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Physicochemical characteristics of fat blend from hydrogenated coconut oil and acyl migrated palm mid-fraction

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ABSTRACT

Palm mid-fraction (PMF), which has a high content of symmetric POP, was converted to asymmetric PPO (APMF) via acyl migration. After solvent fractionation, the liquid phase of acyl migrated PMF (APMF-L) was obtained and blended with hydrogenated coconut oil (HCO, 50:50, w/w) to produce a fat blend (namely, an alternative fat blend) which had reduced saturated fatty acid content while having similar melting behavior to HCO. In an alternative fat blend, the major fatty acids were lauric (27.94), palmitic (26.93) and oleic (15.75 mol %) acid. The solid fat index was quite similar to that of HCO, especially at 28–44 °C. Nevertheless, an alternative fat blend had lower saturated fatty acid content, by 18%, compared to HCO. The content of highly atherogenic myristic acid was reduced by approximately 40%. The alternative fat blend in this study could be used as a raw material for non-dairy cream with low saturated fat content.

1. Introduction

It has been reported that excessive intake of saturated fatty acids increases triacylglycerol (TAG) and low-density lipoprotein cholesterol content in the body, which increases the risk of diseases such as obesity, hypertension, hyperlipidemia, and artery hardening (Micha & Mozaffarian, 2010; Siri-Tarino, Sun, Hu, & Kruss, 2010; Willett, 2012). Additionally, it is known that lauric acid (C12:0), myristic acid (C14:0), and palmitic acid (C16:0) cause hypercholesterolemia (Ulbricht & Southgate, 1991). Among them, lauric and myristic acid are major fatty acid components of hydrogenated coconut oil (HCO), the latter of which is more atherogenic. HCO maintains a solid-state at room temperature but is abruptly melted within a narrow temperature range close to that of body temperature (Young, 1983). HCO also has high oxidation stability due to its high saturated fatty acid content, and thus is widely used as a raw material for non-dairy cream (Anihouvi, Danthine, Kegelaers, Dombree, & Blecker, 2013).

Palm mid-fraction (PMF) is a widely used source for the confectionary industry. PMF is produced by the multi-stage fractionation of palm oil (*Elaeis guineensis*), which has a high content of symmetrical POP. PMF shows a steep solid fat content curve, resulting in wide application as a confectionary fat (Kellens, Gibon, Hendrix, & De Greyt, 2007).

Acyl migration is the reversible shifting of the fatty acids which compose TAG between the *sn*-1,3 and *sn*-2 positions. For example, during the interesterification catalyzed by *sn*-1,3 specific lipase, TAG molecules are first hydrolyzed into 1,2 (2,3)-DAG as intermediates which subsequently change into 1,3-DAG through acyl migration until a dynamic balance is reached. Therefore, 1,2 (2,3)-DAG and 1,3-DAG are utilized as substrates for further interesterification reaction, after which new TAG molecules having different positional distribution of fatty acids can be formed. Therefore, TAG molecules containing fatty acids with different positional arrangements are produced after acyl migration. This phenomenon is affected by reaction temperature, amount and type of enzyme used, reaction time, and moisture content (Xu et al., 1998; Laszlo, Compton, & Vermillion, 2008). Therefore, lipids that are restructured after acyl migration have different physicochemical characteristics than they did before the reaction.

Generally, fats obtained by blending can have applications in the food industry because they have desirable physical properties. For examples, palm kernel oil with cocoa butter and milk fat has the physical characteristics required for compound coatings, while vegetable oil and palm stearin (PS) can be blended for use in margarine or spreads (Pease, 1985; Reddy & Jeyarani, 2001; Toro-Vazquez, Briceno-Montelongo, Dibildox-Alvarado, Charo-Alonso, & Reyes-Hernández, 2000; Williams, Ransom-Painter, & Hartel, 1997).

The aim of this study was to prepare an alternative fat blend with low saturated fatty acid content but also solid fat content at 25–37 °C

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that is similar to HCO, because such melting behavior is important to provide the rheological characteristic and mouthfeel of fat. In order to prepare an alternative fat blend, the fractionated liquid phase from the palm mid-fraction after inducing asymmetric TAG molecules via acyl migration (APMF-L) catalyzed by an immobilized Lipozyme® TLIM lipase was obtained for blending with HCO (50:50, w/w). Then, the physicochemical characteristics were investigated. Furthermore, the emulsion stability of the alternative fat was assessed by coffee cream preparation in order to determine the possibility of its practical application.

2. Materials and methods

2.1. Materials and reagents

HCO was provided by Dongseo Co. (Incheon, Korea). Palm mid-fraction (PMF) was obtained from CJ Co. (Seoul, Korea). Acyl migration of the PMF was performed using Lipozyme® TLIM (Novozymes-Korea, Seoul, Korea), which is a silica-immobilized lipase prepared from *Thermomyces lanuginosa*. According to the manufacturer, Lipozyme® TLIM is *sn*-1,3 specific lipase with an activity of 250 IUN/g, where 1 IUN is the amount of enzyme activity which generates 1 μmol of propyl laurate per minute. All solvents used for analyses were analytical grade. Lipase from porcine pancreas was purchased from Sigma-Aldrich (Yongin, Korea). Commercial grade sodium caseinate, Almax 6900 (sodium stearoyl lactylate), and Almax 1000 (distilled monoglyceride) were provided by Ilshin Wells Co. (Cheongju, Korea).

