Chemical Properties and Fatty Acid Composition of Mangifera pajang and Mangifera indica Kernel Fats

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ABSTRACT

Introduction: This study aimed to determine chemical properties and fatty acid composition of kernel fats of *Mangifera pajang* (MP) and *Mangifera indica* (MI), and compare the results with that of cocoa butter from literature. **Methods:** Chemical properties of the extracted crude fats were determined for iodine value, peroxide value and saponification value using AOAC methods, whereas acid value of the mango kernels was determined based on AOCS method. Saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) were also determined using gas chromatography-flame ionisation detection method. **Results:** The results showed that kernel fats of MI and MP had low chemical values. The fatty acid compositions of MP kernel fat comprised 55.4%, 39.3% and 5.3% of SFA, MUFA and PUFA, respectively. The total PUFA of MP kernel fat (5.3%) was lower than the total PUFA of MI kernel fat (6.1%). **Conclusion:** Due to the similarity of the fatty acid composition between mango kernel fat and cocoa butter, it is suggested that the kernel fat of MP has potential as a substitute for cocoa butter or hydrogenated fat in confectionary products.

Keywords: Chemical characteristics, cocoa butter, fatty acid, kernel fat, *Mangifera indica*, *Mangifera pajang*

INTRODUCTION

Mangifera plants are from the Anacardiaceae family which consists of over 2,500 species. Among the Mangifera species, eight species originated from Southeast Asia. These species are Mangifera caesia (binjai), M. foetida (bacang), M. horsefielda (hambawang), M. indica (commercial mango), M. lagenifera (machang), M. odorata (kuini), M. pajang (bambangan) and M. torquenda (kemantan) (Khoo & Ismail, 2008; Porcher, 1995). Among the eight Mangifera

species, the trees of *M. pajang* (MP) are found only in the Borneo Island, including Kalimantan (Indonesia), Sarawak and Sabah (Malaysia). The trees of *M. indica* (MI) are found throughout the region of Southeast Asia.

MP has a unique fruit that is huge in size compared with other mango species. The fruit weighs up to 1 kg, and has a thick peel (Bakar & Fry, 2013). The peel of MP is about 12% of the total weight of the fruit while the kernel of MP is about 27% of the

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fruit (Ibrahim, Prasad & Hamid 2010). The local people in Borneo Island, especially those in Sabah use MP fruits in cooking dishes and as a pickle (Hasnah & Mamot, 2004). The kernel of MP is considered waste but has potential as a nutraceutical ingredient. The proximate composition of MP kernel has been determined previously by Hasnah and Mamot (2004). Their results showed that MP kernel has 38.68% carbohydrate, 9.85% fat, 4.79% coarse fibre, 3.08% protein and 2.23% total ash. On the other hand, the kernel fat of MI has similar thermal behaviour as that of cocoa butter (Solis-Fuentes & Durán-de-Bazúa, 2004). For that reason, it is suggested that the fat obtained from MI kernel has potential for use as a cocoa butter substitute Moharram & Moustafa, 1982.

Comparison of the chemical characteristics and fatty acid compositions between kernels of MP and MI has not been determined in the past. A previous study reported that the kernel fat of mango has potential as a cocoa butter substitute (Baliga & Shitole, 1981). Kabuki et al. (2000) also mentioned that kernel fat from mango is promising as a fat and cocoa butter substitute. The kernel fat of MP has certain chemical qualities that show potential for use as a cocoa butter substitute. In this study, the chemical characteristics and fatty acid compositions of kernels of MP and MI were determined and compared with cocoa butter

METHODS

Chemicals and reagents

Ethanol (analytical grade) and chloroform (analytical grade) were purchased from HmbG Chemicals (Hamburg, Germany) and Fisher Scientific (Selangor, Malaysia), respectively. Phenolphthalein, potassium iodide and sodium thiosulfate were obtained from Analar (England, UK) while fatty acid methyl ester (FAME) standard was purchased from AccuStandard (CT, USA). All the other chemicals and solvents

of analytical grade were supplied by Merck (Selangor, Malaysia).

