

PHYSICOCHEMICAL PROPERTIES AND FATTY ACID COMPOSITION OF MANGO SEED KERNEL OIL

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ABSTRACT

Mango seed kernel oil has been extracted from Arumanis mango by multistage extraction with hexane (stage 1) followed with ethanol (stage 2) with the aim that all non-polar and polar components can be extracted. In the present paper, we provide data on physicochemical properties and fatty acid composition of mango seed kernel oil needed by the food, pharmaceutical, and cosmetics industries who are interested in using mango seed oil as its raw material. Extraction was performed at 50°C, for 5 h with mango seed kernel and solvent ratio of 1:4. The physicochemical properties include water content, specific gravity, refractive index, melting point, density, color, acid value, iodine value, peroxide value and saponification value were examined. Oleic, stearic, linoleic and palmitic acids were found dominant in first stage, it has had 27.27% of saturated fatty acid and 72.73% of unsaturated fatty acid as monounsaturated fatty acid 67.51% and 5.22% of polyunsaturated fatty acid. In the second stage extracted with ethanol, palmitic, linoleic, stearic, and lauric acid were dominant extracted. The saturated fatty acid contained of 77.26%, monounsaturated fatty acid of 1.87%, and polyunsaturated fatty acid of 20.87%. In addition, antioxidant activity of mango seed kernel oil has also been shown.

KEYWORDS: Edible oil, extraction oil, solvent extraction, physicochemical properties, fatty acid**I. INTRODUCTION**

Mango (*Mangifera indica* L.) is one of the most important fruit of the nutrition and economic aspect. Mango is consisted of edible peel and seed. The seed consists of a tenacious coat enclosing the kernel [1]. It is present about 18% of the total fruit [2]. The kernel content of the seed ranges from 45.7% to 72.8% [3]. MSK contain almost 15% of oil [4]. Variation in oil content in some reports due to the differences in variety of plant, cultivation (soil, climate, etc), ripening stage, the harvesting time and the extraction method of oil used [5, 6]. This can be as a result of difference in climatic conditions of their geographical locations [7].

Mango seed kernel (MSK) is an important source of nutrients and oil [8]. It has mainly been on the lipid component of the kernel, and of its potential application in the confectionery industry as a source of cocoa-butter substitute [9], and could potentially be used in the formulations of food products such as chocolate and biscuits as a natural nutritional additive [10]. The fatty acid (FA) profile is a main determinant of the oil quality, it is important as nutritional substances and metabolites in living organisms, the high quality and nutritional value of mango seed kernel oil (MSKO) has potential application in human foods [11].

MSKO is a promising edible oil, safe and natural source of edible fat, it is not contain trans FA [12]. MSKO is a promising and a safe source of edible oil and was found to be nutritious and non-toxic, so that it could be substitute for any solid fat without adverse effects [9]. Toxicity was not noticed in rats fed with diets containing 100g/kg crude of MSKO [13]. The lipids were extracted from several varieties of mangoes, free of toxic materials such as hydrocyanic acid [14].



Related to method of oil extraction, solvent extraction result [28] sesame revealed that polar solvent such as ethanol is better than non-polar solvent such as hexane. It can be explained by the interaction between unsaturated fatty acids and polar solvent, compared with non-polar solvents [15]. On the other hand, hexane is the most commonly used, and preferably for oil extraction from seed, due to its availability at reasonable cost and its functional characteristics suitable for oil extraction. Hexane is capable of dissolving triglycerides at sufficiently low temperatures, not reactive with oil and miscella, and requires simple equipment.

In a previous study, we have reported the optimum conditions of the MSKO extraction process [16] that are urgently needed by the food, pharmaceutical, and cosmetic industries that use MSKO as their raw material. Furthermore, to complement the MSKO study, in this paper, we provide data on the physicochemical properties and the fatty acid composition of MSKO obtained from multistage extraction with hexane followed by ethanol, so that all polar and non-polar components and especially long chains and short chain fatty acids of MSKO can be perfectly extracted. Throughout our literature studies on oil extraction, studies have been reported only on the use of one type of solvent and repeated several times, or using two types of solvent with a certain ratio, no studies have used multistage extraction using non-polar and polar solvents, especially on MSKO extraction. In addition, we also provide data on the antioxidant activity of MSKO in relation to the potential of MSKO to be processed as an oil-based functional food.