2.2. Acyl migration reaction and preparation of alternative fat

PMF (30 g) and Lipozyme® TLIM (6 g) were weighed into a 250 mL Erlenmeyer flask with a screw cap. Acyl migration of the PMF was carried out for 3, 6, and 9 h at 80 °C in a shaking water bath at 180 rpm. TAG was separated from by-products such as free fatty acid (FFA) and mono- and di-acylglycerol (MAG and DAG) by passage through a column (Lee & Foglia, 2000) with modification. After the acyl migration, the reaction product (about 30 g) was mixed with 10 mL of *n*-hexane. The column (3.5 cm diameter, 20 cm length) was packed with silica gel (30 g) and Florisil (30 g). Then, the reaction product in *n*-hexane was loaded on the column and eluted with 250 mL *n*-hexane. If necessary, this step was repeated until isolation of TAG was confirmed with thin-layer chromatography. Hexane was then removed under vacuum with a rotary evaporator at 40 °C. Traces of *n*-hexane were removed by flushing with nitrogen. Isolation of TAG was confirmed through analysis of thin-layer chromatography via the absence of DAG, MAG, and FFA bands. Then, solvent fractionation was conducted to remove high melting TAG (i.e., PPP, tripalmitin) from the reactants as follows: reactants and acetone (1:9, w/v) were placed into a 50 mL vial and stirred until completely melted. Crystallization was carried out in a 25 °C incubator for 24 h and was followed by separation into solid and liquid fractions using filter paper. Acetone was entirely removed from the liquid fraction by a rotary evaporator and nitrogen gas to obtain the fractionated liquid phase of the PMF (APMF-L). Finally, the alternative fat blend was prepared as follows: the APMF-L was blended with HCO at a ratio of 50:50 (w/w) and stirred for 1 h in a double-jacket water bath kept at 50 °C for complete mixing.

2.3. Differential scanning calorimetry (DSC) and solid fat index (SFI)

Melting and crystallization curves and the solid fat index (SFI) of samples were obtained by DSC (Model DSC 2010, TA Instruments, New Castle, USA). Samples (8 ± 1 mg) were sealed in an aluminum pan, and an empty pan was used as a reference. The following time-temperature program was used: the temperature was held at 80 °C for 10 min to completely destroy the crystal memory of the sample, cooled at 10 °C/min to −60 °C to obtain the crystallization curve, held for 10 min at this temperature, then heated at 5 °C/min to 80 °C to obtain the melting curve. The SFI, which is used to determine solid-liquid content at a specific temperature, was calculated from the melting curve as follows: [100% − {energy% at specific temperature/total △H (J/g, enthalpy)}].

2.4. Fatty acid composition and TAG species

The total and positional fatty acid compositions of the samples were analyzed by gas chromatography (GC) (Adhikari et al., 2010). Analysis was performed in duplicate and the results are expressed as the average value and standard deviation. As briefly described, positional fatty acid composition was determined by pancreatic hydrolysis. To determine the positional fatty acid composition at the *sn*-2 position, TAG was hydrolyzed with *sn*-1,3 specific pancreatic lipase (Sigma-Aldrich, Yongin, Korea). TAG (7 mg) was mixed with 7 mL of 1 M Tris-HC1 buffer (pH 7.6), 1.75 mL of 0.05% bile salts, 0.7 mL of 2.2% $CaC1_2$, and 7 mg pancreatic lipase. Initially, the mixture was vortexed vigorously for 1 min. Then, it was incubated in a water bath at 37 °C for 3 min and vortexed again for 30 s. This procedure was repeated 2 times. Finally, it was warmed again to 37 °C and held at that temperature for 2 min. Then, the hydrolysis product was extracted with 4 mL diethyl ether (two times) and then the upper layer was obtained after centrifugation (2500 rpm, 3 min). Finally, the organic phase (upper layer) including the hydrolysis products was eluted in an anhydrous sodium sulfate column. Thin layer chromatography (TLC) was used for the separation of 2-monoacylglycerol (2-MAG) from diacylglycerol (DAG) and TAG on a silica gel 60 F_{254} plate (Merck KGaA, Darmstadt, Germany) developed with hexane/diethyl ether/acetic acid (50:50:1, v/v/v). The band corresponding to 2-MAG was scraped and methylated for the conversion of fatty acid in the 2-MAG molecule into the fatty acid methyl ester (FAME). Finally, it was analyzed by gas chromatography. Methylation was accomplished by adding 1.5 mL of 0.5 N methanolic NaOH solution and 2 mL of 14% BF_3-methanol solution (Sigma-Aldrich, Yongin, Korea). The scraped 2-MAG band was mixed with alkaline reagent and heated in a boiling water bath for 5 min. Then, BF_3-methanol solution was added for a 3 min incubation in boiling water. After cooling, 2 mL isooctane and 1 mL saturated NaCl solution were added. The upper isooctane phase that included the FAME was isolated and passed through an anhydrous sodium sulfate column. Fatty acid composition at *sn*-1,3 position was calculated using the following equation (Anderson, Bottino, & Reiser, 1970): fatty acid composition at *sn*-1,3 position (%) =

$$[(\text{Total fatty acid composition} \times 3) - \text{fatty acid composition at } sn-2]/2$$

