Blending of mango kernel fat and palm oil mid-fraction to obtain cocoa butter equivalent

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Abstract Cocoa butter equivalent (CBE) was produced from a blend of mango kernel fat (MKF) and palm oil mid-fraction (PMF). Five fat blends with different ratios of MKF/PMF (90/10, 80/20, 70/30, 60/40 and 50/50 (%wt)) and pure MKF, PMF and cocoa butter (CB) were characterized. Similar to CB, all fat blends contained palmitic (P), stearic (S) and oleic (O) acids as the main fatty acid components. The triglyceride compositions of all blends were significantly different from CB. However, blend 80/20, which contained higher content of SOS, similar content of POP and lower content of POS compared to CB, exhibited a slip melting point, crystallization and melting behavior most similar to CB and hence it was recommended as CBE. The chosen CBE was then mixed with CB in a ratio of 1:5.64 (wt), mimicking that of typical dark chocolate where 5 % of CBE is added to the finished product. The crystallization behavior, the crystal morphology and bloom behavior of the mixture was investigated and was found to be not significantly different from CB.

Keywords Mango kernel fat · Palm oil mid-fraction · Cocoa butter · Cocoa butter equivalent · Solid fat content

Introduction

Cocoa butter (CB) is an important major constituent of chocolate and other confectionery products. It is one of the most expensive vegetable fats. The typical fatty acid composition of CB is (in mol-%) palmitic acid (C_{16}) 24.4, stearic acid (C_{18}) 33.6, oleic acid ($C_{18:1}$) 37.0, linoleic acid ($C_{18:2}$) 3.4 and others 1.6 (Xu 2000). CB is predominantly composed of three monounsaturated triglycerides—POP, POS and SOS (where P = palmitic acid, O = oleic acid, and S = stearic acid). It is the unique symmetrical positioning of the fatty acids in these triglycerides, with saturated fatty acids in the *sn*-1,3 positions, and an unsaturated fatty acid in the center or *sn*-2 position, that gives CB its desirable characteristics. The fat is mainly solid at temperatures below 25 °C but is almost entirely liquid at body temperature (~37 °C) (Beckett 2008). CB helps to bind the other key ingredients in chocolate and confectionery (cocoa powder, sugar, etc.) and thus has a major impact on sensory and physical properties. CB can crystallize into six polymorphic forms (Wille and Lutton 1966). Moreover, it is resistant to oxidation (Minifie 1970). However, only form V (β_2) gives the optimal melting behavior for CB in chocolate (Beckett 2008). It also confers chocolate products proper snap (ability to break apart easily), good demoulding properties (contraction) and a good quality finish in terms of color and gloss. In addition, it exhibits resistance to fat bloom, a physical defect that appears during storage as undesirable white spots or a streaky grey-white finish on the chocolate surface (Beckett 2008). Shortage of supply, poor CB quality of individual harvests, economic advantages and some technological benefits have prompted the chocolate and confectionery industries to look for alternative fats that are fully compatible with the physical and sensory properties of CB but cheaper.

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Cocoa Butter Equivalents (CBE) have been used in chocolate products for many years. They are vegetable fats which have chemical and physical properties similar to CB (Samsudin and Rahim 1996). They can be added to CB in any proportion without causing significant softening effect, nor altering the melting, rheological and processing characteristics of CB (Zaidul et al. 2007). To be able to achieve this CBE must crystallize in the same way as CB (Beckett 2008). CBE are designed to contain a glyceride composition similar to that of CB, i.e. the majority of fatty acids contained in CBE are palmitic, stearic and oleic acids. The transformation of low-cost fats and oils through chemical and enzymatic interesterification processes as well as fractionation have been used to produce CBE (Tchobo et al. 2009). For example, CBEs have been produced through enzymatic interesterification of palm oil mid-fraction (PMF) with stearic acid (Undurraga et al. 2001), of tea seed oil with methyl palmitate and methyl stearate (Wang et al. 2006) and of *Pentadesma butyracea* butter with ethyl palmitate (Tchobo et al. 2009). In these works, the effect of several parameters such as initial ratio of fatty acid-fat, initial humidity of the enzyme preparation and the enzyme-substrate ratio were studied. The triglycerides produced from these works exhibited thermograms obtained by scanning differential calorimetry similar to CB.

Fractionation as a means to produce CB-like fats with and without other techniques has been studied by many researchers (see, for example, Hashimoto et al. 2001; Calliau et al. 2005). Apart from interesterification and fractionation, fat blending or mixing has also been used in the preparation of CB-like fats. The fat manufacturer can obtain CBE with the appropriate ratio of POP, POS and SOS by mixing different vegetable fats together (Beckett 2008). Conventionally, CBE are produced by blending PMF with one or more types of exotic fats which are high in SOS content such as sal, shea, and Borneo tallow. For example, Md Ali (1996) prepared CBE from sal fat blended with co-fractionated palm oil. The palm oil was dry fractionated to obtain palm olein, which was then mixed with sal fat and the fat blend was solvent fractionated using acetone to obtain CBE.

Mango is one of the most important fruit crops of Asia. Mango fruits are considered to be a good source of carotenoid, Vitamin C, fair source of organic acids, carbohydrates and minerals (Gupta and Jain 2012). In addition, the fat extracted from the mango seeds, called mango kernel fat (MKF), has received attention in recent years due to the resemblance between its characteristics and those of CB. Solis-Frentes and Duran-de-Bazua (2004) reported notable similarities between the melt and crystallization profiles and solid fat content of MKF and CB. MKF has been reported to contain high content of stearic and oleic acids (Narasimha Char et al. 1977; Van Pee et al. 1980; Lakshminarayana et al. 1983; Ali et al. 1985; Solis-Frentes and Duran-de-Bazua

2004). The fat has also been reported to have a high SOS content with $C_{18:1}$ being most abundant acid in the middle *sn*-2 position (Holcapek et al. 2005). According to the EU chocolate directive (2000/36/EC), MKF is one of only six vegetable fats which are allowed to be used for up to 5 % in chocolate (Wilson 1999).

