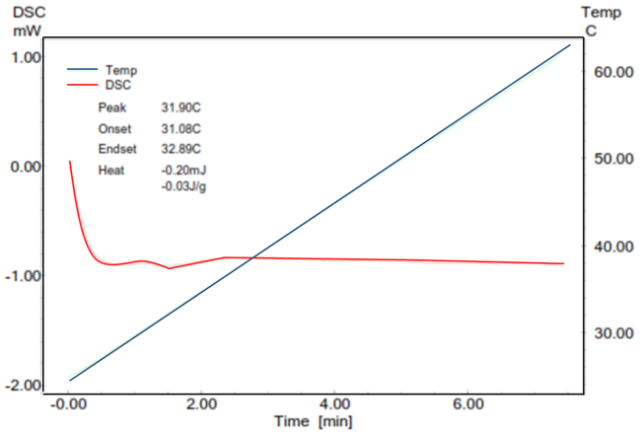

Fig. 2: *DSC analysis of Celebes RBO*

4 CONCLUTIONS

The results of this study show that the fatty acid profile of Celebes RBO contains a high percentage of oleic and linoleic acid indicates that this oil is excellent for consumption as one of the best sources of fatty acids in food, and potentially can be used as a feedstock in the production of functional foods. RBO extraction using hexane is better than using ethanol associated with the concentration of fatty acids that can be obtained. Celebes RBO also contains some minerals which its needs special attention related to its effect on the quality and stability of the oil. DSC analysis has shown that RBO has a melting point of 31.90^{o}C with a heat capacity value of 0.03 J/g.

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