II. MATERIALS AND METHODS

Arumanis mango, a local mango Indonesia, as samples were obtained from South Sulawesi. Standards of fatty acid methyl ester (FAME) were from Supelco Inc., Bellefonte, PA (Supelco 37 Component FAME Mix), all chemical/reagents were from Merck, Germany, and DPPH were from Sigma Aldric.

Preparation of MSK and extraction oil

Preparation of MSK according to Mahale and Giri [8] with minor modification. The seeds were washed, and the kernel enclosed in the hard cover was separated manually. The kernels were dried in the oven at 50°C for 12 h to a constant weight in order to reduce its moisture content. Separation of thin cover from the kernel was carried out using tray to blow away the cover in order to achieve very high yield. Stainless steel grinder was used to powdered form, sealed in a plastic container and stored in a freezer until extraction to prevention of its oxidation.

At experimental unit, 50 grams of MSK powder were weighed in the reactor 1.0 L four neck flasks, MSK was extracted with 400 mL solvent, using a heating mantle connected with the thermometer setting at 50°C for 5 h. Motor on the top, speed of 200 rpm, residue was separated by centrifugation (refrigerated AX-521 centrifuge) at a speed of 3500 rpm for 20 min. The liquid part accommodated in the flask evaporator, solvents removed on a rotary evaporator Buchi R-215 incorporates vacuum Pump V-700. MSKO obtained was packaged in a dark glass bottle and stored in a freezer for analysis. The MSK residue from hexane extraction subsequently extracted with ethanol.

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Preparation and analysis of fatty acid methyl esters (FAME)

FA composition of MSKO was verified by gas chromatography. Lipids were esterified by method adapted from Metcalfe [17], which consists of lipid saponification with KOH 0.5 M in methanolic solution and catalyzed BF₃-MeOH reagent. The sample was solubilized by dichloromethane, from which 1 µL was injected for GC analyses. To separate and quantify the esterified FA mixture, GC-MS QP 2010 by Shimadzu was used equipped with split/split less injector, capillary column RTX®-1 (30 m x 0.25 mm ID x 0.25 µm) and flame ionization detector (FID). Helium was used as the carrier gas at flows of 1.25 mL/min. The injector and detector temperature were set to 260°C. The chromatographic conditions for separation were column initial temperature of 50°C, raising to 200°C at a flow rate of 6°C/min, holding during 4 min, the second step consisted in increased at a heating rate of 2°C/min to 240°C, and held for 10 min. FAME peaks were identified by comparing their retention time and equivalent chain length with respect to standard FAME.

Determination of physicochemical properties and antioxidant activity of MSKO

Physical properties of oil include water content, specific gravity, refractive index, melting point, and chemical characteristics including free fatty acid (FFA), peroxide value, iodine value and saponification value were determined according to AOCS [18]. Antioxidant activity of MSKO was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. The DPPH radical scavenging activity of oil extracts



was quantified according to Hatano et al. [19]. 0.0016 g of DPPH weighed and diluted with methanol in a 50 mL volumetric flask to obtain a solution of 10 mM. Preparation of standard BHT solution: 100 mg of BHT dissolved with methanol in 100 mL volumetric flask, in order to obtain a solution of 1000 ppm. Create BHT stock solution with a concentration of 25, 50, 100 and 200 ppm. Preparation of polyphenol extract: 100 mg of oil was dissolved in 50 mL methanol, sonication for 20 min and concentrated until methanol evaporated. Preparation of stock solution sample: 100 mg of polyphenol extract dissolved with methanol in a 100 mL volumetric flask, in order to obtain a 100 ppm standard solution. Created stock solution with a concentration of 80, 200, 400, 800 ppm. Measurement of antioxidant activity: 4 mL of BHT solution and 4 mL of sample solution were put into a tube and added 1 mL of 1 mM DPPH solution, the mixture is homogenized and incubated for 30 min at 37°C. Absorbance was measured at a wavelength of 516 nm. The radical scavenger activity was expressed in terms amount of antioxidants necessary to decrease the initial DPPH absorbance by 50% (IC50). The IC50 value for each sample was determined graphically by plotting the percentage disappearance of DPPH as a function of the sample concentration.