The Kaew mango variety (*Mangifera indica*, L., cv. Kaew) is widely cultivated in Thailand with an annual production of ca. 400,000 tons. Compared to other varieties of mangoes in Thailand, Kaew mangoes have been processed the most in the canning industry into fruit juice, fruits in syrup, jam, jelly, etc. This has created extensive amount of seeds as agroindustrial waste because only the pulp of mangoes is needed and the mango seeds are thrown away after being separated from the pulp. The seed represents up to 25 % of the fruit (Lakshminarayana et al. 1983). Consequently, the total annual production of discarded seeds from Kaew mangoes alone in Thailand is ca. 100,000 tons. Since the average fat content of mango seeds is ca. 10 % (Ali et al. 1985), if collected properly with an assumption that all of the fat can be removed, ca. 10,000 tons of MKF could be produced from Kaew mango seeds.

To help reduce the agricultural waste from the canning industry, in this work, attention has been drawn towards the possible utilization of the discarded Kaew mango seeds as a non-conventional source of vegetable fat for the chocolate and confectionery products. The aim of this research was to examine the possibility of producing CBE from mixtures of MKF and PMF for use in chocolate products. PMF is obtained by double fractionation of palm oil, resulting in a fraction that exhibits melting points between those of palm stearin and palm olein (Sakamoto et al. 2004). Its main characteristic is a very high content in symmetrical disaturated triglycerides (mainly POP) leading to a very steep solid fat content versus temperature curve. PMF is regarded as a suitable raw material for the production of CBE in terms of cost, availability and composition (Undurraga et al. 2001). In addition, it could be used to increase solid fat content and promote β' crystal formation in margarines and spreads. MKF was mixed with PMF in many ratios. The fat mixtures and the pure fats including CB were characterized using various methods and techniques in order to find a fat blend that was compatible with CB.

Materials and methods

Materials

Mango seeds, Kaew variety, were obtained from canned fruit factories in Ratchaburi province, Thailand. The seeds were cut open and the seed kernels were removed. The kernels were kept in polyethylene bags at -18°C until

further use. CB was purchased from Sino-Pacific Trading (Thailand) Co., Ltd. PMF was supplied by Morakot industries PCL (Thailand). The standard fatty acid methyl esters for fatty acid analysis using gas chromatography (GC) were purchased from AccuStandard, Inc. (USA). OOO and POP as standards for triglyceride analysis using high performance liquid chromatography (HPLC) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acetone and acetonitrile were of HPLC grade from Burdick and Jackson (Muskegon, MI, USA). All other chemicals and solvents used were obtained commercially and were of the highest purity available.

Crude fat extraction and purification

The frozen mango seed kernels were removed from the polyethylene bags and defrosted at a room temperature for at least 8 h before being dried at 65 °C in a vacuum oven until the moisture content was less than 10 % (Solis-Frentes and Duran-de-Bazua 2004). Then, the dried kernels were finely ground into powder and stored in polyethylene bags at 4 °C until extraction. The mean particle diameter of the powder, as determined by a laser diffraction particle size analyzer (LS 100Q, Beckman Coulter Inc., CA, USA), was 330 µm. Crude MKF was extracted using the Soxhlet extraction method and n-Hexane as a solvent at 60 °C for 6 h, and the fat was purified using the method described by Solis-Frentes and Duran-de-Bazua (2004). The purified fat exhibited a cream color and a solid consistency at room temperature (25 °C). The fat was kept away from light and air at 4 °C until further processing and analysis.

Fat blending

The purified MKF was blended with PMF into five proportions of MKF/PMF: 90/10, 80/20, 70/30, 60/40 and 50/50 (%wt). Each mixture was melted at 80 °C for at least 10 min under constant stirring at 200 rpm and then it was stored at 4 °C until further analysis. The fatty acid and triglyceride compositions, physicochemical properties, crystallization and melting profiles and solid fat content as a function of time of all blends including MKF, PMF and CB were characterized using various techniques and methods as described below.

Characterization of fatty acid composition

Fat was converted into fatty acid methyl esters using AOAC official method 969.33 (AOAC 1995). In brief, 0.4 g of each sample was added to 6 mL of methanolic NaOH solution (0.5 M) in a flask with a boiling chip. The mixture was refluxed for 10 min. Then, 7 mL of BF₃ solution (125 g BF₃/L methyl alcohol) was added and the boiling was continued for

2 more minutes. Heptane (5 ml) was added and the mixture was boiled for 1 min longer. The mixture was cooled down and 15 ml of saturated NaCl solution was added. While it was still tepid, the mixture was shaken vigorously for 15 s. Additional saturated NaCl solution was added to float heptane solution into the neck of the flask. An upper part of the heptane solution was transferred into a glass-stoppered test tube and a small amount of anhydrous Na₂SO₄ was added to remove water.

The fatty acid methyl esters analysis was performed in a Shimadzu GC with flame ionization detector (GC-FID). The system had an AT™-WAX capillary column (50 m long, 0.25 mm internal diameter and 0.20 mm film thickness). Compound identification was carried out using external standards of fatty acids methyl esters. Helium was used as a carrier gas with a flow rate of 0.5 mL/min and with a controlled initial pressure of 93.2 kPa at 120 °C. N₂ and air were makeup gases. The injection temperature was 210 °C, and the oven temperature program was holding at 120 °C for 3 min before increasing at a rate of 10 °C/min to 220 °C, holding at this temperature for 30 min, increasing at a rate of 5 °C/min to 240 °C, followed by holding at 240 °C for 30 min. The split ratio was 100:1, the injection volume was 1 µL, and the detector temperature was 280 °C. All the fat mixtures, MKF, PMF and CB were analyzed after which their chromatograms were acquired. All fatty acid contents were given based on percentage area.

Characterization of triglyceride composition

The triglyceride compositions of the mixtures were determined by HPLC (Shimadzu LC-20 AD, Shimadzu Corp, Kyoto, Japan) with system controller CBM-20A and diode array detector SPD-M20A. Two C-18 columns (Inertsil ODS-3; 4.6×250 mm; 5 µm particle size; by GL Sciences Inc., Japan) were used in series. The mobile phase consisted of acetone and acetonitrile (70:30, vol/vol) with a flow rate of 0.72 mL/min. The column temperature was set at 35 °C with a column heater (Shimadzu CTO-10AS column oven). The injection volume was 20 µL. Peaks for POP and OOO were identified by comparing their retention times with those of authentic standards. Peak identification for POS and SOS was based on the POS and SOS components of natural CB. Peak identification for other triglycerides was performed by comparison with the HPLC pattern of MKF reported by Holcapek et al. (2005). Calculation of triglyceride compositions was based on area percents from the chromatograms.

