

THE EXPLOITATION OF UNDERUTILISED *Mangifera indica* L. SEED AS COCOA BUTTER ALTERNATIVE

(Exploitasi Biji *Mangifera indica* L. Yang Tidak Di Gunakan Sebagai Alternatif Mentega Koko)

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Received: 11 September 2020; Accepted: 30 December 2020; Published: xx February 2021

Abstract

Cocoa butter, also known as theobroma oil, is a common fat that can be attained from cocoa seeds. It is the main ingredient used in the cosmetic industry for making ointments, toiletries, essential oils, and pharmaceutical products. However, the cocoa price is increasing continuously due to limited supply. Pest attacks, diseases, and ageing plantations are a few causes which reduced the supply of cocoa seeds. Hence, this study is focussed on utilising the mango seed biomass waste as cocoa butter alternative. The oil from the mango seed or kernel part was extracted and its important properties were determined. The mango kernel oil (MKO) was extracted by using the Soxhlet extractor with hexane which reported to have the highest oil yield $7.32\% \pm 1.25$. The presence of triglyceride in the MKO was confirmed by significant peaks of CH_2 , CH_3 , $\text{C}=\text{O}$ and $\text{C}-\text{O}$ in the FTIR spectrum. Meanwhile, the thermal and fatty acid profiles found that the MKO had a high thermostability, and had a mixture of triglyceride group with a combination of several fatty acids, such as palmitic acid ($\text{C}_{16:0}$), stearic acid ($\text{C}_{18:0}$), and linoleic acid ($\text{C}_{18:2}$). The physical and chemical properties analysis, such as total carotene content ($147.27 \mu\text{g}\%$), melting point ($30\text{--}34^\circ\text{C}$), refractive index (1.7447), saponification value (191.40 mg KOH/g), and acid value (2.40 mg KOH/g) were compared with the cocoa butter properties. The results showed that the MKO properties were comparable with cocoa butter. Thus, it could be a high industrial potential as an alternative to cocoa butter, to be used as an ingredient in pharmaceutical, cosmetic, and food productions.

Keywords: mango kernel oil, cocoa butter, physical properties, chemical properties

Abstrak

Mentega koko juga dikenali sebagai minyak tengkawang adalah lemak biasa yang dapat diperolehi daripada biji koko. Mentega koko merupakan ramuan utama digunakan dalam industri kosmetik untuk membuat minyak gosok, peralatan mandian, minyak pati dan produk farmasuetikal. Namun begitu, harga koko terus meningkat kerana bekalan yang terhad. Serangan haiwan perosak, penyakit dan penuaan tanaman adalah beberapa penyebab yang mengurangkan bekalan biji koko. Oleh itu, kajian ini difokuskan pada memanfaatkan sisa biomas mangga sebagai alternatif mentega koko. Minyak daripada biji mangga atau bahagian isirung diekstrak dan sifat pentingnya ditentukan. Minyak isirung biji mangga (MKO) diekstrak dengan menggunakan pengekstrak Soxhlet dengan heksana dilaporkan mempunyai hasil minyak tertinggi $7.32\% \pm 1.25$. Kehadiran trigliserida dalam MKO dibuktikan oleh kehadiran puncak yang penting iaitu CH_2 , CH_3 , $\text{C}=\text{O}$ dan $\text{C}-\text{O}$ dalam spektrum FTIR. Sementara itu, profil haba dan asid lemak mendapati bahawa MKO mempunyai kebolehtahanan haba yang tinggi, dan mengandungi campuran kumpulan trigliserida dengan

gabungan beberapa asam lemak, seperti asam palmitik ($C_{16:0}$), asam stearik ($C_{18:0}$), dan asam linoleik ($C_{18:2}$). Analisis sifat fizikal dan kimia, seperti jumlah kandungan karotena ($147.27 \mu\text{g}\%$), takat lebur ($30-34^\circ\text{C}$), indeks biasan (1.7447), nilai saponifikasi (191.40 mg KOH/g), dan nilai asam (2.40 mg KOH/g) telah dibandingkan dengan sifat mentega koko. Hasil kajian menunjukkan bahawa sifat MKO setara dengan mentega koko. Oleh itu, **mentega koko** berpotensi perindustrian yang tinggi sebagai alternatif bagi mentega koko untuk digunakan sebagai bahan dalam produk farmasi, kosmetik, dan makanan.