The Agilent 6890 Gas Chromatograph (Santa Clara, USA) equipped with an autoinjector and a flame-ionization detector was used for fatty acid composition analysis. The column (SP™-2560, 100 m × 0.25 mm i.d., 0.2 μm film thickness; Supelco, Bellefonte, USA) was held at 150 °C for 5 min, and increased in temperature to 220 °C at a rate of 4 °C/min, and then held at 220 °C for 30 min. The carrier gas was helium, and its flow rate was 1 mL/min in constant flow mode. The spilt ratio was 1:50. The injector and detector temperatures were 250 °C and 260 °C, respectively. Peak identification of each fatty acid was carried out by comparing their retention time with those of the standards of the Supelco 37 component FAME mixture (10 mg/mL, Sigma-Aldrich/Merck KGaA, Darmstadt, Germany). Fatty acid composition (%) was expressed as percentage of total fatty acids in duplicates. The fatty acid composition was used to calculate the atherogenicity index (AI) (Ulbricht & Southgate, 1991).

$$AI = [(C12{:}0\ \text{mol}\%) + 4 \times (C14{:}0\ \text{mol}\%) + (C16{:}0\ \text{mol}\%)]/\sum \text{USFA mol}\%.$$

TAG composition was analyzed by reversed-phase high-performance liquid chromatography (RP-HPLC) with slight modifications (Adhikari et al., 2010). TAG species were identified by comparison of

retention time and partition number (PN) with various TAG standards (Tricaprylin, CpCpCp; Trilaurin, LaLaLa; Trimyristin, MMM; Tripalmitin, PPP; Triolein, OOO; Trilinolein, LLL) obtained from Nu-Chek-Prep (Waterville, USA); PN was calculated using the following equation: PN = total carbon number (CN) − 2 × total number of double bonds (ND). The analysis of TAG species was performed with a Yonglin SP930D HPLC (Yonglin, Anyang, Korea) connected with a Sedex 75 evaporative light-scattering detector (Sedere, Alfortville, France). The nebulizing temperature and gas pressure were 40 °C and nitrogen of 2.2 bar, respectively. Nova-Pak® C18 column (150 × 3.9 mm, 4 μm, Waters, Milford, Ireland) was used. The separation was obtained by gradient elution that consisted of acetonitrile (A) and isopropanol/*n*-hexane (2:1, v/v) (B) at a flow rate of 1 mL/min with the following elution program (elapsed time, ratio of solvent B): 0 min, 20% B; 45 min, 46% B; 60 min, 46% B; 65 min, 20% B; and 70 min, 20% B.

2.5. Preparation of oil-in-water emulsions and stability

Each oil-in-water emulsion of HCO and an alternative fat blend was prepared as follows: Almax 1000 (monoglyceride, 0.4 g) was stirred with (10 g) of HCO and with (10 g) of the alternative fat blend at 40 °C. The aqueous phase of each was prepared with 20 mM sodium phosphate buffer (pH 7.0, 80 mL), sodium caseinate (1.5 g), and corn starch syrup (DE 20.0–24.0, 7.9 g). Sodium azide (0.1 wt%) was added to the buffer to prevent microbial spoilage. All mixed materials were stirred for homogenization. Thereafter, Almax 6900 (sodium stearoyl lactylate, 0.2 g) was added to the aqueous phase of each sample. After mixing the oil and aqueous phases of each sample, a pre-emulsion of each sample was prepared using a Silverson mixer (L4RT, Silverson Machines Ltd., Chesham, UK) at 5000 rpm for 2 min. Each sample was then passed through a microfluidics processor (Model M-110Y, Microfluidics, Newton, USA) with two passes at 3000 psi to obtain the final emulsions. Each emulsion was stored for 20 days in a 20 °C incubator.

The droplet size distribution of the emulsions was measured in duplicate using a Malvern MasterSizer instrument (Worcestershire, UK). The results are expressed as volume-surface mean diameter (d_{32}) and weight mean diameter (d_{43}).

2.6. Statistical analysis

Significant differences in the analytical results were evaluated using the Statistical Analysis System (SAS, version 9.2, SAS Institute Inc., Cary, USA). Duncan's multiple range tests were conducted to determine the statistical significance with differences at 95% confidence interval ($P < 0.05$).

