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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