Kata kunci: minyak isirung biji mangga, mentega koko, sifat fizik, sifat kimia

Introduction

Mango (*Mangifera indica* L.) is being commercialised in more than 90 countries, including Malaysia. In Malaysia, mango is cultivated widely, and abundantly at the northern states of Peninsular Malaysia due to the suitable climate and favourable soil conditions [1]. It is recognised for its varieties, striking colour, flavourful taste, health benefits, and also high nutritive values [2]. A report estimated about 16,913 million tons of mangoes were exported for domestic and international demands [3]. Apart from direct consumption, mangoes could be converted into various products, such as juices, puree, leather, chutney, jam, slices, powder, and ready-to-serve beverages [4].

It is estimated annually that 35-60% of the total fruit weight was discarded as waste after processing them in the form of peel and seed. As a result, the waste might pose as a threat to environmental pollution if not properly managed [5]. It was reported that the seed or mango kernel had the potential for a sustainable secondary product. The fat content (7-15% of dry matter) from the seed could be a good source for the recovery of edible fats [6]. Researchers found that the mango kernel oil (MKO) from the mango seeds could be an alternative to cocoa butter usage in food, cosmetic, and pharmaceutical industries as it contained common fatty acids, such as palmitic acid, stearic acid, and linoleic acid [7, 8]. Cocoa butter is an important ingredient in such products because it is rich in antioxidants, and also has a natural emollient moisturiser that is useful in dealing with dry skin. Due to limited sources, which were only cultivated in a few countries, and had tempering difficulties, the price of cocoa butter increased yearly [9-11]. Therefore, the economical source that has similar properties like cocoa butter is needed as an alternative in the future.

In this study the MKO from Malaysia's variety was extracted through the Soxhlet extraction by using hexane, ethanol, and petroleum ether. The structure of extracted MKO was then analysed by using the Fourier Transform Infrared Spectroscopy (FTIR). The physical and chemical properties of the MKO was analysed and compared with cocoa butter in order to determine its suitability as an alternative to cocoa butter.

Materials and Methods

Materials

The mango kernel sample was prepared as follows. The mangoes were purchased from the NSK supermarket located in Seremban, Negeri Sembilan, Malaysia. The mangoes were supplied directly from the Federal Agricultural Marketing Authority's (FAMA) farm, and known as *Mangga Susu*, in the local name. The mango seeds were obtained from 10 kg of mangoes and cracked after utilising the flesh. Then, the kernel was dried, grounded to powder form, and stored at a temperature of below 4°C . The storage at low temperature maintained the humidity and prevented the oxidation process on the dried mango kernel where the powder caused a decrease of extracted MKO yield [12].

The chemicals in this research, used without future purification, were hexane (QREC-98%), ethanol absolute (HMBG), petroleum ether (R&M-analytical grade), diethyl ether (R&M-99.5%), methanol (HMBG-analytical grade), phenolphthalein solution 1% (BENDOSIN), sodium hydroxide-50% w/v solution (MERCK), potassium hydroxide (QREC), sulphuric acid (R&M-95%), hydrochloric acid-fuming 37% (R&M), acetone (R&M-analytical grade), acetonitrile (FRIENDEMANN SCHMIDT-analytical grade), and hexane (HPLC grade). Besides, three standards were used, which were palmitic acid ($C_{16:0}$), stearic acid

(C_{18:0}), and linoleic acid (C_{18:2}). All standards were purchased from the R&M chemicals.

Extraction of the MKO

The Soxhlet extraction was performed in triplicate with hexane, ethanol, and petroleum ether, respectively. The mango kernel sample (20 g) was extracted with 200 mL of solvent for 4 hours separately at 60 °C (hexane), 70 °C (petroleum ether), and 50 °C (ethanol). The temperature chosen was different for each solvent to avoid thermal degradation on bioactive components [12]. Finally, the resulting extracts were separated by evaporating the solvents used in a rotary evaporator under low pressure. The significant functional group in the structure of the MKO was confirmed by using the Perkin Elmer Spectrum 100 FTIR over the range 4,000-650 cm⁻¹, performed in attenuated total reflection (ATR) mode.

