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Crystallization and Melting Profiles of Blends of Palm Mid Fraction, Virgin Coconut Oil and Canola Oil as Cocoa Butter Substitutes as Determined Using Differential Scanning Calorimeter and Pulse Nuclear Magnetic Resonance Techniques

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Abstract: The isothermal melting and crystallization behavior of cocoa butter substitute (CBS) formulations, consists of oil blends among palm mid fractions (PMF), virgin coconut oil (VCO) and canola oil (CO) were determined using a Differential Scanning Calorimeter (DSC), while their melting and crystallization profiles were measured as the CBS solid fat content (SFC), using pulses Nuclear Magnetic Resonance (pNMR), Bruker Minispec. The CBS formulations were developed through ternary mixture of the three oils with different ratios created using a Mixture Design, Design Expert version 6.0. Selected formulations were compositionally modified through immobilized *Thermomyces lanuginose* lipase column which catalyzed the interesterification under controlled conditions: temperature (40-60°C), catalyst concentration (1-10%), time (4-48h), C8:0 (1-5 mole) at 200 rpm orbital shaking. DSC data showed the melting behavior of the CBS was in the range between ÄH$_f$6.1491J/g-62.7894J/g. The melting peak for all blends was between 25.88- 33.78°C. The SFC for ternary blends at 25°C was between 5.84-46.85%. While at 37.5°C the SFC was in the range between 0.00-0.03%. Based on the analysis, a ternary blend constitutes of PMF:VCO:CO ratio of 90:5:5 (sample S3 or S10) with SFC (25°C) of 46.84 or 46.40% was selected for modification through enzymatic acidolysis to produce low calorie cocoa butter substitute.

Key words: Palm Mid Fraction · Virgin Coconut Oil · Canola Oil · Solid Fat Content · Melting and Crystallization · CBS · Pulsed Nuclear Magnetic Resonance · Differential Scanning Calorimeter

INTRODUCTION

Cocoa butter has a complex polymorphism which is manifested by six polymorphs called form I to VI [1]. It is a fat-phase material used in products like chocolate. At about 28 to 30% of all ingredients used [2]. This fat is important for a successful production of chocolate as well as in obtaining a high quality product to suit consumer's expectation[3]. The crystallization and melting properties of cocoa butter are of major important characteristics in the production of chocolate and related confectionery products. A steep melting profile with high solid fat content at 30°Cis capable of being fully melted at mouth or bodytemperature, resulting in a glossy chocolate with good mouth feel, flavor release and easy remolding [3].

The crystallization and melting behaviors of fats and oils are important properties in several prepared food products. Differential Scanning Calorimetry (DSC) is a thermal-analytical technique widely used in fats and oilsanalyses [4]. The SFC is affected by temperature and it is considered as an important property of fats and oils. It is used to determine the matching of fats and oils, individually or their blends, in suitable applications. Meanwhile, much attention has been given to the production of CBRs from various fats and oils via fractionation, enzymatic interesterification and blending methods [5, 6].

In this study, palm mid fraction (PMF) was chosen because it has similar triglyceride compositions of cocoa butter which are POS (2-oleo palmitostearin), SOS (2-oleo distearin) and POP (2-oleo dipalmitin) [7]. This makes it suitable as a candidate for producing a cocoa butter substitute (CBS) and could also be modified through blending and enzymatic interesterification with other

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fat/oil. Virgin coconut oil (VCO) has many advantages, which include the health benefits from the retained vitamins and antioxidants, possessing the antimicrobial and antiviral activitiesdue to lauric acid component and its readily digestible medium chain fatty acid (MCFA) fractions[8]. The lauric acid, a MCFA component of VCO has been shown to havepotential application in obesity treatment [9, 10] as it increases energy expenditure, directly absorbed and burnt as energy in the liver, resulted in early satiety and thus leading to weight loss. Canola oil (CO) is characterized by its low level of saturated fatty acids (7% total and < 4% palmitic acid). It contains a relatively high level of oleic acid (6%) and medium percentage of linolenic acid which makes up approximately one-third of fatty acid composition of the oil. Diets containing canola oil have been shownas equally effective in reducing total plasma and LDL-cholesterol as those of corn oil, soybean oil and sunflower oil [11].

Appropriate measurement processing parameters that distinguish the melting and crystallization thermodynamics of the ternary blend studied must be conducted. Commonly, theisothermal behavior of oil blends is determined using a differential scanning calorimeter (DSC) which provides the type of crystal polymorph formed. The solid fat content (SFC) of crystallized oil to its melting point was appropriately measured using pulse Nuclear Magnetic Resonance (pNMR) [12, 13].

MATERIALS AND METHODS

Materials: The PMF used in this study was supplied by Alami Group Sdn. Bhd. Panglima Garang, Klang, Malaysia. The VCO and CO were purchased from a local market.