3. Results and discussion

3.1. Fatty acid composition and triacylglycerol species

The changes in total and positional fatty acid composition in PMF after acyl migration for 3, 6, and 9 h are presented in Table 1. PMF is used in a variety of fields but mainly as a substituent fat in cocoa butter (Bloomer, Adlercreutz, & Mattiasson, 1990). The total fatty acid content (area%) of PMF showed palmitic acid and oleic acid as the most abundant fatty acids at 55.5 and 33.9%, respectively. PMF was expected to be composed mainly of symmetric POP since 81.9% oleic acid was located at the *sn*-2 position while palmitic acid was 78.2% at *sn*-1,3 position of TAG molecules. Symmetric POP was expected to be converted to asymmetric PPO by acyl migration. The total fatty acid content of PMF after acyl migration (3, 6, and 9 h) consisted of 55.1–56.2% palmitic acid and 33.5–34.2% oleic acid, which were not significantly changed when compared with those of PMF. However, the fatty acid composition at *sn*-2 position consisted of 57.0–61.3% palmitic acid, which was greatly increased compared with PMF (10% palmitic acid at *sn*-2 position) ($P < 0.05$). Oleic acid (C18:1, n-9) content (area%) at

Table 1
Positional fatty acid composition of palm mid-fraction (PMF) and acyl migrated PMF depending on the reaction time (area%).

Fatty acids	TAG	*sn*-2				*sn*-1,3					
	PMF	Acyl migrated PMF			PMF	Acyl migrated PMF			PMF	Acyl migrated PMF		
		3 h	6 h	9 h		3 h	6 h	9 h		3 h	6 h	9 h
C14:0	1.1 ± 0.1^{a}	1.0 ± 0.1^{b}	1.1 ± 0.1^{ab}	1.1 ± 0.1^{ab}	–	–	–	–	1.7 ± 0.1^{a}	1.5 ± 0.1^{a}	1.6 ± 0.1^{a}	1.6 ± 0.1^{a}
C16:0	55.5 ± 0.1^{a}	55.4 ± 0.7^{a}	56.2 ± 0.5^{a}	55.1 ± 0.9^{a}	10.0 ± 1.0^{c}	57.0 ± 1.7^{b}	61.3 ± 0.1^{a}	59.9 ± 0.3^{a}	78.2 ± 0.6^{a}	54.7 ± 1.9^{b}	53.6 ± 0.8^{b}	52.7 ± 1.1^{b}
C18:0	6.2 ± 0.1^{a}	6.0 ± 0.1^{a}	6.0 ± 0.2^{a}	6.2 ± 0.1^{a}	0.8 ± 0.1^{c}	5.1 ± 0.1^{b}	5.6 ± 0.1^{a}	5.9 ± 0.2^{a}	8.8 ± 0.1^{a}	6.5 ± 0.3^{b}	6.2 ± 0.4^{b}	6.4 ± 0.1^{b}
C18:1 (n-9)	33.9 ± 0.1^{a}	34.2 ± 0.3^{a}	33.5 ± 0.1^{a}	34.1 ± 0.7^{a}	81.9 ± 1.1^{a}	34.4 ± 1.2^{b}	30.0 ± 0.1^{c}	30.7 ± 0.1^{c}	10.0 ± 0.7^{b}	34.1 ± 1.0^{a}	35.3 ± 0.1^{a}	35.8 ± 1.0^{a}
C18:2 (n-6)	3.3 ± 0.1^{a}	3.3 ± 0.2^{a}	3.2 ± 0.2^{a}	3.6 ± 0.2^{a}	7.3 ± 0.1^{a}	3.5 ± 0.4^{b}	3.1 ± 0.1^{b}	3.5 ± 0.1^{b}	1.2 ± 0.1^{b}	3.2 ± 0.5^{a}	3.3 ± 0.3^{a}	3.6 ± 0.2^{a}

TAG, triacylglycerol; –, not detected.
All values are expressed as mean ± standard deviation of duplicated determinations and different letters within the same row are significantly different at $P < 0.05$.

Table 2
Plausible triacylglycerol (TAG) species from reversed-phase HPLC separation (area%).

PN	TAG species	HCO	PMF	APMF	APMF-L	Alternative fat blend
30–34	CaCaCa/CaCaLa/ CaCaM/CaLaLa/ CpLaM/CpLaLa CpCaLa	31.2	–	–	–	5.9
36	LaLaLa	22.8	–	–	–	5.0
38–40	LaLaM/LaMM/LaLaP	30.6	–	–	–	6.8
42–44	LaLaS/MMM/LaMP/ LaPP/LaMS	10.0	–	–	–	–
46	LaPS/LOO/OLO/ LLS/PLO/POL/PLP/ PPL	1.4	–	–	–	–
48	POO/OPO	–	0.4	22.7	25.9	20.8
48	POP/PPO	–	93.5	63.0	69.1	55.6
48	MPS /PPP/LaSS	1.4	0.2	9.9	1.1	1.9
50	SOO/OSO/POS/PSO/ PPS/PSP/MSS	1.4	5.0	3.2	2.4	2.2
Others		1.2	0.9	0.9	1.5	1.8

PN, partition number = total carbon number (CN) – 2 (total number of double bonds); Cp, caprylic acid; Ca, capric acid; La, lauric acid; M, myristic acid; L, linoleic acid; O, oleic acid; P, palmitic acid; S, stearic acid; HCO, hydrogenated coconut oil; PMF, palm mid-fraction; APMF, acyl migrated PMF for 6 h; APMF-L, APMF liquid phase after solvent fractionated at 25 °C; –, not detected. APMF-L was blended with HCO at a ratio of 50:50 (w/w) for preparing an alternative fat blend.