Physical properties of MKO: Thermal profile

The Perkin-Elmer Diamond Different Scanning Calorimetric (DSC) instrument was calibrated by using indium and zinc. The MKO was purged with 99.99% nitrogen with a flow rate of 100 mL/min and pressured at 20 psi. Frozen oil samples were heated at 40°C in an oven until it completely melted. Thereafter, the MKO was placed in an aluminium volatile pan and cooled to -80 °C and held for 2 minutes. It was heated from -80 °C to 40 °C at a rate of 5 °C/min, and held isothermally for 2 minutes [11, 13].

Melting point

The MKO was inserted around 1 cm in a capillary tube, and was cooled at a temperature of less than 0 °C for 2 hours. The frozen capillary tube was submerged in hot water to make sure the top end of the MKO was 1 cm below the water level. The temperature was recorded when the range of the frozen MKO started to slip until it totally slipped out from the capillary tube [14].

Refractive index

Refractive index indicates the ratio of speed of light at a specific wavelength in the air to the speed of light in the MKO. The determination of the refractive index was carried out by using a refractometer and a sodium lamp. Two to three drops of distilled water were placed on the

main prism surface and covered with secondary prism that was visible through an eyepiece. The refractometer was set at 1.3330 when the temperature reaches 20 °C. Then, the 2-3 drops of MKO were inserted at the centre of the main prism. The boundary line was observed by turning the measurement knob slowly. The colour compensator knob was turned to remove the colour of the boundary line, and the refractive index was recorded [14].

Chemical properties of MKO: Total carotene content

The total carotene content in the MKO was analysed by using the Ultraviolet-Visible (UV-Vis) spectrophotometer at 450 nm. The MKO was homogenised by using a homogeniser, and weighed accurately to 0.1 g in the 25 mL volumetric flask, and diluted to the mark with n-hexane solution [15]. The total carotene content of the MKO was calculated as β-carotene in part per million (ppm) by using the equation 1, where V = volume used for analysis (mL), As = absorbance of the sample, Ab = cuvette error, W = weight of MKO used (g), and 2,592 = the extinction coefficient.

$$\text{Carotene content} = \frac{[V \times 2592 \times (A_s - A_b)]}{(100 \times W)} \quad (1)$$

Saponification value

The MKO was melted at 90 °C and homogenised by using a homogeniser for 2 minutes, and then filtered to remove any impurities. The 2 g of MKO was added into a conical flask which contained 30 mL of 0.5 N ethanolic potassium hydroxide solution. The reaction mixture was refluxed for 1 hours stirring continuously, and cooled at room temperature. Then, the phenolphthalein solution was added to the reaction mixture as an indicator followed by titration with 0.5 N hydrochloric acid until the pink colour disappear. A blank determination was also carried out omitting the MKO, and the saponification value was calculated by using the equation bellow, where a = volume of HCl titrated in reaction mixture (mL), b = volume of HCl titrated in blank solution (mL), M = molarity of HCl, 56.1 = molecular weight of KOH, and w = weight of MKO used (g) [14].

$$\text{Saponification value} = \frac{[(b-a) \times M \times 56.1]}{w} \quad (2)$$

Acid value

The MKO was melted at 90 °C, weighing approximately 5-10 g, and transferred into the Erlenmeyer flask. Similar volume (25 mL) of diethyl ether, and methanol were added followed with a few drops of 1% phenolphthalein solution as an indicator. The temperature of the mixture was controlled at 40 °C and was titrated against 0.1 M NaOH along with a gentle shake until the first permanent pink colour appears. The free fatty acid value was calculated as oleic acid, where 1 ml of 0.1 M NaOH = 0.0282 g of oleic acid [14]. The formula was used, where b = volume of NaOH titrated in the sample (mL), and w = weight of MKO used (g).