Characterization of physicochemical properties

Iodine value was analyzed using automatic titrator (Mettler Toledo DL58 titrator). Saponification value was analyzed

following Palm Oil Research Institute of Malaysia (PORIM) Test Method no. p3.1 (1995). Briefly, each sample was melted at 80 °C and filtered through a filter paper. Then, 0.2±0.005 g of the sample was put inside a conical flask and 25 ml of the ethanolic potassium hydroxide solution was added. The reflux condenser was connected and the content was gently boiled for at least 60 min. 1 ml of phenolphthalein solution was added and the solution was titrated with the 0.5 N hydrochloric acid until the pink color just disappeared. Slip melting point was analyzed using PORIM Test Method no. p4.2 (1995). Each sample was melted at 80 °C and filtered thorough a filter paper. At least three clean capillary tubes were dipped into the liquid sample so that columns of fat ~10 mm high were obtained in the tubes. The fat columns were cooled immediately by holding and rolling the ends of the capillary tubes pressed against a piece of ice, until the fat had solidified. The tubes were wiped dry as rapidly as possible and placed in a test tube. The test tube was transferred to a chiller set at 10±0.1 °C for 16 h. The capillary tubes were removed from the test tube and attached to a thermometer with a rubber band. The thermometer was suspended in a beaker containing 400 ml of distilled water. The starting temperature of the water was set at 5–10 °C below the expected slip melting point of the sample. The beaker was stirred with a magnetic stirrer and the heat was applied to increase the temperature at a rate of 1 °C per min, slowing down to 0.5 °C per min as the expected melting point was attained. The heating was continued until the fat column in each tube rises. The temperature of the water was recorded and the average was calculated from all tubes.

Characterization of crystallization and melting profiles

The crystallization and melting profiles of the fat samples were determined with a Perkin-Elmer differential scanning calorimeter (DSC) (model DSC 8000, PerkinElmer Co., Norwalk, CT) following procedure Cj 1–94 recommended by AOCS (AOCS 1997). The heat flow of the instrument was calibrated with indium (mp 156.6 °C) as a reference standard. A fat sample of 6–8 mg was placed in an aluminum pan (30 µL capacity) and hermetically sealed. An empty pan served as reference. The samples were heated from room temperature to 80 °C and held for 10 min to ensure homogeneity and to destroy any crystal memory. Then, the samples were cooled to –40 °C at the rate of 5 °C/min and held at this temperature for 30 min. This was followed by heating the sample at the rate of 5 °C/min to 80 °C. The crystallization and melting profiles were generated during the cooling and heating, respectively. The crystallization onset (T_{CO}) and melting completion temperatures (T_{MC}) were obtained from the peaks located at the highest temperature of each fat sample. T_{CO} and T_{MC} were considered to be the temperatures at which the

crystallization began and the melting ended, respectively (Kim et al. 2008).

Characterization of solid fat content

Changes in the solid fat content (SFC) as a function of temperature between 15 °C and 40 °C and melting behavior of the pure fats and the fat mixtures were determined by pulse-nuclear magnetic resonance (p-NMR) spectrometer (Minispec-mq20, BRUKER, Karlsruhe, Germany) following a method for measuring SFC of CB and similar fats developed by the “Joint Committee for the Analysis of Fats, Oils, Fatty Products, Related Products and Raw Materials (GA FETT)” as described by Fiebig and Luttke (2003).

Compatibility test

All the results obtained from the studies in all the previous sections were used to decide which ratio of the fat blends would have the highest potential to be used as a CBE fat, e.g. the blend that exhibited the fatty acid composition, triglyceride composition, crystallization and melting behavior, SFC, and slip melting point closest to those of CB. Once the blend was recommended, its compatibility with CB was tested by mixing it with CB in different ratios (100/0, 80/20, 60/40, 40/60, 20/80 and 0/100 for the chosen CBE/CB (%wt)). Then, the SFC at 20 °C for each ratio was measured using the p-NMR following the procedure given by Smith (2001).

Crystallization behavior under static isothermal condition

The recommended fat blend from the above section was mixed with CB in a ratio of 5.64:1 (wt) for CB/CBE which mimicked that of typical dark chocolate where 5 % of CBE is added to the finished product (Sonwai and Rousseau 2006). Assuming that the total fat content of typical dark chocolate is 33.2 %wt of the final product, chocolate that contains 5 % CBE will contain 28.2 % CB and this will give a ratio of 5.64:1 for CB/CBE in the product. The crystallization behavior of the recommended fat blend, the mixture of selected fat blend and CB (called MIX), pure CB, PMF and MKF under static conditions at 20 °C was investigated using p-NMR and x-ray diffraction (XRD) techniques.

For the p-NMR study, the fat samples, which were contained in p-NMR tubes, were heated to 80 °C for 15 min in a waterbath and then were transferred to a cooling bath set below 20 °C. Once the temperature of the samples inside the tubes dropped to 20.5 °C, the tubes were removed from the cooling bath, wiped dry and rapidly put into the p-NMR sample port with the temperature set at 20 °C. The timing then started and the SFC was recorded once every min for 3 h, allowing in-situ observation of the crystallization of the fat samples.

For the XRD study, each fat sample was put inside an XRD capillary sample holder using a pipette. The sample was then heated to 80 °C for 15 min by dipping in a water-bath, after which it was transferred to a cooling bath set at 20 °C and the timing started immediately. Each crystallization run lasted for 1 h. The XRD characterization was performed once every 5 min using an x-ray diffractometer (Rigaku TTRAX III, Rigaku Corporation, Tokyo, Japan). Scans were performed in wide angle x-ray scattering (WAXS) from 15° 2 θ to 25° 2 θ with a scan speed and a step width of 2.7° 2 θ /min and 0.02° 2 θ , respectively.