Preparation of Oil Blends: Initially, PMF was melted in an oven at 60°C for 1 h. Ten different ratios of ternary blends (S1-S7, S11, S12, S14) which consisted of PMF, VCO and CO, were prepared based on the D-Optimal design, Design-Expert Version 6 as shown in Table 1. The mixture was then homogenized using a magnetic stirrer as described by [14]. Other ternary blends (S8, S9, S10 and S13) were included as replicates for S5, S7, S3 and S11 blends, respectively.

Solid Fat Content Determination: Solid fat contents (SFC) of 10 different ratios of ternary blends of PMF, VCO and CO were determined using pulse nuclear magnetic resonance spectrometer (p-NMR,Bruker Minispec pNMR Analyzer model 120). Prior to analysis, fat mixture was melted at 60°C for 30 min and subsequently placed into a NMR tube and chilled at 0°C for 90 min. Then, solid fat content of the samples were taken at temperature 0, 5, 10, 15, 20, 25, 30, 35 and 37.5°C (held for 30 min). The NMR tubes were equilibrated at the same temperatures for 30 min before measurement recorded [15].

Isothermal Profile and Thermodynamic (Differential Scanning Calorimeter): Isothermal analysis was carried out using Perkin Elmer Differential Scanning Calorimeter (DSC), where 3-5 mg sample of the PMF: VCO: CO blend was placed and sealed in an aluminium pan at 60°C for 20 min with the rate temperature increment of 5.0°C/min. This followed by cooling the system at a rate of 10°C/min to -60°C for 2 min and heat again to 60°C (5.0°C/min) and retain for 1 min to prevent recrystallization create melting profile. Then, the system was cooled again to-60°C at 1°C/min and left for 30 min for polymorphism transformation until the exothermic was complete; that is heat capacity returned to the baseline. Finally, the melting thermogram was determined by heating from 0 to 60°Cat 5°C/min of temperature increment and after 1 min, the melting thermogram was obtained. The melting temperature at the peak of the endothermic in the thermogram was determined using the first derived of the heat capacity. The onset of crystallization was calculated from the crystallization thermogram using the DSC software while the end of melting was calculated from the melting thermogram using the first derivative of the heat capacity of the sample. It was defined as the temperature at which the first derived of the heat capacity of the last endothermic returned to the baseline [16].

RESULTS AND DISCUSSION

A new cocoa butter substitute (CBS) was formulated and selected form 14 ternary blends of palm mid fraction, virgin coconut oil and canola oil. The blends were created using the Mixture Design, Design Expert Version 6.0, as listed in (Table 1). Melting thermogram for the ternary blends isshown in Table 2. The melting peaksof S1-S14 blends were between 25.88°C-33.78°C and begin to crystallize at temperature between-7.91°C until-8.56. The melting and crystallization enthalpies were between $ÄH_f$6.1491-62.7894J/g and $ÄH_f$-42.2291 until-68.2675J/g respectively. The sharp melting peak for cocoa butter (CB) required higher endothermic energy of $ÄH_f$108.8096J/g. Among the blends studied, S3/S10 (PMF:VCO:CO, 90:5:5) was closer to CB.

Table 1: Direct blending ratio between palm mid fraction, virgin coconut oil and canola oil

Fat blend sample	Palm mid fraction (PMF) (PMF)	Virgin Coconut Oil (VCO)	Canola Oil (CO)
S1	82.50%	12.50%	5.00%
S2	63.75%	12.50%	23.75%
S3	90.00%	5.00%	5.00%
S4	62.50%	20.00%	17.50%
S5	75.00%	20.00%	5.00%
S6	70.00%	12.50%	17.50%
S7	50.00%	20.00%	30.00%
S8	75.00%	20.00%	5.00%
S9	50.00%	20.00%	30.00%
S10	90.00%	5.00%	5.00%
S11	65.00%	5.00%	30.00%
S12	57.50%	12.50%	30.00%
S13	65.00%	5.00%	30.00%
S14	80.00%	8.75%	11.25%

S8, S9, S10 and S13: duplications were prepared and analyzed for experimental accuracy confirmation.

