

Chemical Properties and Fatty Acid Composition of Palado Seed Oil (*Aglaia sp*) Extracted Using Chloroform Solvent

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Abstract

Palado (*Aglaia sp*) is a plant that grows wild in the forest around Mamuju regency of West Sulawesi, Indonesia. This plant is locally known as palado. Palado seeds (*Aglaia sp*) can be used as a source of vegetable oil because it contains approximately 14.75 % oil, and it has the potential to be used as food ingredients or as raw material for oil production. The purpose of this study was to determine the chemical properties and the composition of fatty acids contained in palado seed oil (*Aglaia sp*). The employed method involved the use of palado fruit that had been processed to be palado seed and undergoing flouring process. Palado flour was produced by the extraction process by using chloroform solvent with the soxhlet method. The characteristics of the chemical properties in the oil produced were analyzed by using a standard method, including iodine, saponification, and acid values. The analysis of fatty acid composition was conducted by using gas chromatography. The results showed that palado oil extracted with hexane had an iodine value of 15.38 mg/g, saponification value of 190.01 mg KOH/g, and acids value of 1.961 mg KOH/g. The fatty acid composition of the palado seed oil consisted of saturated fatty acids (41.601 %), which included palmitic acid (41.062 %), myristic acid (0.539 %), and unsaturated fatty acids (45.949 %), which included mono-unsaturated fatty acids (MUFA) such as (22.929 %), oleic acid and poly-unsaturated fatty acids (PUFA), which was linoleic acid (23.020 %).

Keywords: Chemical properties, Extracted in chloroform solvent, Fatty acid composition, Palado seed oil

Introduction

Indonesia is a tropical country with great biodiversity. Indonesian forests are rich of potential sources of edible plants, such as fruits, seeds, and nuts. Palado (*Aglaia sp*), locally known as palado in West Sulawesi, is a fruit beeping tropical forest tree. The palado (*Aglaia sp*) fruits generally contain 2 seeds rich in carbohydrates and fat. At the national level, the use of palado seeds as food ingredients is far from popular. However, people living around forest area in West Sulawesi have been using the palado seeds as a boiled snack [1].

Palado (*Aglaia sp*) trees grow well on moist soil. They can live up to 100 years and can reach up to 50 m in height. These plants only come into fruit-bearing at the age above 10 years. They only bear fruit once a year, bloom in around September and drop their fruits around early February to late March. The fallen fruits are then collected by village dwellers for food. Each tree produces around 250 - 500 kg of wet palado fruit for a year [2].

Palado seeds serve as a considerably potential source of food products or as a source of vegetable oil. Research by [1] suggested that palado seed (*Aglaia sp*) as a processed product contains crude oil at

around 14.75 % by dry weight. The oil content is relatively larger than the other seeds, such as grape seeds with 12.20 % and pear seeds with 14.10 % [3]. However, the oil content of palado seeds is still lower than the fat of rambutan seed with 17.39 % as reported by [4]. Oil separation from the surrounding tissue using solvent extraction is an important method to extract oil from some types of grains. There have been some reports on tree extraction methods which include blight and dryer, soxhlet, and pressing that are used to extract *Clarias microcephalus* oil. The best method among these methods is soxhlet since it can produce lipids up to 36.71 %, as compared to blight and dryer method that only produces lipids up to 26 % and pressing method is 17 % lipids [5]. On the basis of the separation by using solvent extractions are the differences in solubility of each component in the solids with a solvent [3].

This study aims to determine the chemical properties and composition of fatty acids in palado seed oil (*Aglaia sp*) which is extracted using chloroform solvent. The portrayal of the seeds of palado is shown in **Figure 1**.



Figure 1 Seeds of palado (*Aglaia sp*), [6].

Materials and methods

Material preparation

The materials used in this study were the Palado fruits (*Aglaia sp*) collected from forests around the area of West Sulawesi, Indonesia. Palado fruits (*Aglaia sp*) as much as 15 kg were obtained from the forest, then they were sorted out based on the level of intactness. Afterward, the fruits were peeled with simple equipment of small logs to press the tip of the fruit and the board with the size of 20×40 cm² as its foundation. Then, the peeled palado seeds (*Aglaia sp*) were dried at a temperature of 30 - 40 °C for 40 h until the beans of palado (*Aglaia sp*) reached 4.87 % and their water content amounted to 6 kg.