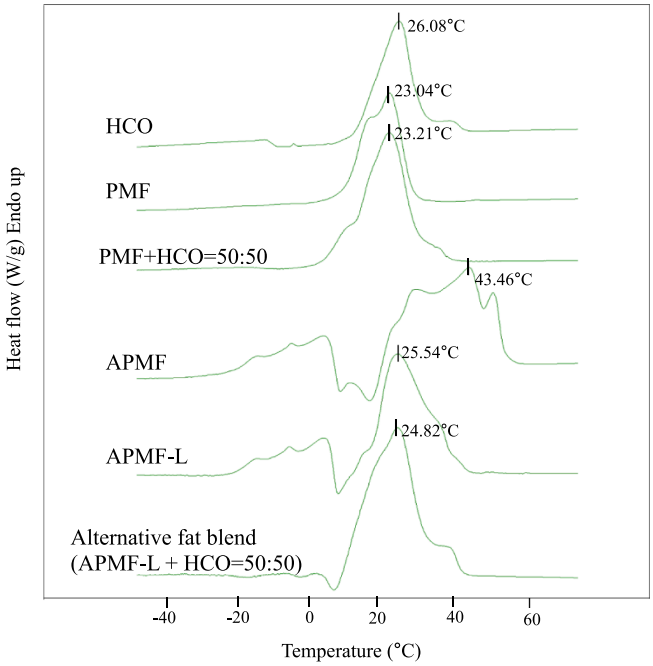

Fig. 1. DSC melting thermogram of hydrogenated coconut oil (HCO), alternative fat blend, and other fats. HCO, hydrogenated coconut oil; PMF, palm mid-fraction; APMF, acyl migrated PMF for 6 h; APMF-L, APMF liquid phase after solvent fractionated at 25 °C. APMF-L was blended with HCO at a ratio of 50:50 (w/w) for preparing an alternative fat blend.

the *sn*-2 position of PMF (81.9%) was greatly reduced after PMF underwent acyl migration (30.0–34.4%) ($P < 0.05$). Acyl migration time did not greatly affect the fatty acid composition, even after the 6 h reaction ($P > 0.05$). Therefore, PMF underwent acyl migration for 6 h was used for subsequent experiments. After the acyl migration reaction, palmitic acid content was reduced to 52.7–54.7% at the *sn*-1,3 position, while oleic acid content significantly increased to 34.1–35.8%. The results indicate that the acyl groups of TAG molecules in PMF shifted between the *sn*-1,3 and *sn*-2 positions (Laszlo et al., 2008; Xu et al., 1998), demonstrating that symmetric POP, a major TAG component of the PMF, was partly converted to asymmetric PPO.

The TAG composition (area%) of HCO is shown in Table 2. The TAG molecules in the HCO were distributed over the range PN 30–50, with the major components of TAG having PN = 30–44. CaCaLa, CaCaM/CaLaLa, LaLaLa, and LaLaM are examples of major components of TAG. The TAG compositions (area%) of PMF, 6 h acyl migrated PMF (APMF), and the liquid phase obtained by fractionation of APMF (APMF-L) are presented in Table 2. The major component of PMF was POP/PPO (93.5%) with PN = 48, which may be mostly symmetric POP in accordance with the fatty acid composition (Table 1). The major TAG components of APMF were POO/OPO (22.7%), PPO/POP (63.0%), and PPP (9.9%) with PN = 48, showing changes in TAG composition due to acyl migration. Therefore, the results of TAG and fatty acid analysis (Tables 1 and 2) indicate that symmetric POP and asymmetric PPO are the major components in PMF and APMF, respectively. Meanwhile, the results of TAG composition for APMF-L, obtained by fractionation of APMF, showed 25.9% POO/OPO, 69.1% PPO/POP, and 1.1% PPP.

3.2. Melting characteristics by differential scanning calorimetry

Differential scanning calorimetry (DSC) has been proposed to offer an alternative method for presenting solid-liquid ratio in fats (namely, solid fat content), in which pulsed nuclear magnetic resonance would usually be applied. Additionally, DSC provides a thermogram including the temperature range from which melting and crystallization behavior of fats can be considered (Marquez, Perez, & Wagner, 2013). Since DSC values (namely, the solid fat index) of fats are calculated from the thermograms, melting peak temperature and total enthalpy of melting, (ΔH) can be measured, in which ΔH is expressed as energy per unit of mass (J/g). Generally, an increase in the acyl chain length and degree of saturation in fats provokes an increase in melting enthalpy.

Melting curves and thermal characteristics (T_{on}, T_{peak}, and ΔH) of HCO and PMF are presented in Fig. 1 and Table 3. The melting curve of HCO showed one sharp peak (T_{peak} = 26.08 °C) with one small peak at 39.85 °C. This is mainly due to the TAG molecules (LaLaLa, LaLaCa, etc.) consisted of medium chain saturated fatty acids, which are major components of HCO (Young, 1983). In the melting curve of PMF, T_{peak} was shown at 23.04 °C, and 69.1% was melted at that temperature. Such melting behavior suggests that PMF has lower SFI than HCO between 20 and 30 °C. The SFI at 20–30 °C would be important to retard oil exudation in the powered type of non-dairy cream for which HCO is generally used. Overall, the required total melting ΔH of PMF was 89.63 J/g and that of HCO was 108.80 J/g, suggesting that HCO melted at a higher temperature range with an increasing ΔH compared with PMF due to the different TAG composition between the two fats (Table 2).