$$\text{Acid value} = \frac{b \times 5.61}{w} \quad (3)$$

Fatty acid profile

Fatty acid composition was determined by the conversion of fatty acid to fatty acid methyl ester. The 0.05 g of melted MKO was trans-esterified by using 2 mL of methanol:sulphuric acid (20:80) and heated at 90-100 °C for 30 minutes in a water bath. Then, the solution was cooled at room temperature and added with n-hexane HPLC grade (2 mL), and distilled water (8 mL). The mixture was agitated vigorously for 1 minute, and continued with the separation of hexane soluble and insoluble layer by using centrifuge at 3,500 rpm for 2 minutes. The fatty acid methyl esters (FAME) that was soluble in the hexane was collected and the fatty acid profile of the MKO was determined quantitatively and qualitatively by using a GC-FID. The 0.5 µL of FAME was injected into the system with the injector temperature of 220 °C with helium as gas carrier. The oven temperature was set at 175 °C for 30 min, and gradually increased at 3.0 °C/min up to 220 °C [16].

Results and Discussion

The MKO extract was pale yellow in colour and the percentage yield of extraction for each solvent used was summarised in Table 1. The selection of solvent used in this study were suitable for the extraction of oil from plants [12]. Among the three solvents used, hexane gave the highest percentage yield, followed by petroleum

ether, and ethanol. The differences of percentage yield among the solvents were due to the different polarity between solvents and solutes. Since triglyceride is a non-polar compound, the extraction by using hexane, which was the most non-polar solvent compared to petroleum ether and ethanol, gave the highest percentage yield [7]. Thus, hexane was chosen as a preference solvent in the extraction of the MKO for this study.

Table 1. The percentage yield of MKO extract

Solvent	% Yield
Hexane	7.32% ± 1.25
Petroleum Ether	3.40% ± 0.84
Ethanol	1.16% ± 0.21

FTIR spectroscopy

The FTIR spectrometer is an important analysis to determine the presence of triglycerides based on its significant functional group in the MKO. The triglycerides were ester derived from fatty acid, and a trifunctional alcohol-glycerol. The functional group that was found in the triglycerides were CH₂, CH₃, C=O and C-O. The FTIR spectrum of the extracted MKO was illustrated in Figure 1, and showed a similarity of the FTIR at its peak [17]. Significant absorption peaks of CH₂, CH₃, and C-O were observed in the medium intensity at 2921, 2852, and 1260 cm⁻¹, respectively. While the absorption peak of C=O at 1747 cm⁻¹ with a high intensity was probably because it contained more than one ester in the molecule.

Thermal profile

The DSC was used to determine the thermal profile of the MKO. The crystallisation and melting profile of the MKO was obtained during the cooling and heating process, respectively. Both thermograms were shown in Figure 2. The crystallisation onset (T_{CO}) and melting completion (T_{MC}) were considered to be the temperature at which the crystallisation began and the melting ended, respectively. The MKO started to crystallise at 26 °C (T_{CO}) which was considered a high temperature because

it consisted of a long-chain fatty acid. The crystallisation was followed by the phase transition at temperatures of 18 °C (T_1), 16 °C (T_2), 11 °C (T_3), 4 °C (T_4), and ended at -10 °C (T_5). Meanwhile, the melting process of the MKO started at -40 °C (T_1) with the phase transition at 1 °C (T_2), 6 °C (T_3), 12 °C (T_4), 20 °C (T_5), and ended at 30 °C (T_{MC}). The transition temperatures observed in the thermogram were an evidence of the complex feature of the MKO and corresponded to the mixture of triglycerides group with a different melting point [18]. The melting behaviour indicated the presence of triglyceride formed by poly unsaturated acid appearing at -40 °C, mono unsaturated at 1 °C, and saturated acid at a temperature above than 12 °C [13]. The melting and crystalline values were slightly higher than the cocoa butter values as reported previously [1]. These may be contributed to the higher saturated fatty acid content in the MKO which comprised of saturated long hydrocarbon chain of palmitic, stearic, and oleic acid. Besides, the higher value of T_{MC} showed that the MKO had higher thermostability [13, 17, 19].