III. RESULTS AND DISCUSSION

Physicochemical properties of MSKO

Physical properties including refractive index, melting point, color and odor of MSKO were determined (Table 1). MSKO extracted with hexane was creamy pale yellow in color. Extracted of MSKO was semi-solid, pale yellow colored fat [8]. Refractive index of both MSKO at 30°C was 1.46 and 1.53, respectively. The refractive index associated with yield. The longer the extraction time, the more the oil yield, so that the refractive index is higher. Refractive index is an indication of quality assurance, analyzing the stability of oil during thermal treatment and the level of saturation of oil [20]. The refractive index of MSKO at 40°C is about 1.359 to 1.559 [21].

Table 1. Physical properties of MSKO^a

Physical properties	Value	
	Stage 1	Stage 2
Refractive index at 30°C	1.46±0.01	1.53±0.01
Melting point (°C)	33±0.35	31±0.26
Density (g/mL)	0.83±0.007	0.85±0.003
Color	Creamy yellow	Brown
Odor	Pleasant	Pleasant
Physical state at 27°C	Semi-solid	Semi-solid

^aResults are average of duplicate determinations ±S.D.

The density of both MSKO was 0.83 and 0.85 g/mL, respectively. Density is a measurement of the mass per unit volume. The greater the density of oil, the greater the mass of each volume. The oil has a density of 0.8 g/mL [22]. Each type of oil has a density that is typical, depending on the FA composition of the oil. The melting point of both MSKO was 32°C and 31°C, respectively. The lower the melting point of oil, the better the oil is for making oil creams [23]. The highest melting point of oil due to the presence of long-chain SFA [24]. MSKO had the lowest melting point as it contains high amount MUFA and PUFA such as oleic and linoleic acids. The melting point of MSKO from different countries ranged from 25 to 47°C [25], 39 to 40°C [26], 25 to 33°C [27].

The chemical properties of oil are the most important properties that determine the present condition of the oil. Chemical properties including the total acid, iodine, peroxide, FFA and saponification values of MSKO were determined (Table 2). FFA and peroxide value are the major characterization parameters for oil quality [11]. The low FFA content is indicative of low enzymatic hydrolysis. The oil with high FFA can develop off flavor during storage. Low FFA of oil indicate that the oil almost free from hydrolytic rancidity brought almost by lipases, and enables the direct use of such an oil in industries without further neutralization [5]. FFA was determined in both MSKO of 2.11 and 2.39%, respectively. The FFA of MSKO was about 2.26-3.76% [27].

MSKO had a high quality due to the low level of peroxide value [11]. MSKO founded high quality, the peroxide value of both MSKO were 1.83 and 2.39 mg/kg, respectively. This value were lower than that expected of rancid oil which ranges from 20.00-40.00 mg/g oil [28]. Generally in the fresh oil, the peroxide value should be less than 10 mg/g oil [11]. The peroxide value of MSKO was 1.95 to 1.99 mEq/kg [26]. The peroxide value is a good indicate for the stability of the oil and use as indicator of deterioration of oil. It is



measure of the concentration of peroxide and hydroperoxide formed in the initial stages of lipid oxidation. The peroxide value is also as a measure of the extent oxidation or oxidative rancidity of oil. Oxidative rancidity is the addition of oxygen across the double bonds in unsaturated FA in the presence of enzymes or certain chemical compounds. High peroxide values are associated with higher rate of rancidity [7].

Table 2. Chemical properties of MSKO^a

Chemical properties	Value	
	Stage 1	Stage 2
FFA, as oleic acid (%)	2.11±0.2	2.39±0.2
Peroxide value (mg/kg)	1.83±0.2	2.35±0.2
Acid value (mg KOH/g oil)	3.44±0.16	4.33±0.59
Iodine value (gram I ₂ /100 g oil)	53.19±0.45	31.27±0.44
Saponification number (mg KOH/g oil)	191.21±0.99	135.27±1.9

^aResult are average of duplicate determinations ±S.D.