Palado flour processing

Palado (*Aglaia sp*) flour processing was conducted by adopting the methods done by [7], which began with washing the palado seeds, soaking them for 30 min using *Sodium bisulphite* 0.2 %, flour milling using a disc mill Model of AGC 23 (Jakarta, Indonesia), then they were sifted using Tristar 237 (Surabaya, Indonesia) so that the flours produced through a 60 mesh sieve of 250 g. Palado flour (*Aglaia sp*) was, then, packaged in a plastic container and stored under dry conditions at room temperature until it was used for further applications.

Palado oil extraction

Palado oil (*Aglaia sp*) extraction was conducted by using the method applied by [8] which with some modification. In this method, 100 g of palado flour (*Aglaia sp*) was extracted using chloroform solvent (bp 40 - 60 °C) for 8 h in a soxhlet apparatus. Sodium sulfate anhydrite was, then, added to remove residual moisture, then oil was filtered using Whatman No. 1 to produce palado oil (*Aglaia sp*) of 10.97 mL/100 g sample with a pale yellow color, which was, later, used for further analysis.

Determination of iodine value

Iodine value derived from palado oil (*Aglaia sp*) was determined by the method applied by [9] in which 0.130 g of oil was put in a conical flask with a stoppered glass sized 500 mL. An empty flask that contained no oil was prepared as well. About 15 ml of cyclohexane and acetic acid solution were mixed in a ratio of 1:1, and then added to the flask containing the sample and the empty flask. Then, 25 ml of the oil that had been added to both flasks were closed with a stoppered glass and stirred well. The thermos bottle was left in the dark for 1 h. After that, 20 ml of potassium iodine and 150 ml of distilled water were added to remove iodine from the non-reacted iodine monochloride. Finally, all mixtures were titrated with sodium thiosulfate solution until the yellow color almost disappeared before 1 - 2 ml starch solution was added as an indicator and titration could be continued. The process ended when the blue color of the starch solution completely disappeared [9].

Determination of saponification value

The fat of saponification value obtained from palado oil (*Aglaia sp*) was determined by the method of AOAC 920.160 [10]. In brief, 2 g of fat sample was put into a 500 ml sized flask cone. Then 25 ml of sodium hydroxide solution (40 g per 1 liter of 95 % ethanol) was added into the tube, and then the flask was connected to the reflux condenser and heater for 1 h. Then the flask was cooled down in an ice container. Titration was done by adding 1 ml of phenolphthalein (as the indicator) into the mixture and titration was conducted using 0.5 M chloroc acid until the color changed from pink to colorless at the endpoint. A titration control was prepared without adding fat sample and used to determine the saponification value based on this formula;

$$\text{Saponification value} = \frac{[(BS) \times 5.28]}{[\text{sample weight (g)}]} \quad (1)$$

where B is the volume (mL) of chloric acid needed to control titration and S is the volume (mL) of chloric acid needed for the titration sample.

Determination of the acid value

The fat acid value obtained from palado oil (*Aglaia sp*) was determined by the AOCS method of Cd3d-63 [10]. In brief, 5 g of fat was put into a 500 mL sized cone and added with 50 mL of ethanol. Then, 50 mL of phenolphthalein (as the indicator) was added to the mixture and stirred for 20 s. The mixture was titrated with 0.5 of N potassium hydroxide until the color changes to pink at the end point. The acid value of the sample was calculated as follows;

$$\text{Acid value} = \frac{(56.1 \times V \times N)}{[\text{sample weight (g)}]} \quad (2)$$

where V is the volume (ml) of potassium hydroxide needed for titration and N is potassium hydroxide normality.