Sample preparation and extraction

The fruit of MP (~5 kg) was obtained from the Department of Agriculture Sarawak, Sarawak, Malaysia, Kuching, MI fruit (Chokanan, one of the mango varieties originated from Thailand, ~5 kg) was purchased from a local market in Sri Serdang, Selangor, Malaysia. All fruits were in a fully ripe stage prior to use as samples. The peels and pulps of these fruits were removed using a sharp household knife. The fruit kernels were cut into small pieces (~3 cm² size) and stored at -80°C before freeze-drying. The kernel samples were freeze-dried using a bench-top freeze dryer (Virtis, NY, USA) for four days. The lyophilised samples were ground into powder, sieved using a 20-mesh sieve and stored at -80°C before further analysis.

The fat of MP and MI kernels was extracted using Soxhlet method. Briefly, 10 g of the lyophilised powders of MP and MI kernels were weighed into cellulose thimbles and placed inside the Soxhlet apparatus. Using 250 ml of petroleum ether, the fat extraction process was carried out for 6 h. The petroleum ether was removed using a rotary evaporator (BOECO, Hamburg, Germany) and the fat from MP and MI kernels were collected to determine its chemical characteristics and fatty acid composition. Extraction of each sample was done in triplicate.

Determination of chemical characteristics *Iodine value*

Iodine values of the fat obtained from MP and MI kernels were determined based on AOAC method 920.159 (Nielsen, 1994). Briefly, 0.2 g of fat sample was inserted into a 500 ml conical flask. Then 20 ml cyclohexane and 25 ml of 0.1 M Wijs solution were added to the flask. The mixture was swirled for 20 s and kept in the dark for 30 min at room temperature

(25°C). Then, 20 ml of 0.1 M potassium iodide and 100 ml distilled water were added to the mixture and swirled for 20 s. Titration was done by addition of 0.1 N sodium thiosulfate and 2.0 ml starch solution (1%) as the indicator of titration until the blue colour became colourless at the end point. A control analysis was performed based on the same procedure without the addition of the fat sample. The control was used for calculation of iodine value based on the formula as follows:

Iodine value =
$$\underline{((B - S) \times N \times 12.69)}$$

(Sample weight (g))

where B is the volume (ml) of sodium thiosulfate needed for control titration, S the volume (ml) of sodium thiosulfate needed for sample titration, and N the normality of sodium thiosulfate.

Saponification value

Saponification values of the fats obtained from MP and MI kernels were determined based on AOAC method 920.160 (Nielsen, 1994). Briefly, 2 g fat sample was inserted into a 500 ml conical flask. Then 25 ml sodium hydroxide solution (40 g in one litre of 95% ethanol) was added to the flask. The flask was connected to a reflux condenser and heated for 1 h. Then, the flask with the mixture was cooled in an ice container. Titration was performed by addition of 1 ml phenolphthalein (as an indicator) into the mixture and titrated with 0.5 M hydrochloric acid until the colour changed from pink to colourless at the end point. A control titration was prepared without the addition of fat sample, and was used to calculate the saponification value based on the following formula:

Saponification value =
$$((B - S) \times 28.05)$$

(Sample weight (g))

where B is the volume (ml) of hydrochloric acid needed for control titration and S is the volume (ml) of hydrochloric acid needed for sample titration.

Peroxide value

Peroxide values of the fats obtained from MP and MI kernels were determined based on AOAC method 965.33 (Nielsen, 1994). Briefly, 5 g fat sample was inserted into a 500 ml conical flask to which was added a mixture of 50 ml acetic acid and chloroform (3: 2). Then potassium iodide was added to the mixture until saturation, and swirled for 20 s and kept in the dark at room temperature for 2 min. This was followed by the addition of 100 ml distilled water and three drops of starch indicator to the mixture. The final mixture was titrated with 0.1 N sodium thiosulfate until the blue-grey colour of the mixture become colourless at the end point. A control titration was prepared without the addition of fat sample for calculation of peroxide value based on the formula as follows:

Peroxide value =
$$\underline{((S - B) \times N \times 1000)}$$

(Sample weight (g))

where B is the volume (ml) of sodium thiosulfate needed for control titration, S the volume (ml) of sodium thiosulfate needed for sample titration and N the normality of sodium thiosulfate.