Acid value serves as an indicator for edibility of oil. It is the mg KOH required to neutralize the FFA in 1 g of oil. The acid value is used to measure the extent at which glycerides in oil are decomposed by lipases and other actions such as light and heat, that its determination is often used as general indication of the condition and stability of oils. Acid value was determined in both MSKO of 3.44 and 4.33 mg KOH/g oil, respectively, indicates that the oils were edible because it falls within the recommended codex of 0.6 and 10 for virgin and non-virgin edible fats and oil, respectively [20]. In general, the lower the acid value, the more its acceptability for edibility purpose. The acid value of MSKO is about 4.49-7.48 mg KOH/g [27]. The acid values varied from 1.22% for the *Egyptian* mango variety to 7.48% for the *Kenyan Kent* mango variety [25].

The iodine value of oil indicates the unsaturation of FA, it was found to be 53.19 and 57.59 g I₂/kg oil for both MSKO, respectively. Iodine value is the amount of iodine necessary to saturate 100 g of oil and is a measure of the amount of unsaturation in oil. It is used to determine in assessing the stability of oil in industrial applications [29]. The iodine value of MSKO ranged from 51.08-56.79g I₂/kg oil [27], 40.90 to 56.79 g I₂/kg oil [25]. The iodine value of the *Kaew* mango variety was 40.90 cultivated in Thailand, while the *Kagege* mango variety was 56.79 cultivated in Kenya, the iodine values of different mango varieties cultivated in India, Bangladesh, Egypt, Mexico and Malaysia were comparable [27, 30]. The MSKO of *Arumanis* mango had the lowest iodine value, which reflected its characteristic such as higher resistance to oxidation, longer shelf life and higher quality. Iodine value maybe due to the differences of FA compositions [11]. A highly SFA level of oil is confirmed to be of benefit in terms of storage ability when compared to more unsaturated oil. The iodine value is also an index for assessing the ability of the oil to be rancid [7]. The iodine value obtained from this study indicates that the oil contained appreciable level of unsaturated FA present especially oleic acid.

MSKO *Arumanis* mango has saponification value of 191.21 and 206.73 mg KOH/g oil, respectively. The saponification values ranged from a low of 185.4 mg KOH/g for the *Thailand* and *Kaew* mango variety to a high of 197.0 mg KOH/g for the *Bangladesh Ranipas* mango variety [25]. The saponification value is the milligram of KOH necessary to saponify 1 g of oil and shows the capacity of forming soaps of oil, it shows the average mass long-chain molecules or FFA [31]. The saponification value was a useful tool for the evaluation of the chain length or molecular weight of FA occurring in the triacylglycerol in oil [11]. Its value represents the average molecular weight, or chain length of all the FA. Saponification value is inversely proportional to the molecular weight of the glycerides of the oil. The lower saponification value indicates a very high content of low molecular weight triacylglycerol. Saponification value indicates the size or nature of FA chains esterified to glycerol. It gives information about characteristic of the FA and the solubility of their soaps in water. The higher the saponification value of fat free from moisture and unsaponifiable matter, the more the solubility of the soap produced from it [7].

Fatty acid composition of MSKO

The FA profile of MSKO was analyzed by GC-MS (Table 3). The results showed that the group of saturated fatty acid (SFA) was predominantly of MSKO, there were valeric, lauric, myristic, palmitic, margaric, stearic, arachidic, behenic, tricosanoic and lignoceric acids. The data showed that the main FA of MSKO extracted with hexane (first step) was oleic (67%) and stearic acids (19.22%), they together constituted 86.68% of total FA.



MSKO had 27.27% of SFA and 72.73% of unsaturated fatty acid (UFA) as MUFA 67.51% and PUFA 5.22%. On the other hand, all of FA could be well extracted though the result of the second stage with ethanol was still founded.