Chromatography gas analysis of fatty acid

The fatty acid composition of the oil palado (*Aglaia sp*) was determined using chromatography gas of Agilent 6890 (CA, USA) based on the method explained by [10]. Gas chromatography is a system equipped with HP EL-980 with Flame Ionization Detector (FID) and split/separated port injection. Individual FAME separation was reached by the column of HP88 GC (100×0.25 mm², ID 0.2 µm). The temperature of GC was set at 125 °C. Then, it was increased to 145 °C for 8 °C/min for 26 min and from 145 °C for 200 °C at 2 °C for 1 min. The injection volume was 1 mL with a split ratio of 50:1. The detector temperature was set at 260 °C and a carrier gas was helium (30 cm/min at 150 °C and 303 kPa). Chromatography data were recorded using Chemstation software (version 6.0). FAME standard mixture was used for the identification and quantification of the fatty acid composition of the palado oil. Identification of fatty acid methyl ester (James) was done by comparing the retention times of FAME standard and FAME from the sample.

Statistical analysis

The determination of palado oil (*Aglaia sp*) content, iodine value, saponification value and acid value was done in 3 repetitions with the average value, and standard deviation (mean + SD) calculated using Ms. Excel 2010. Meanwhile, the analysis of methyl ester fatty acid composition was done by using gas chromatography that was conducted in 2 repetitions and the average value was not presented.

Results and discussion

Iodine value

Iodine value gives the estimated value of unsaturated fatty acids in the triglyceride molecule fats and oils [11]. The amount of absorbed saturated value indicates the value of double bonds/unsaturated bond [12]. Palado oil (*Aglaia sp*) iodine value obtained 15.38 mg iodine/g, as shown in **Table 1**. These results are much lower than that reported by [13] on walnut oil iodine value from species of *Canarium indicum* and *Canarium vulgare* that were extracted with hexane of 58.63 and 58.38 mg iodine/g respectively. Similarly, the results are comparable to the iodine value of varieties of tea seed oil of *Assamica* reported by [14] with 83.38 - 88.89 mg iodine/g.

Table 1 Characteristics of chemical properties of oil palado (*Aglaia sp*).

Chemical characteristics	Value
Iodine (mg Iodine/g)	15.380±0.29
Saponification (mg KOH/g)	190.010±1.26
Acid value (mg KOH/g)	1.961±1.05

The high iodine value of oil shows high unsaturated fatty acids in the oil [15]. The decreased iodine value on a dry material allegedly is caused by oxidation in the drying process causing the bound oxygen to the double bonds of unsaturated fatty acids. These processes resulted in reduced oil unsaturation, thereby diminishing iodine value oil [15]. The iodine value serves as an important parameter in the trade that can determine the quality of oil based on the value of double bonds in the fatty acid as revealed by [13]. However, the more double bonds in oil, the easier the oil damage, because it is easily oxidized by oxygen from the air, chemical, or thermal process as indicated by [13].

Saponification value

The saponification value is inversely proportional to the molecular weight or average chain length of the fatty acids in oils and fats oils [11]. Likewise, [10] explained that the saponification values can be used to detect the long chain of fatty acids in the oil or fat. This study obtained, the value of saponification of palado oil (*Aglaia sp*) of 190.01 mg KOH/g (**Table 1**). This result approaches the saponification value of fat cocoa with 190.191 mg KOH/g [10], close to the results of [16], which stated that the saponification value of sesame oil (*Sesamum indicum* L.) is 188 - 191 mg KOH/g, and standards results as revealed by [16] which also mentioned the same thing, that the saponification value of sesame oil is 187 - 195 mg KOH/g. This fact shows that palado oil saponification value is higher than the saponification value reported by [10] for mango kernel fat of *Mangifera pajang* and *Mangifera indica* respectively with 169.70 and 138.15 mg KOH/g.

Oil that has a lower molecular weight will have a higher saponification value than oil with a high molecular weight [15]. In the dry material and methods of extraction using longer heating and high temperatures, the short-chain free fatty acids will evaporate, leaving the long-chain fatty acids. The longer the chain of free fatty acids is, the higher the molecular weight will be, and thus the lower the saponification oil value is [15].