Fatty acid profile

The MKO consisted of both saturated and unsaturated fatty acid. The major difference between saturated and unsaturated fatty acid is the presence of double bond. The double bond is only present in the unsaturated fatty acid which means there are less hydrogen atoms present in the molecule's structure. Three major fatty acid presented were palmitic, stearic, and linoleic acid, similar to studies conducted previously [1]. The separation of the compounds was based on the different strengths of interaction of the compounds with the stationary phase. The stronger the interaction, the longer the compound interacted with the stationary phase, and the more time needed to migrate through the column. Stearic acid took a longer time to separate (13.651) than palmitic acid (11.710), and linoleic acid (13.409), as it contained the longest carbon chain which made them to be less polar than the others [2, 7, 12, 20].

Total carotene content

The total carotene content found in the MKO was 147.27 µg%. The lower value was related to the pale yellow colour of the extracted MKO. Carotene is a natural pigment that represent the orange colour of a

substance, mainly found in fruits and vegetables. Carotenoid is a bioactive substance in food with powerful antioxidant activity. However, due to the unsaturated nature of the carotene, it is easily degraded due to the oxidation and severe processing conditions, such as thermal process [14]. In the human body, they could act as a precursor of vitamin A, which is a pigment essential for good vision and healthy eyes. Studies showed that regular consumption of β -carotene in food lowered the risk of certain chronic diseases, such as cataract, cardiovascular diseases, and cancers [2, 15].

Comparative study of MKO and commercial cocoa butter

The physical and chemical properties of extracted MKO was analysed and compared with the commercial cocoa butter. The results of the melting point, refractive index, saponification value, and acid value were summarised in Table 2.

The melting point of the MKO was observed at a range of 30 to 34°C. Meanwhile, cocoa butter had a melting point between 27 to 35°C [21]. This result could classify that the MKO and cocoa butter had comparable melting point. The MKO started to melt at a higher temperature compared to cocoa butter due to the long-chain length of saturated fat in the structure of the MKO, thus increasing the melting point, as well as its thermostability [17, 21].

The refractive index of the MKO was found at a value of 1.7447 at 40 °C. This data showed that the MKO had higher refractive index value compared to commercial cocoa butter which was 1.4540 to 1.4590 [22]. Higher refractive index indicated an increase in the chain length and the number of double bond in the MKO. The value was proportional to the length of carbon chain, the number of double bond presented, and the molecular weight of the substance. However, refractive index was found to decrease linearly with increasing temperature. The correlation concluded that the substance with the higher value of refractive index would have a higher density. The refractive index measured the bending ray of light as it passed from one medium to another that showed the information about the behaviour of light, and intermolecular interaction of the substance [23, 24].

The saponification value data for the MKO and cocoa butter were comparable at 191.41 mg KOH/g, and 194.4 mg KOH/g, respectively [25]. The finding indicated the chain length of the MKO carbon molecule was similar to the chain length of commercial cocoa butter. The saponification value found in the MKO was considered high and showed the indicator that it was suitable for use in the cosmetic industries [26, 27]. It represented the amount of potassium hydroxide, or sodium hydroxide (in mg) required to saponify 1.0 g of fat under specific conditions. The average molecular weight or chain length of all the fatty acid might vary depending on the choice of substance and solvent used in the study [2, 12, 24].

Another parameter measured for the physicochemical study is the acid value. The acid value for the MKO was found as 2.40 mg KOH/g, while for cocoa butter was 1.68 [11]. The higher value of the MKO compared to

commercial cocoa butter might indicate the increased susceptibility of fat to oxidation due to high degree of unsaturation. The result was in accordance with the previous finding [21]. Even though the MKO acid value was higher compared to cocoa butter, it was still considered acceptable. The acid value was a measure of the total acidity of the lipid, involving contribution from all the constituent fatty acid that made up the glyceride molecule, and it also measured the free fatty acid. The acid value was defined as the weight of potassium hydroxide (in mg) needed to neutralise the free fatty acid present in 1.0 g of fat, and it was also necessary in determining the quantity of free fatty acid present in fat or oils. It indicated its quality, age, edibility, and its oil suitability for cosmetic usage. The hydrolysis of triglycerides occurred and increased in rancidity when there was an increase in the free fatty acid value in a substance [28].