To improve the SFI profile, PMF was blended with HCO. The resulting melting curve when intact PMF and HCO are blended (50:50, w/w) is shown (Fig. 1). In the melting thermogram, PMF + HCO (50:50, w/w) showed a distinct endothermic peak (T_{peak} = 23.21 °C, ΔH = 97.88 J/g), indicating lower melting characteristics than those of HCO (T_{peak} = 26.08 °C, ΔH = 108.80 J/g).

Because the blended fat showed a lower T_{peak} compared to HCO, acyl migration of PMF was performed. Since asymmetric TAG may have a higher melting point than symmetric TAG, it is possible to increase SFI at the designated temperature without increase in saturated fatty acid content. For the β' form, it is believed that the melting temperature of symmetric POP has a range from 30.3–33.5 °C, while that of asymmetric PPO is 34.5–35.2 °C. Regarding the general tendency of polymorphic behavior, the thermodynamically stable crystal form of symmetric POP, the β form, does not occur in PPO; in PPO the most stable crystal form is β'. Usually, β' provides creaming properties, whereas the β form is

Table 3
Solid fat index (%) and thermal characteristics (T_{on}, T_{peak}, and ΔH) of hydrogenated coconut oil (HCO), alternative fat blend, and other fats.

	Solid fat index (%) at different temperature (°C)															T_{on}	T_{peak}	ΔH (J/g)	Range (°C)
	10	24	28	30	32	34	36	38	40	42	44	46	48	50	52				
HCO	100.0	60.2	27.1	17.2	11.3	8.2	5.9	4.0	2.1	0.6	0.1	–	–	–	–	14.64	26.08	108.80	9.50–46.00
PMF	97.6	22.1	3.0	0.8	0.2	–	–	–	–	–	–	–	–	–	–	12.49	23.04	89.63	1.70–35.50
PMF + HCO blend (50:50, w/w)	95.6	32.2	11.7	8.1	5.7	3.4	1.7	0.3	–	–	–	–	–	–	–	12.08	23.21	97.88	1.30–41.60
APMF	74.9	67.6	59.0	53.8	48.4	43.5	38.7	33.7	28.3	22.8	17.2	12.1	8.2	4.3	1.2	−18.38	5.87	32.66	−20.50–17.80
																42.81	43.46	94.90	17.80–58.30
APMF 3 h-L	71.6	49.9	31.4	23.3	16.3	10.8	6.7	3.6	1.4	0.4	< 0.1	–	–	–	–	−12.00	5.57	26.19	−19.20–9.50
																16.01	24.80	66.00	9.50–45.20
APMF-L	71.7	49.5	31.3	23.7	17.1	11.9	7.5	4.0	1.9	0.7	0.1	–	–	–	–	−12.19	6.45	27.02	−19.00–10.00
																17.72	25.54	69.06	10.00–46.30
APMF 9 h-L	67.9	47.4	28.7	20.7	13.4	8.1	4.0	1.9	0.9	0.2	< 0.1	–	–	–	–	−10.56	7.07	30.36	−19.20–10.90
																18.18	26.10	63.92	10.90–44.60
APMF 3 h-L + HCO blend (50:50, w/w)	99.4	44.8	21.7	15.3	11.0	8.1	5.5	3.2	1.1	0.1	–	–	–	–	–	8.78	24.24	99.55	7.10–44.30
APMF-L + HCO blend (50:50, w/w)	99.7	46.8	24.2	17.3	12.6	9.2	6.4	3.6	1.4	0.4	0.1	–	–	–	–	9.39	24.82	100.00	8.20–44.00
APMF 9 h-L + HCO blend (50:50, w/w)	99.6	45.3	22.0	15.0	10.3	7.3	4.7	2.4	0.6	< 0.1	–	–	–	–	–	9.18	24.44	101.90	7.90–43.00

–, not detected; HCO, hydrogenated coconut oil; PMF, palm mid-fraction; APMF, acyl migrated PMF for 6 h; APMF-L, liquid phase of acyl migrated PMF for 6 h after solvent fractionation at 25 °C; APMF 3 h-L and 9 h-L, liquid phase of acyl migrated PMF for 3 h and 9 h after solvent fractionation at 25 °C, respectively. Each of liquid phase was blended with HCO at a ratio of 50:50 (w/w) for preparing APMF 3 h-L + HCO blend, APMF-L + HCO blend (alternative fat blend), and APMF 9 h-L + HCO blend.

desirable in chocolate confectionery because the large crystals of β form result in a coarse and grainy texture (Lutton, 1951; Minato, Ueno, Smith, Amemiya, & Sato, 1997; Sato et al., 1989).