Crystal morphological study

Crystal network microstructure of the recommended fat blend, MIX, CB, PMF and MKF crystallized under static conditions at 25 °C was observed by polarized light microscopy (PLM) (Olympus BX51, Olympus Optical Co., Ltd., Tokyo, Japan) equipped with a digital camera (Olympus C-7070, Olympus Optical Co., Ltd., Tokyo, Japan). The fats were crystallized under quiescent conditions following the procedure described by Narine and Marangoni (1999). All fat samples were melted at 80 °C for 15 min to totally eliminate the memory effect. 20 μ L of each molten sample was placed on a glass slide, which was heated to 80 °C prior use, and covered by a cover slip. Then, the samples were transferred to and stored in a temperature-controlled cabinet, the temperature of which was maintained at 25 $\pm$ 0.2 °C, for 48 h. A 10 $\times$ lens was employed to image the gray scale photographs of the fat crystals.

Fat bloom study

Fat bloom formation on the recommended fat blend, MIX and CB was observed by using a Hunter LabScan colorimeter (0°/45° geometry) (Hunter Associates Laboratory, Inc. Reston, VA, USA) to follow changes in the surface whiteness index (WI) of the surfaces during storage at 25 $\pm$ 0.2 °C for 6 months. The samples were tempered using a procedure described elsewhere (Sonwai and Rousseau 2006), then moulded into solidified discs (diameter = 30 mm, thickness 3 mm) and put inside a temperature-controlled cabinet. L*, a* and b* values of the moulded side of the samples were measured once every week and converted to WI values using Eq. (1) (Lohman and Hartel 1994).

$$WI = 100 - \left[(100 - L^*)^2 + a^{*2} + b^{*2} \right]^{1/2} \quad (1)$$

Statistical analysis

All experiments were performed in duplicate or triplicate. All results from the characterization of fatty acid contents,

triglyceride compositions, physicochemical properties, solid fat content and bloom formation were analyzed by Analysis of Variance with Least Significant Difference (ANOVA/LSD) at 95 % confidence interval.

Results and discussion

Fatty acid composition

The fat content of the mango kernels was found to be 7.28 $\pm$ 0.19 % (dry basis). Table 1 shows fatty acid compositions for MKF, PMF, different MKF/PMF blends and CB. CB was high in C₁₈ (36.5 %), C_{18:1} (33.5 %) and C₁₆ (25.8 %). These three fatty acids represented more than 90 % of the fat. Compared to CB, MKF from the Kaew variety can be a potential source for fat high in not only C₁₈ (46.6 %) but also C_{18:1} (41.1 %). However, the fat was much lower in C₁₆ (5.4 %). The C₁₈ content of MKF reported here is higher than that in MKF from Manila variety (39.1 %) (Solis-Frentes and Duran-de-Bazua 2004), and is within the range of C₁₈ (24–57 %) found in various mango varieties cultivated in India (Lakshminarayana et al. 1983). The fatty acid content of PMF was dominated by C₁₆, which amounted to more than 50 % of the total quantity. The fat also contained a relatively high content of C_{18:1} (35.6 %). The three pure fats, CB, MKF and PMF, contained only small amount of fatty acid constituents C₁₂, C₁₄, C_{16:1}, C_{18:2}, C_{18:3}, C₂₀, C₂₂ and C₂₄.

Similar to CB, all fat blends were composed mainly of C₁₆, C₁₈ and C_{18:1}. The measured values of fatty acid compositions in the fat mixtures were consistent with no change in fatty acid composition occurring upon mixing (i.e. the fatty acid compositions were similar to those estimated from averaging values of MKF and PMF). As the quantity of MKF decreased in the fat mixtures, the amount of C₁₈ and C_{18:1} decreased proportionally with a corresponding increase in C₁₆ content. The fatty acid compositions of all fat blends were significantly different ($p < 0.05$) from one another and from those of CB. However, among the fat blends, blends 60/40, 80/20 and 50/50 exhibited the content of C₁₆, C₁₈ and C_{18:1}, respectively, closest to that of CB.

Triglyceride composition

Triglyceride compositions of all fat samples are given in Table 2, where it can be seen that CB was rich in POP, POS and SOS. Similar to the triglyceride content of MKF from other mango varieties reported in the literature (e.g., Holcapek et al. 2005), MKF from Kaew mangoes was high in SOS, followed by SLS and SOO. PMF was high in PLP, POL and POP but much lower in POS and SOS. As the amount of PMF increased in the fat blends, the contents of POS and SOS in the

Table 1 Fatty acid compositions for mango kernel fat (MKF), palm oil mid-fraction (PMF), different MKF/PMF blends (%wt) and cocoa butter (CB) ($n=3$)