Acid value

Acid values of the fat obtained from MP and MI kernels were determined based on AOCS method Cd 3d-63 (Nielsen, 1994). Briefly, 5 g fat sample was inserted into a 500 ml conical flask to which was added a mixture of 50 ml ethanol. Then 50 ml phenolphthalein (as an indicator) was added to the mixture and swirled for 20 s. The mixture was titrated with 0.5 N potassium hydroxide (KOH) until the colour changed at the end point to a persistent pink colour. Acid value of the sample was calculated based on the following formula:

Acid value =
$$(56.1 \times V \times N)$$

(Sample weight (g))

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

Preparation of fatty acid methyl esters (FAMEs)

FAMEs of the fat sample were prepared by addition of 10 ml hexane to a 0.1 g fat sample in a centrifuge tube. The procedure was adapted from the method described by David, Sandra & Wylie (2002). Briefly, 100 µl of 2 N KOH in methanol was added to a conical flask, and the tube was vortexed for 30 s. Then the tube was centrifuged at 3600 rpm at 25°C for 15 min. The supernatant was transferred to a 2 ml GC vial and analysed using a gas chromatography-flame ionisation detector.

Gas chromatography analysis of fatty acids

Fatty acid composition of MP and MI kernels were determined using an Agilent 6890 gas chromatography (CA, USA) based on the method described by Vickers (2007). The gas chromatography (GC) system was equipped with HP EL-980 flame ionisation detector (FID) and a split/splitless injection port. The separation of individual FAME was achieved using an HP88 GC column (100 mm \times 0.25 mm, ID 0.2 μ m). The temperature of GC oven was set at 125°C. It was then increased to 145°C at 8°C/min for 26 min and from 145°C to 200°C at 2°C for 1 min. The injection volume was 1 µl with a split ratio of 50:1. The detector temperature was set at 260°C and the carrier gas was helium (30 cm/min at 150°C and 303 kPa). The chromatographic data was recorded using the ChemStation software (version 6.0). A mixture of FAME standards was used for identification and quantification of fatty acid compositions of MP and MI kernel fats. Identification of FAMEs was done by comparing the retention times of FAME standard and FAME from the sample.

Statistical analysis

All data are presented as a mean \pm standard deviation. Independent sample t-test was used for comparison of the values for chemical characteristics and fatty acid compositions of MP and MI kernel fats. The values for cocoa butter from previous studies were used for comparison without any statistical analysis. The significant value was set as p<0.05. Statistical Package for Social Science (SPSS) version 17 was used for the statistical analyses.

RESULTS AND DISCUSSION

Iodine value

Iodine values determined for the kernel fats of MP and MI are shown in Table 1. The iodine value for kernel fat of MP was 387.0 mg iodine/g fat, but the iodine value for the kernel fat of MI (368.0 mg iodine/g fat) was not significantly lower than the kernel fat of MP. The results revealed similar degrees of saturation for both kernel fats but the fatty acids of *Mangifera* kernels had a lower degree of saturation. Iodine value is an important indicator of the level of saturation for fatty acid because the iodide molecules react with the double bonds of

Table 1. Comparison of chemical characteristics of fats from mango kernel and cocoa butter

Chemical characteristics	MP kernel	MI kernel	Cocoa butter²
Iodine value (mg iodine/g)	387 ± 26.9	368 ± 53.8	384ª
Saponification value (mg KOH/g)	169.7 ± 1.98^{1}	138.15 ± 0.99^{1}	190.2ª
Peroxide value (μEq/g)	2.0 ± 2.83	1.0 ± 1.41	1.78 ^b
Acid value (mg KOH/g)	2.81 ± 0.79	4.77 ± 0.39	$1.75^{a}/2.11^{b}$

Significant difference between MP and MI kernels.