Peaks in the melting curve of the PMF that underwent acyl migration for 6 h (APMF) are distributed over a wide temperature range (Fig. 1). In the PMF, the major components of TAG are POP/PPO (93.5%), showing a simple composition (Table 2). However, the composition of TAG has changed during acyl migration, showing several peaks over a wide temperature range. In the melting curve of APMF, distinct endothermic peaks were even observed at high temperature (40–50 °C) and SFI at 40 °C was 28.3%. This is due to the presence of high melting TAG molecules which were produced during the acyl migration reaction. Thus, acetone-fractionation was performed, and the liquid phase was obtained to remove high melting TAG molecules. In the melting curve of the APMF-L (i.e., liquid phase of acyl migrated PMF for 6 h after solvent fractionation at 25 °C), a major peak was shown at 25.54 °C (Fig. 1). The peak of APMF-L was shifted to a lower temperature and the temperature range was narrowed when compared to the melting curve for APMF. The melting curve showed a broad melting range in the lower temperature zone (−19.0–10.0 °C, T_{peak} = 6.45 °C, and ΔH = 27.02 J/g) and in the higher temperature zone (10.0–46.3 °C, T_{peak} = 25.54 °C, and ΔH = 69.06 J/g), for a total ΔH = 96.08 J/g. Melting characteristics of APMF-L obtained from 3 (APMF 3 h-L) and 9 h (APMF 9 h-L) reactions are referentially presented in Table 3; total ΔH was 92.19 J/g (T_{peak} = 5.57, 24.80 °C) and 94.28 J/g (T_{peak} = 7.07, 26.10 °C), respectively, indicating that the liquid phase of acyl migrated PMF showed similar melting characteristics regardless of reaction time. Therefore, APMF-L would be a more suitable material than PMF or APMF for preparing an alternative fat blend with a lower degree of saturation.

3.3. Characteristics of the alternative fat blend

To develop a fat blend with reduced saturated fatty acid content while having similar melting characteristics to HCO, APMF-L was blended with HCO (50:50, w/w) to produce an alternative fat blend. Thereafter, total and positional fatty acid, TAG composition, SFI, and melting behavior were further analyzed.

The SFI profiles of an alternative fat blend are presented in Table 3. Generally, various TAG species are mixed in the fat/oil, and the melting points of these differ from each other; thus, the fat/oil does not have a clear melting point. Therefore, degrees of solidification and melting are generally represented by the solid fat content (SFC) or SFI. The SFI/SFC shows the solid content of solidified fat in a temperature range, which is regarded as an important indicator for assessing the quality of fat products (Karabulut, Turan, & Ergin, 2004; Md. Ali & Dimick, 1994).

A narrow temperature range with a peak was also observed in the melting curve of an alternative fat blend (Fig. 1). One distinct peak appeared in the melting curve at 24.82 °C (ΔH = 100.00 J/g), which was slightly lower than that of HCO. Generally, a change of melting attribute can be observed after the blending of fats and oils (Noor Lida, Sundram, Siew, Aminah, & Mamot, 2002). The SFI of an alternative fat blend were 99.7 (10 °C), 46.8 (24 °C), 24.2 (28 °C), 12.6 (32 °C), 9.2 (34 °C), 6.4 (36 °C), and 0.4 (42 °C), and showed quite similar, whose solid fat content abruptly decreased as the temperature was increased. The SFI of HCO in those temperature ranges were 100.0, 60.2, 27.1, 11.3, 8.2, 5.9, and 0.6, respectively. For reference, melting characteristics of the HCO blend with the liquid phase of acyl migrated PMF obtained from 3 and 9 h reactions are presented in Table 3, in which total ΔH was 99.55 J/g (APMF 3 h-L + HCO blend, T_{peak} = 24.24, °C) and 101.90 J/g (APMF 9 h-L + HCO blend, T_{peak} = 24.44 °C), respectively (Table 3).

After the alternative fat blend (APMF-L + HCO blend, 50:50, w/w) was prepared, its physicochemical characteristics were observed. As shown in Table 4, lauric acid (C12:0) is the major component of HCO at 48.40 mol%, while unsaturated fatty acid was not detected. However, the content of ΣUSFA in an alternative fat blend was approximately 17.92 mol%. Further, the content of myristic acid (C14:0) was lowered by approximately 40% compared with that of HCO. Based on the fatty acid composition, the atherogenicity index (AI), related to how the coronary artery is negatively affected by the consumption of lauric, myristic and palmitic acid, was considered. As suggested by Ulbricht and Southgate (1991), AI is the sum of the amounts of lauric (C12:0), palmitic (C16:0), and four times that of myristic acid (C14:0) divided by the amounts of total unsaturated fatty acids. Therefore, even though the AI value of HCO could not be calculated because ΣUSFA is 0 (not detected), it can be assumed that the AI of HCO is much higher than that of the alternative fat blend (AI = 5.36) due to high content (124.6 mol %) of Σ [(C12:0) + 4 × (C14:0) + (C16:0)] (Table 4).

Meanwhile, TAG composition of an alternative fat blend was

Table 4
Fatty acid composition (mol%) of hydrogenated coconut oil (HCO) and alternative fat blend.