Fatty acid	Fatty Acid Content (area %)							
	MKF	Blend 90/10	Blend 80/20	Blend 70/30	Blend 60/40	Blend 50/50	PMF	CB
Lauric (C ₁₂)	0.01±0.001 ^g	0.05±0.002 ^f	0.08±0.002 ^e	0.09±0.004 ^d	0.12±0.004 ^c	0.13±0.000 ^b	0.25±0.004 ^a	Trace
Myristic (C ₁₄)	0.03±0.001 ^h	0.14±0.003 ^f	0.26±0.003 ^e	0.33±0.008 ^d	0.43±0.003 ^c	0.52±0.001 ^b	1.0±0.01 ^a	0.07±0.002 ^g
Palmitic (C ₁₆)	5.4±0.03 ^h	10.3±0.10 ^g	16.3±0.07 ^f	19.8±0.15 ^e	24.9±0.05 ^d	29.1±0.05 ^b	51.6±0.13 ^a	25.8±0.13 ^c
Palmitoleic (C _{16:1})	0.05±0.036 ^e	0.04±0.000 ^e	0.08±0.000 ^{bc}	0.07±0.001 ^{cd}	0.06±0.001 ^{de}	0.05±0.002 ^{de}	0.09±0.000 ^b	0.17±0.001 ^a
Stearic (C ₁₈)	46.6±0.09 ^a	42.8±0.07 ^b	37.2±0.05 ^c	33.9±0.06 ^e	28.6±0.07 ^f	24.7±0.03 ^g	4.2±0.01 ^h	36.5±0.04 ^d
Oleic (C _{18:1})	41.1±0.09 ^a	40.0±0.11 ^b	39.6±0.02 ^c	39.2±0.03 ^d	39.1±0.07 ^d	38.7±0.05 ^e	35.6±0.08 ^f	33.5±0.10 ^g
Linoleic (C _{18:2})	3.8±0.01 ^f	3.6±0.01 ^g	3.9±0.01 ^e	4.2±0.02 ^d	4.6±0.01 ^c	5.0±0.01 ^b	6.6±0.03 ^a	2.4±0.01 ^h
Linolenic (C _{18:3})	0.51±0.003 ^a	0.39±0.002 ^b	0.36±0.001 ^c	0.32±0.00 ^d	0.28±0.002 ^e	0.27±0.008 ^f	0.10±0.001 ^h	0.14±0.001 ^g
Arachidic (C ₂₀)	1.7±0.008 ^a	1.6±0.05 ^b	1.4±0.02 ^c	1.3±0.017 ^d	1.1±0.01 ^e	0.97±0.012 ^f	0.28±0.004 ^h	0.93±0.004 ^g
Behenic (C ₂₂)	0.35±0.029 ^b	0.43±0.087 ^a	0.31±0.011 ^b	0.31±0.041 ^b	0.28±0.033 ^{bc}	0.22±0.040 ^{cd}	0.05±0.003 ^e	0.20±0.007 ^d
Lignoceric (C ₂₄)	0.54±0.132 ^{ab}	0.57±0.153 ^a	0.48±0.038 ^{ab}	0.44±0.068 ^{abc}	0.42±0.058 ^{bc}	0.32±0.070 ^{cd}	0.10±0.016 ^e	0.20±0.016 ^{de}

Similar letters in the exponential in the same row show there are no significant differences ($p>0.05$)

blends decreased while the content of POP increased proportionally. The triglyceride compositions of all fat blends were different from one another and from those of CB. However, the contents of POP and SOS in blend 70/30 were most similar to those of CB, while none of the fat blends exhibited the POS content close to that of CB.

Physicochemical properties

Table 3 shows the iodine value (Iv), saponification value (Spv) and slip melting point (SMP) of all the fat samples. The Iv is useful in determining the degree of unsaturation and hardness of fats. The higher the Iv, the more unsaturated fatty acid bonds present in a fat. From the table, CB exhibited the lowest Iv whereas the Iv of PMF was the highest, and the Iv of MKF was in between the two. This indicates that CB and PMF contained lowest and highest amount of unsaturation, respectively. The Iv of MKF from Kaew mangoes reported here was ~14 % lower than that of MKF from Manila mangoes cultivated in Mexico (Iv=47.7) (Solis-Frentes and Duran-de-Bazua 2004) and ~32 % lower than that of MKF from Indian mangoes (Iv=50) (Narasimha Char et al. 1977). It was also lower than those of various mango varieties grown in Bangladesh (Iv range 44–55) (Ali et al. 1985). This implies that MKF from Kaew mangoes is less unsaturated and hence harder than other mango varieties. This was probably due to its high and low C_{18} and $C_{18:2}$ contents, respectively. The Iv of all MKF/PMF fat blends, although not significantly different from one another ($p>0.05$), were in between but significantly different from those of MKF and PMF ($p<0.05$).

Saponification value is a measurement of the average molecular weight (or chain length) of all the fatty acids present in a fat. Spv increases when molecular weight decreases. From Table 3, pure PMF had the highest Spv. As the amount of PMF in the MKF/PMF fat blends increased, Spv increased proportionally. Compared to CB, PMF and all MKF/PMF fat blends, Spv of pure MKF from Kaew mangoes was the lowest. The value reported here was lower than those of MKF obtained from different mango varieties provided in the literature (Solis-Frentes and Duran-de-Bazua 2004; Narasimha Char et al. 1977; Ali et al. 1985). This could be due to higher content of C_{18} in Kaew MKF compared to MKF from other varieties. It can be seen from Table 3 that Spv of CB was not significantly different from the Spv of 70/30, 60/40 and 50/50 blends ($p>0.05$), but significantly different from those of MKF, 90/10, 80/20 blends and PMF ($p<0.05$).

Slip melting point of a fat is the temperature at which a column of fat in an open capillary tube softens or becomes sufficiently fluid to slip or run up the tube when it is subjected to a controlled heating. SMP of all samples were significantly different from one another ($p<0.05$). CB and

Table 2 Triglyceride compositions for mango kernel fat (MKF), palm oil mid-fraction (PMF), different MKF/PMF blends (%wt) and cocoa butter (CB) ($n=3$). *P* palmitic, *O* oleic, *S* stearic, *L* linoleic, *L_n* linolenic

Triglyceride	Triglyceride Content (area %)							
	MKF	Blend 90/10	Blend 80/20	Blend 70/30	Blend 60/40	Blend 50/50	PMF	CB
POL	4.1±1.03*	5.1±0.33	4.3±0.06	9.0±0.69	11.0±0.47	11.6±0.53	19.0±0.25	2.6±0.32
PLP	1.8±1.34	4.3±0.37	5.9±0.28	9.6±0.80	12.4±0.52	14.2±0.59	22.3±0.25	8.4±0.28
OOO	2.5±1.26	3.1±0.16	3.4±0.18	3.2±0.13	3.2±0.04	3.1±0.08	2.8±0.04	0.6±0.06
SLO	—	7.1±0.45	4.8±0.18	5.8±0.59	5.2±0.25	3.7±0.53	—	2.1±0.16
POO	10.8±0.52	2.7±0.42	4.9±0.10	5.5±0.54	6.9±0.04	8.6±0.06	13.4±0.38	2.4±0.06
POP	8.9±0.19**	10.7±0.87	12.7±0.78	13.6±1.24	15.5±0.32	16.5±0.93	17.8±0.13	13.8±0.42
SOO	14.6±0.29	13.9±1.15	12.7±0.52	10.3±1.15	8.8±0.40	7.6±0.25	1.2±0.28	3.9±0.19
SLS	14.6±0.04	12.7±0.79	11.4±0.52	9.2±0.83	7.9±0.18	6.1±0.16	—	6.8±0.31
POS	5.7±0.18	6.2±0.57	6.7±0.35	5.2±0.48	5.1±0.24	4.6±0.17	2.9±0.64	26.3±0.04
SOS	29.4±1.20	26.9±2.30	26.9±1.28	18.9±1.99	16.1±0.39	13.0±0.62	0.5±0.12	19.2±0.05
Others	7.7±0.48	7.4±1.22	6.2±1.18	9.8±3.5	7.9±2.57	11.0±0.23	20.1±0.86	13.9±1.65