Table 2: Isothermal behavior for each relevant sample based on differential scanning calorimeter (DSC) analysis

	Melting behavior				Crystallization behavior			
Sample		Melting peak temperature (°C)			Cooling enthalpy	Crystallization peak temperature (°C)		
PMF:VCO:CO	Melting enthalpy (endothermic) (J/g)	Onset	1	Ended	(exothermic (J/g)	Onset	1	Ended
CB	108.8096	27.92	34.26	36.92	-97.5577	12.05	4.57	-5.07
S1	46.8969	26.80	32.80	34.41	-60.6613	-5.81	-8.56	-11.12
S2	14.7083	27.90	30.81	33.72	-54.1189	-5.33	-8.07	-10.58
S3	62.7894	26.30	33.28	35.24	-72.4078	-5.15	-7.91	-10.39
S4	13.3986	26.51	30.30	32.80	-45.8457	-5.18	-7.96	-10.43
S5	35.0306	26.05	31.50	33.20	-62.8600	-5.04	-7.86	-10.44
S6	22.6610	30.06	30.29	32.94	-57.1512	-5.22	-8.04	-11.05
S7	7.1456	22.40	27.88	30.21	-42.4581	-5.61	-8.22	-10.89
S8	34.6631	26.58	31.30	33.81	-63.9201	-5.33	-8.04	-10.64
S9	6.0098	21.89	25.88	28.21	-42.2291	-5.48	-8.12	-10.70
S10	60.7469	26.89	33.78	35.00	-68.2675	-5.14	-8.00	-10.65
S11	23.5777	27.43	31.81	34.79	-47.4728	-5.20	-7.99	-10.60
S12	6.1491	27.49	30.33	33.33	-46.7746	-5.69	-8.34	-10.92
S13	25.9394	26.99	31.30	34.09	-49.9878	-5.29	-8.05	-10.61
S14	43.4696	27.08	32.20	34.23	-67.4035	-5.31	-8.15	-10.67

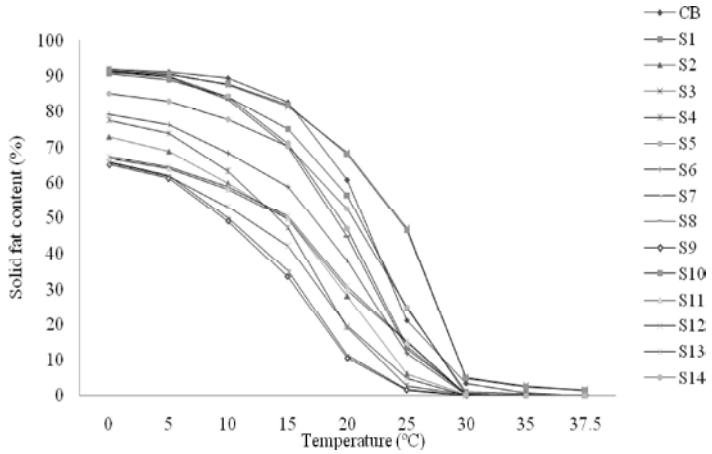

Fig. 1: Solid fat content (%) of 10 ternary blends (S1-S7, S11, S12 and S14) of palm mid fraction (PMF), virgin coconut oil (VCO) and canola oil (CO) with different ratios and cocoa butter, CB. S8-S10 and S13 are the duplicates for S3, S5, S7 and S11, respectively.

Figure 1 shows the solid fat content (SFC) of all 14 ternary blends. On the whole, SFC decreased with the increase in temperature. The SFC of individual blend differs even at the temperature due to the difference in the ratio of PMF, VCO and CO. In general, there was a decrease in solid fat content starting from 0°C to 37.5°C. All the formulated blends were completely melted with SFC of 0.00% at 30°C-37.5°C. These decreases perhaps were due to the existence of thin ratio in temperature range and thus the fat became much softer [6]. By comparing the SFC at 0°C, 25°C and 37.5°C to the one of CB, S3/S10 was the best candidate for CBS. As shown in Fig. 1, the SFC of S3 and/S10 was91.76 and/91.35%, 46.85 and/46.40% and 0.00 and/0.00%, while for CB was 91.72%, 20.99% and 0.00% at the corresponding temperatures. Thus, S3 or/S10 blend was selected for modification using an enzymatic acidolysis involving controlled parameters such as temperature (40-60°C), catalyst concentration (1-10%), time (4-48 hours), C8:0 (1-5 mole) and orbital shaking at 200rpm.

CONCLUSIONS

Analyzing the crystallization profile of oil/fat blends intended on producing a CBS is a key in deciding which type of fat crystal formed during tempering process of a confectionary chocolate. A cocoa butter substitute should be able to form mostly type V crystals during tempering to produce a chocolate with glossy, firm, best snap and melts near body temperature. This require melting point of approximately 34.26°C similar to cocoa butter polymorphous crystallization. Hence, isothermal of oil blends was studied using differential scanning calorimeter (DSC) to determine the potential type of crystal polymorph formed through melting and crystallization behavior thermogram. Whereas, the solid fat content (SFC) are appropriately measured using pulse Nuclear Magnetic (pNMR). As revealed in this study, the yield of S3 and S10 blends would be able to form stable β crystals during tempering as both have melting peak at 33.28°C and 33.78°C respectively which was closer to melting point of cocoa butter that assure best chocolate appearance, texture and stability and was selected for modification through enzymatic acidolysis.

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