In general, stearic acid was the main SFA in MSKO extracted with hexane, while oleic acid was the major UFA. The ratio of SFA to UFA were 1:2.67, this ratio indicated that the oil stable and tolerant to rancidity due to high content of UFA. In the second stage extracted with ethanol, MSKO had 77.26% of SFA and 22.74% of UFA as MUFA 1.87% and PUFA 20.87%. The data showed that the main FA of total lipid was palmitic (37.64%), linoleic (20.87%) and lauric acid (11.97%), they together constituted 70.48% of total FA. In addition, valeric, lauric, myristic, palmitoleic, margaric, arachidic, behenic, tricosanoic and lignoceric acids were minor FA present in MSKO.

Table 3. Fatty acid composition on MSKO extracted by multistage extraction

Fatty acid	Lipid Numbers	Group	Value	
			Stage 1(mg/L)	Stage 2 (mg/L)
Valeric	C5:0	SFA	0.759	0.139
Lauric	C12:0	SFA	3.614	3.818
Myristic	C14:0	SFA	2.744	1.82
Palmitic	C16:0	SFA	213.511	12.006
Palmitoleic	C16:1	MUFA	1.698	0.131
Margaric	C17:0	SFA	2.86	0.243
Stearic	C18:0	SFA	699.803	6.343
Oleic	C18:1	MUFA	2456.043	0.464
Linoleic	C18:2	PUFA	190.178	6.658
Arachidic	C20:0	SFA	48.981	0.222
Behenic	C22:0	SFA	8.395	0.02
Tricosanoic	C23:0	SFA	1.952	0.011
Lignoceric	C24:0	SFA	10.07	0.025

MSKO is more stable than many other vegetable oils, because it is rich in PUFA. Such oils seem to be suitable for blending with other vegetable oils, stearin manufacturing, confectionery industry or in the soap industry [32]. The PUFA is very important for the stability of oil because of the chemical reactions, due to occurring at the double bonds. The rates of those oxidation reactions depend on the number of double bonds in the carbon chain. Therefore, oils with a high proportion of oleic acid are more stable than others. Oleic acid is less susceptible to the oxidation than PUFA from the n-6 series (linoleic acid). Linoleic acid is important for the stability of oil, due to chemical reactions occurring at the double bonds. It contributes to health benefits of human body and was preferred by industries when oil hydrogenation was required [11].

The mango fats were rich source of stearic, oleic, palmitic, and linoleic acids [25]. The Egyptian mango contained stearic and oleic acids of total lipid and neutral lipids constituted 84.4% and 86.2% of total FA, respectively [21]. The major FA in MSKO were stearic acid (31.3-41.3%), oleic acid (38.7-42.3%) and palmitic acid (8.311.3%) [33]. The major FA in all the three mango varieties (*Saigon*, *Edward* and *Julie*) evaluated were stearic (37.57-38.41%), palmitic (8.61-9.06%), oleic (43.71-44.91%), linoleic (5.87-7.00%) and linolenic (0.63-0.95%). *Saigon* had the highest stearic acid content while *Edward* had the highest palmitic, linoleic and linolenic acid [1].

Oleic acid is an 18-carbon MUFA, essential in human nutrition and helps to reduce triglycerides, LDL-cholesterol, total cholesterol and glycemic index [11]. The increase in stability over oxidation of vegetable oil is attributed to oleic acid [34]. Stearic acid, a long C18 straight-chain SFA, has been found to bind and plasticize composites [35], human serum albumin [36] and α -helical sites in bio-molecules [37], indicating the usefulness



of the kernels of these mangoes for those purposes. The high quality and nutritional value of MSKO has potential application in human foods. The high content of oleic and linoleic acids make MSKO a potential source of nutrient rich food oil.