*shows contents of POL + SLL + SOL_n, and**shows contents of POP + SLP + SL_nS

PMF exhibited the lowest and the highest SMP, respectively. SMP of the MKF/PMF fat mixtures appeared to increase as the content of PMF in the mixtures increased. However, compared to other samples, SMP of the 80/20 blend was closest to that of CB.

Characterization of crystallization and melting profiles

MKF and CB exhibited one crystallization peak each, with MKF showing a slightly higher T_{CO} (18.98 °C) than CB (18.64 °C) (Fig. 1a). The crystallization thermogram of PMF contained two peaks. T_{CO} of PMF (25.25 °C) was significantly higher than those of MKF and CB, indicating the presence of higher melting triglycerides in PMF. As the amount of MKF in the fat blend decreased from 90 % to

80 % and 70 %, T_{CO} decreased from 18.14 °C to 17.97 °C and 17.46 °C, respectively. The crystallization peak heights also diminished. When the amount of MKF in the fat blends dropped to 60 %, the crystallization thermogram began to show two peaks, with an increase in T_{CO} to 19.83 °C. As the amount of MKF decreased further (blend 50/50), the two crystallization peaks shifted outwards from each other, resulting in a higher T_{CO} (23.39 °C) and a widened crystallization range.

For pure fats, PMF exhibited the highest T_{MC} (49.25 °C) followed by MKF (34.58 °C) and CB (29.49 °C) (Fig. 1b). In additional, PMF demonstrated the broadest melting range, indicating that the fat had a wide range of triglyceride content (i.e. having some triglycerides with low melting temperatures and some with high melting temperatures). When the amount of MKF in the fat blends decreased from 90 % to 80 %, T_{MC} decreased from 32.88 °C to 31.02 °C with a diminished melting range. After that, the trend reversed with an increase in T_{MC} as the MKF amount decreased (35.93 °C, 38.14 °C, 40.68 °C for blends 70/30, 60/40, 50/50, respectively) and a widened melting range. It can be seen from the figure that T_{MC} of blend 80/20 was closest to that of CB.

Solid fat content

Plots of SFC (%) versus temperature (15–40 °C) for MKF, PMF and CB are given in Fig. 2a and the SFC plots for different fat blends are given in Fig. 2b. A SFC versus temperature plot generally gives not only the information on how hard or soft a fat is at different temperatures but also the melting behavior of the fat. From Fig. 2a and b, the SFC

Table 3 Iodine value (Iv), saponification value (Spv) and slip melting point (SMP) of mango kernel fat (MKF), palm oil mid-fraction (PMF), cocoa butter (CB) and different MKF/PMF blends (%wt) ($n=3$)

Fat Blend	Iv (g I ₂ /100 g fat)	Spv (mg KOH/g fat)	SMP (°C)
MKF	40.9±0.31 ^c	185.4±2.79 ^c	35.7±0.1 ^b
90/10	42.2±0.48 ^b	187.4±1.55 ^c	30.8±0.0 ^f
80/20	42.0±0.09 ^b	187.8±0.89 ^c	29.3±0.0 ^g
70/30	42.3±0.65 ^b	190.9±1.04 ^b	32.1±0.1 ^e
60/40	42.0±0.12 ^b	191.6±1.55 ^b	32.9±0.1 ^d
50/50	42.3±0.30 ^b	192.4±0.38 ^b	33.2±0.1 ^c
PMF	43.9±0.48 ^a	196.6±0.81 ^a	39.8±0.1 ^a
CB	34.0±0.47 ^d	190.7±1.13 ^b	27.8±0.0 ^h

Similar letters in the exponential in the same column show there are no significant differences ($p>0.05$)

Fig. 1 DSC crystallization thermograms (a) and melting thermograms (b) of mango kernel fat (MKF), palm oil mid-fraction (PMF), cocoa butter (CB) and the fat mixtures with different ratios of MKF/PMF (%wt). T_{CO} and T_{MC} indicate crystallization onset temperature and melting completion temperature, respectively

of all fat samples decreased as the temperature increased, however, with different rate of decrease. CB exhibited the most rapid SFC drop with temperature, implying that it was the fastest melting fat when consumed. At temperatures below 22 °C CB was the hardest fat among the three pure fats, presumably due to its high contents of both SOS and POS (Table 2), followed by MKF and PMF. The high content of SOS but low content of POS in MKF was probably responsible for its lower SFC than CB. The lack of SOS but abundance of POP in PMF was the likely cause for its lowest SFC in this low temperature range.

Between 25 and 30 °C, the SFC of CB dropped dramatically below 10 % while the SFC of MKF became the highest with those of PMF in between the two pure fats. This implies that at room temperature in most countries MKF would exhibit a harder consistency compared to CB

Fig. 2 Solid fat content measured at 15–40 °C of (a) mango kernel fat (MKF), palm oil mid-fraction (PMF), cocoa butter (CB) and (b) the fat mixtures with different ratios of MKF/PMF (%wt) ($n=3$)

and PMF. It also implies that CBE that is produced by mixing MKF to PMF with the appropriate ratio would be able to withstand a higher room temperature than CB. Above 32 °C, SFC of both CB and MKF approached 0 % whereas the SFC of PMF decreased slowly towards ~5 %. At 40 °C, ~4 % of the fat in PMF was still solid, suggesting that chocolate or confectionery products that use unmodified PMF as a fat ingredient could end up having a waxy mouth-feel.