The data were obtained from literature (aMoharram & Moustafa, 1981; Hamid & Damit, 2004). MP: Mangifera pajang; MI: Mangifera indica.

the fatty acids (Nielsen, 1994). The quality of fat or oil can also be predicted based on the iodine value.

Though Hasnah and Mamot (2004) reported a iodine value of 329.7 mg iodine/g fat for bambangan (MP) kernel, our study obtained a lower iodine value. The iodine value of MI kernel obtained from Malaysia is lower than the value reported by Nzikou et al. (2010). Their study results found the iodine value of MI variety Chokanan to be 368.0 mg iodine/g fat compared with 430.0 mg iodine/g fat of MI variety Congo Brazzaville. The difference in iodine value could be due to weather and seasonal variability (Pereyra-Irujo et al., 2009). During heavy rain, the quality of most fruits produced is lower than those harvested during the hot and dry weather. Besides, the species of mango fruit (Mangifera spp.) or the variety of a mango species could affect the degree of saturation of fatty acids in the kernel (Abdalla et al., 2007).

The iodine values for MP and MI kernel fats were compared with the iodine value of cocoa butter reported previously. The results showed that the MI and MP kernel fats had higher iodine values than the reported value (293.7 mg iodine/g fat) for cocoa butter (Kaphueakngam, Flood & Sonwai, 2009). However, the iodine value (384.0 mg/g fat) of cocoa butter reported in the literature is close to the value determined (387.0 mg/100 g fat) for MP kernel fat (Moharram & Moustafa, 1982). As a potential cocoa butter substitute, the iodine value of the fat should be comparable to the iodine value of cocoa butter (Kaphueakngam et al., 2009). The data also showed that the fats obtained from Mangifera kernels have potential for use as a cocoa butter substitute in confectionary.

Saponification value

In this study, saponification values of kernel fats of both MP and MI were low. As shown in Table 1, the saponification value of MP kernel fat was 169.7 mg KOH/g fat, but the saponification value (138.15 mg KOH/g fat) for the kernel fat of MI was significantly lower than the kernel fat of MP (*p*<0.05). A previous study reported a saponification value of 192.16 mg KOH/g fat for the kernel fats of MI of other varieties Zebda, Balady and Succary. This value was higher than the values found in the study of Abdalla *et al.* (2007).

The saponification value is applied to detect long-chain fatty acids in an oil or fat. The value is inversely proportional to a chain length of a fatty acid. A lower saponification value indicates a longer carbon chain of a fatty acid. Comparing our study results with the saponification value of 190-191 mg KOH/g fat of cocoa obtained by other studies (Moharram & Moustafa, 1982; Kaphueakngam et al., 2009), the lower saponification values obtained for the kernel fats of MP and MI denote a longer carbon chain of fatty acids extracted from the kernels. Coupled with the iodine values for the samples, it is concluded that the kernel fats of MP and MI contain longchain unsaturated fatty acids.

Peroxide value

Peroxide values of both kernel fats of MP and MI were determined as 2.0 and 1.0 μEq/g fat, respectively (Table 1). The peroxide value for the kernel fat of MP was not significantly higher than the peroxide value of MI kernel fat at a p-value higher than 0.05. According to Hamid & Damit (2004), the peroxide value for cocoa butter is comparable to the peroxide values of both samples. Abdalla et al. (2007) reported that the kernel fat of mango of different varieties Balady, Succary and Zebda contains an average peroxide value of about $0.96 \mu Eq/g$ fat. The peroxide values obtained from the mango samples of our study were similar to the values reported by previous studies. The low peroxide value of these mango kernel fats hints that the fats are stable and have low rancidity.