Fatty acids	HCO	Alternative fat blend
C6:0	0.85 ± 0.00	0.48 ± 0.00
C8:0	9.31 ± 0.02	4.96 ± 0.04
C10:0	6.74 ± 0.01	3.80 ± 0.02
C12:0	48.40 ± 0.01	27.94 ± 0.10
C14:0	16.93 ± 0.01	10.28 ± 0.01
C14:1	–	0.05 ± 0.01
C16:0	8.42 ± 0.00	26.93 ± 0.18
C16:1	–	0.11 ± 0.00
C18:0	9.25 ± 0.01	7.50 ± 0.01
C18:1 (n-9)	–	15.75 ± 0.05
C18:2 (n-6)	–	1.98 ± 0.01
C20:0	0.10 ± 0.00	0.19 ± 0.01
C20:1	–	0.04 ± 0.01
ΣSFA	100	82.08 ± 0.03
ΣUSFA	–	17.92 ± 0.03
Σ [(C12:0) + 4 × (C14:0) + (C16:0)	124.6 ± 0.05	96.0 ± 0.05
AI	N/A	5.36

ΣSFA, total saturated fatty acid; ΣUSFA, total unsaturated fatty acid; –, not detected.
AI: Atherogenicity index.
NA, not available due to ΣUSFA = 0 (not detected).
Alternative fat blend was prepared from APMF 6 h-L (liquid phase of acyl migrated PMF for 6 h) + HCO (hydrogenated coconut oil).
Values are mean ± standard deviation in duplicate analysis (n = 2).

Table 5
Size distribution (d_{32} and d_{43}) of lipid globule in emulsions prepared with hydrogenated coconut oil (HCO) and alternative fat blend.

Days	d_{32} (μm)		d_{43} (μm)	
	HCO	Alternative fat blend	HCO	Alternative fat blend
0	0.29 ± 0.01	0.28 ± 0.01	0.36 ± 0.00	0.34 ± 0.00
2	0.27 ± 0.01	0.30 ± 0.00	0.36 ± 0.00	0.35 ± 0.00
6	0.26 ± 0.01	0.31 ± 0.00	0.36 ± 0.01	0.35 ± 0.00
15	0.26 ± 0.01	0.29 ± 0.04	0.35 ± 0.01	0.35 ± 0.02
20	0.26 ± 0.01	0.31 ± 0.02	0.33 ± 0.00	0.35 ± 0.02

Values are mean ± standard deviation in duplicate analysis (n = 2).
Alternative fat blend was prepared from APMF 6 h-L (liquid phase of acyl migrated PMF for 6 h) + HCO (hydrogenated coconut oil).
Each emulsion was stored for 20 days in an incubator at 20 °C.

associated with a PN distribution of 30–50 and it was found that TAG molecules appeared in both HCO and the APMF-L (Table 2). Overall, when the APMF-L and HCO were blended (50:50, w/w), a fat containing approximately an 18% reduction in the amount of saturated fatty acids could be obtained that had similar melting characteristics to HCO. In addition, the amount of myristic acid (C14:0), which is reported as a highly atherogenic fatty acid causing cardiovascular disease (Ulbricht & Southgate, 1991), was reduced by 40%.

3.4. Emulsion stability

Emulsion characteristics are evaluated by their stability and rheological properties; stability in particular is regarded as an important factor for judging the quality of emulsified foods (Guzey & McClements, 2006). Emulsions were prepared by slightly modifying the mixing ratio of the ingredients to prepare coffee cream in order to investigate the applicability of the emulsions in the food industry. Emulsions prepared from HCO and an alternative fat blends were stored for 20 days in an incubator at 20 °C and changes in particle size (d_{32}, d_{43}) were measured (Table 5). Particle size was measured during a 20-day storage. The particle sizes (d_{32}, d_{43}) measured on the day the emulsion was prepared were 0.29 and 0.36 μm (HCO), and 0.28 and 0.34 μm (alternative fat blend), respectively, and these measurements were not much different from the measured particle sizes (d_{32}, d_{43}) on the 20th day of storage. Therefore, the stability of the emulsions was maintained, regardless of the fat used (HCO or alternative fat blend), over the storage period of 20 days.

4. Conclusion

To develop a fat with reduced saturated fatty acid content and similar melting behavior to HCO, the APMF-L (liquid phase of acyl migrated PMF for 6 h after solvent fractionation at 25 °C) was blended with HCO, and an alternative fat blend (50:50, w/w) was prepared. The melting behavior of an alternative fat blend was similar to that of HCO, in which the SFI were 99.7 (10 °C), 46.8 (24 °C), 24.2 (28 °C), 12.6 (32 °C), and 0.4 (42 °C). However, an alternative fat blend had a lower saturated fatty acid content by 18% compared to HCO. Therefore, blending HCO and APMF-L, after inducing asymmetric PPO formation by acyl migration of PMF containing symmetric POP, affords a melting profile similar to that of HCO. Emulsions prepared using an alternative fat blend maintained a stable condition over a storage period of 20 days in an incubator at 20 °C, as did the emulsion prepared with HCO. The alternative fat blend prepared in this study could be applied in the food industry as a raw material for low saturated fat confectionary. Specifically, this product could be used as a raw material for non-dairy cream with low saturated fat content.

Conflict of interest

All authors declare that they have no conflict of interest.

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