For different MKF/PMF blends, the SFC of blends 90/10 and 80/20 were in between those of MKF and PMF at the temperature of 25 °C and below. At this temperature range the SFC of 70/30, 60/40 and 50/50 blends were all lower than those of MKF and PMF. Above 25 °C, SFC of blends 90/10 and 80/20 reduced to below those of PMF, but still higher than CB, while moving towards 0 % as the temperature approached 37 °C. The SFC of blend 70/30 behaved similarly to those two blends. In this temperature range, the

SFC of the 60/40 and 50/50 blends also diminished as the temperature rose, however, with a smaller rate of decreasing. As a result of this, the SFC of blend 60/40 and 50/50 became higher than those of 90/10, 80/20 and 70/30 blends above 32 °C. At 37 °C, ~2–3 % of the 50/50 fat blend was still in a solid phase.

Selection of CBE from the fat blends

Statistic analysis on fatty acid contents, physicochemical properties and SFC of all MKF/PMF blends reveals that they were all significantly different from CB ($p < 0.05$). However, it can be seen from the SFC results that blends 90/10 and 80/20 had the highest SFC at 15–25 °C, though not as high as CB at temperatures below 22.5 °C. This would give the blends a hard consistency within this temperature range. The two blends melted relatively rapidly between 25 and 30 °C and finally became almost completely liquid at body temperature. In addition, the blends exhibited T_{CO} and T_{MC} that were close to those of CB. Because of this, both blends 90/10 and 80/20 could be used as CBE. Chocolate that is made with either of these two fat blends should exhibit good characteristics with proper melting behavior, e.g. being solid at room temperature and melting quickly in the mouth. However, blend 90/10 contained higher contents of MKF than blend 80/20. Considering that up until now MKF has not been produced in industrial scale in Thailand and therefore PMF is more widely available than MKF, it would be more convenient and more economical to produce a CBE fat that contained as high a content of PMF as possible. Additionally, blend 80/20 exhibited SMP that was closest to that of CB. As a consequence, blend 80/20 was recommended as CBE in this work.

Compatibility between CBE and CB

The compatibility between the recommended CBE and CB is illustrated in Fig. 3, which exhibits the SFC at 20 °C of the mixtures of the CBE and CB. The figure shows that the SFC of the fat mixtures with different CBE/CB ratios lies on the straight line that connects the SFC of the individual fats. The results indicate that the two fats were fully compatible and because of this their mixtures in any proportions did not result in a softening effect.

Crystallization behavior under static isothermal condition—SFC studies

The development of SFC during quiescent crystallization at 20 °C of MKF, PMF, CB, the recommended CBE and the mixture between CBE and CB (called MIX) is given in Fig. 4 as a plot of SFC versus crystallization time. It can be seen that PMF started to crystallize before the temperature

Fig. 3 Solid fat content of mixtures of the recommended cocoa butter equivalent (CBE) and cocoa butter (CB) measured at 20 °C ($n=3$)

reached 20 °C whereas the crystallization of CB began immediately after 20 °C was attained. On the contrary, the crystallization induction time for MKF was almost 10 min. During crystallization, the SFC of PMF increased slowly throughout 180 min. At the end of the experiment, PMF exhibited the lowest SFC value, indicating the softest consistency. However, the SFC of MKF developed very rapidly; immediately after the crystallization set in with a steep slope of the crystallization curve. After 60 min, the SFC of MKF reached a plateau, suggesting that the equilibrium had been obtained.

The crystallization curve of CB is clearly divided into two parts with different slopes, suggesting that the crystallization of the fat was a two-step process. Initially, the fat solidified slowly during the first 20 min with the SFC rising

Fig. 4 Crystallization curves for mango kernel fat (MKF), palm oil mid-fraction (PMF), recommended cocoa butter equivalent (CBE), cocoa butter (CB) and a mixture of CBE and CB (MIX) obtained during isothermal crystallization under static condition at 20 °C ($n=3$)

Fig. 5 X-ray diffraction patterns of (a) mango kernel fat, (b) palm oil mid-fraction, (c) recommended cocoa butter equivalent, (d) cocoa butter and (e) a mixture of cocoa butter equivalent and cocoa butter during crystallization at 20 °C for 60 min under static conditions

to an intermediate plateau. Then, the SFC increased rapidly before slowing down to attain a second plateau towards the end of the experiment. The two-step crystallization curves for CB have been observed before for crystallization at temperatures below 20 °C (Marangoni and Mcgauley 2003). The change in the slope of the crystallization curve could be related to either the formation of a different polymorphic form of the fat, or the crystallization of different fractions (Foubert et al. 2006). It could be possible here that CB crystallized into an unstable form during the first 20 min and then transformed into a more stable polymorph afterwards.

The recommended CBE took approximately the same amount of time as MKF to begin solidification. However, the shapes of the crystallization curves of the two fats were completely different. The crystallization curve of the CBE was more similar to that of CB, with a change in slope at ~40 min of the crystallization time. The crystallization behavior of MIX was also very similar to that of CB with the change in the slope of crystallization curve at ~20 min. At the end of the crystallization time, the SFC of MIX was slightly lower than that of CB. The similarity of the crystallization behavior of MIX and CBE implies that adding the recommended CBE to CB in the ratio of 1:5.64 (wt) did not significantly alter the crystallization behavior of CB.

Crystallization behavior under static isothermal condition—polymorphic behavior studies

The x-ray diffraction pattern of MKF (Fig. 5a) obtained during 60 min of static isothermal crystallization at 20 °C exhibited diffraction characteristics of more than one crystal structure, with four main diffraction peaks in WAXS at 3.86 Å, 4.20 Å, 4.38 Å and 4.55 Å and one diffraction peak with low scattering intensity at 3.60 Å. The peaks at 3.86 Å and 4.20 Å were typical diffraction patterns of β' structure (Larsson 1966). The two peaks showed the strongest scattering intensity, implying that β' was the predominant polymorph for MKF solidified under these conditions. The diffraction peak at 4.38 Å was close to that of pseudo- β' form as reported by Gibon et al. (1986) whilst the one at 3.60 Å could be associated with the sub- β structure (D'Souza et al. 1990). The 4.55 Å peak was a typical main diffraction peak in WAXS of the β structure of fats (Larsson 1966). The x-ray diffraction patterns of MKF indicated that the fat crystallized into a mixture of these polymorphs throughout 1 h of crystallization. Although the scattering intensity of all peaks increased with time, the peaks representing β' remained predominant. The crystallization of

Fig. 6 Polarized light microscope images of (a) mango kernel fat, (b) palm oil mid-fraction, (c) recommended cocoa butter equivalent, (d) cocoa butter and (e) a mixture of cocoa butter equivalent and cocoa butter obtained after static crystallization at 25 °C for 48 h

MKF into a mixture of crystal structures reported here is in agreement with the crystallization behavior of the MKF at 22 °C published by Solis-Fuentes et al. (2005), where MKF was found to crystallize into a mixture of β' , pseudo- β' , sub- β and β structures under quiescent conditions over a long period of 26 days.