Peroxide value is a measure of oxidation level in fat or oil (Brown, 2008). The oxidation process enhances rancidity of the oil or fat. A low peroxide value reflects a low oxidation process of oil or fat. An antioxidant such as butylated hydroxytoluene can be added to oil to reduce oxidation during long-term storage (Al-Neshawy & Al-Eid, 2000). One can expect a decrease in peroxide value caused by the oxidation process.

Acid value

Acid values of both kernel fats of MP and MI were 2.81 and 4.77 mg KOH/g fat, respectively. The acid value of MI kernel fat was significantly higher than the acid value of MP kernel fat (p<0.05). Based on a previous study (Nzikou et al., 2010), a high acid value (7.82 mg KOH/g extract) was found for kernel extract of mango variety Congo Brazzaville. However, the acid values for cocoa butter are 2.11 mg KOH/g fat (Hamid & Damit, 2004) and 1.75 mg KOH/g fat (Moharram & Moustafa, 1982). The acid values of cocoa butter reported in the literature are lower than the acid values of MP and MI kernel fats obtained in our study. It shows that the fats obtained from MP and MI kernels are less stable than cocoa butter.

The acid value of a fat or oil is a useful parameter to determine the quality of

oil or fat. In this method, KOH is used to measure the amount of free fatty acid in the oil or fat. A high level of free fatty in oil or fat denotes a low quality. A high amount of free fatty acid in oil or fat could be due to the high oxidation rate. The kernel fats of MP and MI have higher acid values compared to cocoa butter. It could be attributed to the high level of SFA(48%) and the low level of PUFA (10%) (Hamid & Damit, 2004).

Fatty acid composition

Fatty acid compositions of both MP and MI kernels were analysed using a GC-FID system. GC-FID is the typical equipment used in the determination of fatty acids worldwide (Ulberth & Buchgraber, 2003). The fatty acid compositions of these mango kernels are shown in Table 2. MI kernel contained 51.48%, 42.4% and 6.13% of SFA, MUFA and PUFA, respectively, whereas the SFA, MUFA and PUFA levels in MP kernel were 56.19%, 39.24% and 5.32%, respectively. The results showed that MP kernel fat had significantly higher SFA (%) than MI kernel fat (v<0.05). The MUFA and PUFA levels of MI kernel fat were not significantly higher than the MP kernel fat.

The SFA, MUFA and PUFA levels of MP and MI kernels were comparable to cocoa butter. The levels of these fatty acids in cocoa butter ranged from 50-62%, 33-

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Fatty acid composition	MP kernel¹	MI kernel¹	Cocoa butter²	
SFA (%) Palmitic acid (C16) Stearic acid (C18)	15.8 ± 0.01 40.39 ± 1.02	14.91 ± 0.03 36.57 ± 4.12	24.7 35.1	
MUFA (%) Oleic acid (C18:1)	39.24 ± 0.99	42.4 ± 3.05	36.47	
PUFA (%) Linoleic acid (C18:2) Linolenic acid (C18:3)	4.95 ± 0.02 0.37 ± 0.01	5.49 ± 0.74 0.64 ± 0.37	2.85 0.3	

¹ No significant differences between MP and MI kernels for all fatty acids.

² The data were obtained from literature (Solís-Fuentes & Durán-de-Bazúa, 2004).

³ SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; MP: Mangifera pajang; MI: Mangifera indica.

Sample	Current study Study 1		Study 2		Study 3		
	MI	MI	СВ	MI	СВ	MI	СВ
SFA (%)	51.48	39.6	50.2	48.36	59.37	51.94	62.35
MUFA (%)	42.4	52.9	44.8	40.81	36.47	41.09	33.49
PUFA (%)	6.14	7.56	5.06	10.83	4.16	6.97	4.17

Table 3. Comparison of total SFA, MUFA and PUFA contents of cocoa butter and MI kernel

45% and 4-5%, respectively (Moharram & Moustafa, 1982; Solís-Fuentes & Durán-de-Bazúa, 2004; Kaphueakngam *et al.*, 2009). Comparing our results with those obtained from previous studies, a similar fatty acid composition was found for the mango (MI) kernel (Table 3). Kaphueakngam *et al.* (2009) revealed that mango kernel has 51.94%, 41.09% and 6.97% of total SFA, total MUFA and total PUFA, respectively.