Antioxidant activity of MSKO

The radical scavenging activity is expressed as median inhibitory concentrations (IC50) value which is inversely proportional to the antioxidant activity [38, 39]. Antioxidants are major ingredients that protect the quality of oils by retarding oxidation [40]. The mechanism of action of flavonoids are through scavenging or chelating process [41]. Study on the antioxidant activity in MSKO, it was obtained IC50 value of hexane and ethanol extract of 30.5 µg/mL and 32 µg/mL, respectively, while BHT was used as a comparison had IC50 values of 34.5 µg/mL, higher than the IC50 value of hexane and ethanol extract. The smaller the value of IC50 indicates that the compound has a large antioxidant activity. MSKO could be categorized as oil with considerable antioxidant potential. The DPPH radical scavenging activity of hexane extract was higher than that of ethanol extract, because hexane more extracting other antioxidant components soluble in oil, such as β-carotene and vitamin E (tocopherols and tocotrienols). Studies reported that the IC50 value of selected vegetable oil extracts, such as soy, sunflower, rapeseed and corn ranged from 29.7 to 34.0 µg/mL [42].

Synthetic antioxidants are used at legal limits to reduce deterioration, rancidity and oxidative discoloration. BHA and BHT are volatile and decompose easily at high temperatures [43]. There are some serious problems concerning the safety and toxicity of such synthetic antioxidants related to their metabolism and possible absorption and accumulation in body fluids and tissues. Therefore, the search for preparation of useful natural antioxidants is highly desirable. Many studies showed that natural antioxidants, as flavonoids and other phenolic phytochemicals, present in plants are associated with reduced chronic disease risk [44]. The ability of phenolic compounds to quench free radicals arises because of both their ability to donate protons and to transfer electrons while remaining relatively stable [45].

The extract of MSK exhibited contained the highest degree of free-radical scavenging and tyrosinase-inhibition activities compared with methyl gallate and phenolic compounds [11]. Many studies showed that natural antioxidants, as flavonoids and other phenolic phytochemicals, contained in plants were associated with reduced chronic disease risk [46]. The type of phenolic compounds has been demonstrated to inhibit lipid peroxidation of human LDL in vitro [47]. Some studies indicated that MSKO contained different phenolic compounds and consisted of stable fat-rich with SFA, so it could be good source of natural antioxidants [48]. MSKO can be used as natural antioxidants in a variety of foods, due to the content of some phenolic compounds, FA component that was rich with the SFA and MUFA oleic acid besides tocopherols, squalene and different sterol fractions. The antioxidant property of MSKO is attributed to some extent to its richness in SFA and MUFA as well as fractions of tocopherols, sterols, their esters and other phenolic compounds are present as unsaponifiable matter at MSKO [49].

MSKO can be used as antioxidant in edible oil. Oxidative stability of buffalo fat with the addition of 5% MSK similar to sunflower seed oil stability with added 300 ppm TBHQ during 12 months of storage in darkness, peroxide and anisidine values are at the lowest concentrations [50]. Oxidative stability of refined sunflower seed oil after adding 1% crude MSKO and placing it at 90°C incubator for 36 hours was equal to the antioxidant capacity of added 200 ppm BHT [49]. Comparison of the peroxide value between fat with containing different concentrations of MSKO showed that the addition of crude MSKO could be reduced the oxidation process and as the concentration of the MSKO increased oxidation chain reaction and consequently peroxide formation was reduced. Gallic acid present in MSKO has been known as an anti-inflammatory, anti-mutant and anti-oxidative agent [51].

CONCLUSION

Based on the results of physicochemical properties and fatty acid profile of the MSKO, it could be concluded that the MSKO could be become valuable resource to produce high value of vegetable oil. The MSKO extracted with hexane has better quality. The MSKO was analyzed for refractive index, acid value, saponification value, and iodine value. Most of the values comply with the standard specifications. The oil was good quality and could be recommended suitable for the food, pharmaceutical and cosmetics industries. MSKO is rich in oleic and stearic acid indicating that oil was stable and tolerant to rancidity. This oil may be considered as an important source of UFA and has the potential to be used as nutrient rich food oil. Furthermore, the results also



verified that MSKO have IC50 value that lower than BHT as reference, enabling their application as ingredient of functional or enriched food. The results of present study provide useful information for edible oil and food industry, due to its special composition, rich in PUFA as oleic acids, and in antioxidant compounds.

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