The diffraction patterns of PMF (Fig. 5b) support the results of the SFC studies that the fat started to crystallize before the crystallization temperature was reached. The diffraction patterns of the fat obtained during 1 h of solidification contained three peaks at 3.88 Å, 4.20 Å and 4.38 Å. The two former peaks could be related to β' form (Larsson 1966) and the latter peak was close to that of pseudo- β' structure (Gibon et al. 1986). Unlike MKF, the predominant structure of PMF during 1 h of crystallization appeared to be pseudo- β' .

The diffraction patterns of the recommended CBE (Fig. 5c) are similar to those of MKF. Unlike MKF, when CBE started to crystallize at ~10 min of the crystallization time, only one diffraction peak appeared in WAXS at 4.19 Å. Although this signal could be linked to either α or β' structures of fats (D'Souza et al. 1990), the fact that no any other signature peaks of β' appeared during the first 30 min implies that the fat crystallized into α form first. After 30 min, more diffraction peaks began to emerge at 3.60 Å (sub- β'), 3.88 Å (β'), 4.38 Å (Pseudo- β') and 4.55 Å (β'). At 1 h, β' was a predominant structure. Since the crystallization of CBE was a two-step process (Fig. 4), it was likely here that CBE crystallized into the α structure before transforming into a mixture of polymorphs.

Fig. 7 Changes of whiteness index with time of tempered cocoa butter equivalent (CBE), cocoa butter (CB) and a mixture of CBE and CB (MIX) during storage for 6 months at 25 °C ($n=3$)

The crystallization of CB started with α structure soon after 20 °C was reached (Fig. 5d). X-ray diffraction patterns obtained from the first 30 min revealed one small diffraction peak at 4.18 Å, which is close to that of a signature diffraction peak in WAXS of form II (α -structure) of CB (Marangoni and Mcgauley 2003). After 30 min, the fat structure began to transform to form IV (β') with diffraction peaks at 4.14 Å and 4.31 Å (Wille and Lutton 1966). This coincided with the change in the slope of the fat's crystallization curve observed at ~30 min (Fig. 4). At 1 h of crystallization, the diffraction pattern of the fat showed mainly the diffraction peaks of form IV (β') with two additional peaks at 3.79 Å, 3.95 Å and a peak with low scattering intensity of form V (β) at 4.55 Å, indicating that CB had partially transformed into form V. The phase transition of CB during quiescent crystallization from forms II to IV bypassing form III (β') has been observed before (Sonwai and Mackley 2006). The crystallization behavior of MIX was very similar to that of CB (Fig. 5e) (i.e. the fat initially solidified into α structure before transforming into a mixture of β' and β structures). This suggests that the addition of the recommended CBE to CB did not greatly modify the crystallization behavior of CB.

Crystal morphological study

The PLM micrographs of different fat samples obtained after static crystallization at 25 °C for 48 h are given in Fig. 6. The microstructure of MKF crystals was spherulites with near-perfect spherulitic shape and smooth appearance (Fig. 6a). The black maltese cross could be seen superimposing on the crystals. Some degree of crystal aggregation, presumably by van der Waals forces (Berger et al. 1979), is evident in the image. The crystal size of the fat ranged from 20 to 200 μm . The morphology of PMF crystals was continuous granular (Fig. 6b) whereas the microstructure of the recommended CBE crystallites was a mixture of loosely-packed spherulites and granular texture (Fig. 6c).

The microstructure of CB crystals was spherulites consisting of needlelike crystals radiating and branching outward from central nuclei (Fig. 6d). Some small platelike crystals could be seen in the background. Like MKF, crystal aggregation was also observed with CB spherulites. Finally, similar to CB, the microstructure of MIX crystals was spherulites that were densely-packed together (Fig. 6e). Differences in crystalline structure (size, size distribution, shape, polymorph, surface characteristics, etc.) could be attributed to differences in textural properties between fat-containing products (Ciftci et al. 2009). The addition of CBE to CB, which did not cause any drastic changes in the microstructure, and therefore is not likely to cause any significant modification in the textural properties of CB.

Fat bloom study

WI values of freshly-tempered CB, CBE and MIX were not significantly different from one another ($p>0.05$) (Fig. 7). During a period of 6 months of incubation at 25 °C, the WI of CB remained approximately the same for the first 4 months and increased slowly but continuously during the remaining 2 months. This suggests that the fat only started to mildly bloom after 4 months of storage. On the other hand, the WI of CBE increased continuously during storage before reaching a plateau at around 4 months. The relatively significant increase in WI of CBE indicates that the fat suffered some serious bloom. The changes in WI of MIX with time, however, were very similar to those of CB. This implies that, although exhibiting some strong degree of fat bloom when used on its own, the recommended CBE did not speed up the process of bloom formation in CB when mixed with CB.

Conclusion

MKF obtained from the Kaew mangos could be an alternative source of edible oil. With the right proportion, a fat blend between MKF and PMF could be used as CBE. The 80/20 blend mainly consisted of three fatty acids that were the main fatty acid components of CB. The crystallization and melting behavior of the blend was most similar to that of CB. When mixed with CB with the ratio which mimicked that of typical dark chocolate where 5 % of CBE is added to the finished product, softening due to the eutectic effect was not observed in the mixed fat. In addition, the crystallization curve, polymorphic structure, crystal morphology and bloom behavior of the mixed fat were not significantly different from those of CB. As a result, the 80/20 blend is recommended to be used as a CBE.

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