Among the SFA, palmitic acid and stearic acid were found to be the major SFA in MP and MI kernels. As shown in Table 2. both MP and MI kernels have a similar level of palmitic acid. The stearic acid content of MP kernel was also not significantly higher than that of the MI kernel. Similarly, the levels of oleic acid (MUFA), linoleic acid and linolenic acid (PUFA) of MP kernel were not significantly lower than that of the MI kernel. The data revealed a similar fatty acid composition for both mango kernels. A previous study (Nzikou et al., 2010) reported that the kernel of mango variety Congo Brazzaville contains higher levels of stearic acid (37.73%), oleic acid (46.22%), linoleic acid (7.33%) and linolenic (2.3%) than the MI kernel sample. However, the palmitic acid content of MI kernel (14.91%) determined in this study was far higher than that found in the kernel of mango variety Congo Brazzaville (6.43%).

Cocoa butter contains a higher percentage of palmitic acid than the mango kernels. It has 24-25% of palmitic acid (Solís-Fuentes & Durán-de-Bazúa, 2004; Kaphueakngam *et al.*, 2009). The stearic acid content of cocoa butter is similar to the MI kernel. The levels of MUFA and PUFA in cocoa butter reported previously are slightly lower than that of both MP and MI kernels. Solís-Fuentes & Duránde-Bazúa (2004) reported that cocoa butter has 36.47%, 2.85% and 0.30% of oleic acid, linoleic acid and linolenic acid, respectively.

Variation in fatty acid composition among different varieties and species of mango is probably due to genetic such Preharvest factors factors. weather, temperature, fertiliser usage and stages of maturity could be the major factors affecting the quality of the mango fruit (Nunez-Elisea & Davenport, 1991; Léchaudel & Joas, 2007). Although mango kernel contains a high SFA, the high stearic acid content may not pose any health hazard. A meta-analysis of 60 controlled trials revealed that increased intake of stearic acid through the daily diet slightly improved the lipid profile of the respondents in the study (Mensink et al., 2003).

Stearic acid is one of the long-chain SFAs, and increased intake of stearic acid from food does not pose any adverse health effects. A study reported that consumption of a diet containing cocoa butter rich in SFA for three consecutive weeks reduced plasma LDL-cholesterol to 3.82 mmol/L (Denke & Grundy, 1991). The study also showed that the cholesterol-reducing effect

SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; MI: Mangifera indica; CB: cocoa butter.

² Study 1: Moharram and Moustafa (1982); Study 2: Solís-Fuentes and Durán-de-Bazúa (2004); Study 3: Kaphueakngam et al. (2009).

was comparable to the group consuming olive oil (3.62 mmol/L). Therefore, the level of saturated fat is not the only disease attribute. Oxidation level of fat is another factor that has to be considered.

CONCLUSION

In summary, the kernel fats of MP and MI fruits contained acceptable chemical characteristics (iodine, saponification, peroxide and acid values) ranging from low to moderate levels. The chemical characteristics of MP and MI kernel fats are comparable to the chemical characteristics of cocoa butter. The composition of major fatty acids were also determined in the mango kernels. High levels of SFA were found for both MP and MI kernels, with stearic acid being the major SFA in the kernels. About 40% of MUFA and less than 7% of PUFA were determined for the MP and MI kernels. Since the kernel fats of MP and MI fruit have chemical characteristics and a fatty acid composition similar to cocoa butter, these kernel fats indicate potential as new sources of the ingredient for use as a fat or cocoa butter substitute for confectionary. Further studies on functional effects of MP and MI kernel fats are recommended.

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