Acid value

The value of fatty acids or oils is useful as a parameter to determine the quality of the oil or fat [10]. Likewise, [12] explained that the sum value is a measure of the amount of free fatty acids that are calculated based on the molecular weight of the fatty acids. The acid value is used to determine the level of oil damage. The greater the value of the acid value is, the worse the quality of the oil is. The acid value of palado oil obtained in this study is 1.961 mg KOH/g, approaching the acid value of cocoa butter reported by [10] which is 2:11 mg KOH/g fat. This acid value indicates a lower value than the acid values reported by [10] for mango fat kernel of *Mangifera pajang* and *Mangifera indica* which are respectively 2.81 and 4.77 mg KOH/g fat.

The value of palado oil acid value obtained is smaller than the value of cocoa butter acid and mango fatty kernel. This result means that the oil obtained has a good quality [12]. Additionally [17], described the vegetable oil extracted from seeds stored in a long period of time turn out to contain a high acid value. This is due to the combination of lipase enzymes in tissues and enzymes produced by the combination of microbes.

Table 2 The composition of fatty acids in the oil palado (*Aglaia sp*).

Fatty acid composition	The amount of carbon	Percentage
SFA :		
Palmitic acid	C16 : 0	41.062
Myristic acid	C14 : 0	0.539
MUFA :		
Oleic acid	C18 : 1	22.929
PUFA :		
Linoleic acid	C18 : 2	23.020

Fatty acid composition

The fatty acid composition of palado seed oil (*Aglaia sp*) as a result of gas chromatography analysis can be seen in **Table 2**. The results indicate that palado seed oil contains unsaturated fatty acids (*Saturated fatty acid*/SFA), which are 41.601 % including palmitic acid 41.062 % and acid myristate 0.539 %. Monounsaturated fatty acid (*Monounsaturated fatty acid*/MUFA) consists of linoleic acid of 23.020 %. This result indicates that palado seed oil contains higher unsaturated fatty acids with 45.949 % and saturated fatty acids of 41.601 %. In other words, it is still better than the cocoa fatty acid according to the report by [18] that the unsaturated fatty acid is 26.53 % and saturated fatty acids are 69.47 % (43.23 % stearic acid and 26.24 % palmitic acid).

This fatty acid composition of palado seed oil is nearly the same as the fatty acid composition of pumpkin seed oil (*Cucurbita maxima* Linn.) according to a report by [11] in which unsaturated fatty acid is 55.55 % (40.58 % oleic acid and linoleic acid 14.97 %), while saturated fatty acids is 44.45 % (27.06 % stearic acid and palmitic acid 17.39 %). On the contrary, a report by [10] indicated that the fatty acid composition of mango kernel with the varieties of *Mangifera indica* contains SFA of 51.48 %, MUFA of 42.40 %, and PUFA of 6.13 %, while the fatty acids of mango kernel with the varieties of *Mangifera shelf* contains SFA of 56.19 %, MUFA of 39.24 %, and 5.42 %. This means that the saturated fatty acids of mango kernel are more dominant than its unsaturated fatty acids. In general, the main component of fatty acid in palado seed oil is palmitic acid of 41.062 %, linoleic acid of 23.020 %, oleic acid of 22.929 %, and myristic acid of 0.539 %. The results of this study differ from that reported by [13] regarding the walnut oil (*Canarium Indicum*) extracted with soxhlet hexane methods in which the highest oleic acid is 45.92 %, followed by palmitic acid with 25.12 %, stearic acid with 15.51 %, and linoleic acid 12.58 %. Likewise, [19] reported the fatty acid composition of *Calophyllum inophyllum* L. Seed oil with degumming methods resulting in oleic acid of 41.27 %, linoleic acid of 33.63 %, palmitic acid of 12.23 %, and stearic acid of 11.02 % respectively.

Conclusions

Oil palado (*Aglaia sp*) extracted with hexane solvent at a process temperature ranges from 40 - 60 °C produces the characteristic of chemical properties including; iodine value of 15.38 mg iodine/g, saponification value of 190.01 mg KOH/g, and the acid value of 1.961 mg KOH/g. Meanwhile, the fatty acid composition produced consists of an SFA of 41.601 % including palmitic acid of 41.062 % and myristic acid of 0.539 %, while the unsaturated fatty acids are 45.949 %, including MUFA in which oleic acid is 22.929 % and PUFA linoleic acid is 23.020 %. This result indicates that the palado oil can be potentially developed as a food ingredient both as a source of nutrition and functional food.

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