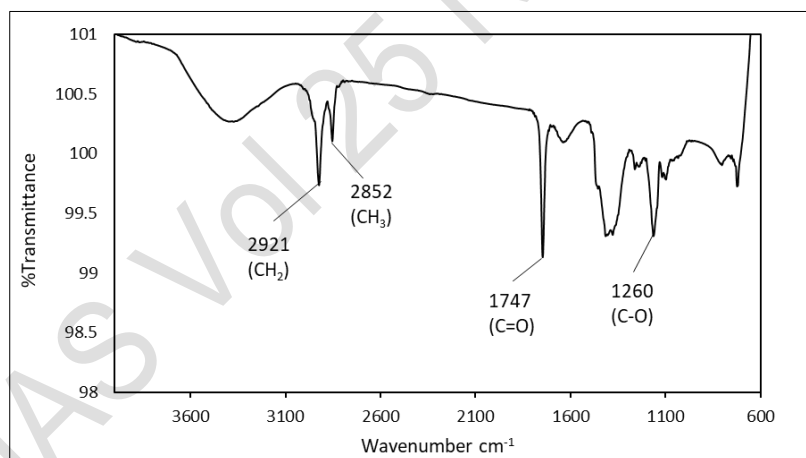


Figure 1. FTIR spectrum of MKO

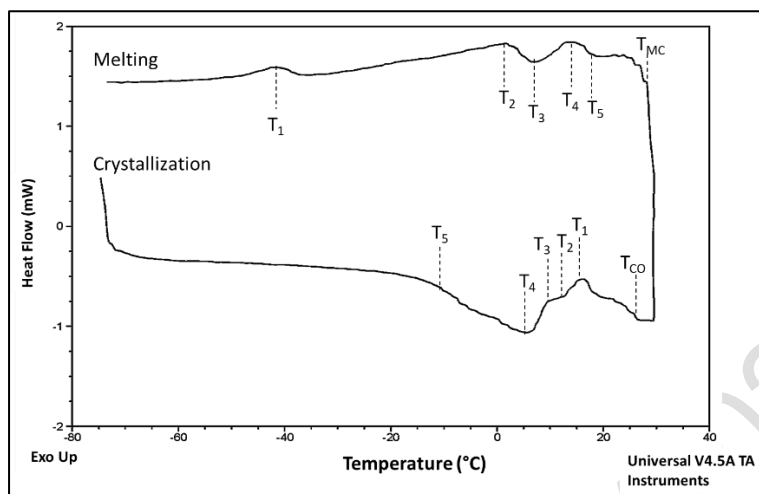


Figure 2. Thermogram of MKO

Table 2. Comparative study between extracted MKO and cocoa butter

Physical or Chemical Properties	MKO	Cocoa Butter [Ref.]
Melting point (°C)	30-34	27-35 [21]
Refractive index	1.7447	1.4540-1.4590 [22]
Saponification value (mg KOH/g)	191.40	194.40 [25]
Acid value (mg KOH/g)	2.40	1.68 [11]

Conclusion

The data obtained showed a few similarities to cocoa butter properties. Thus, it indicated that extraction of the MKO has a potential as a cocoa butter alternative, and can further be used in food, pharmaceutical, or cosmetic industries. However, a few improvisations, such as chemical and physical refining, and blending with other fats and oils sources can enhance the physicochemical properties of the MKO to be on par, or superior than cocoa butter. In addition, the data from this study could contribute to support the uses, health, and economic benefit of the MKO that is completely unexploited commercially in Malaysia. The exploitation of mango seeds by the industry into a valuable product can thrive the agricultural sector by product segment, increase

value-added properties to the by-product, and at the same time help to reduce the environmental pollution.

Acknowledgement

The author wishes to thank Faculty of Applied Sciences, Universiti Teknologi MARA, Cawangan Negeri Sembilan, Kampus Kuala Pilah for the research facilities and financial supported.

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