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Melting Behaviour of Binary Mixtures of Palm Mid Fraction and Rice Bran Oil

(Kelakuan Peleburan Adunan Binari Fraksi Pertengahan Sawit dan Minyak Dedak Padi)

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ABSTRACT

The melting and crystallization profile of cocoa butter substitute (CBS) formulation consisting of oil blends between palm mid fraction (PMF) and rice bran oil (RBO) were measured through solid fat content using pulsed Nuclear Magnetic Resonance (pNMR) Bruker Minispe. The isothermal melting and crystallization behaviour were determined via Differential Scanning Calorimeter (DSC). The formulations were developed through binary mixture of palm mid fraction and rice bran oil with different ratios created using Mixture Design, Design Expert version 6.0. Selected formulations had been studied for compositional modification through immobilized lipase catalyzed by interesterification using Novozyme® 435. Three selected parameters such as temperature (40-60°C), catalyst concentration (2-10%) and time (4-48 h) at 200rpm orbital shaking with 14 formulations acquired by applying D-Optimal experimental design had been applied. The results showed that blends of 75% palm mid fraction with 25% rice bran oil and its 14 enzymatic interesterification (EIE) yields have the potential to perform the desired behaviour as the crystals are completely melted at 32.48 - 38.28°C. However, the yields of formulation with EIE condition of 60°C, 10% lipase and 48 h incubation time (labeled N) formed the stable β crystals during tempering as it had melting peak at 34.19°C which was closer to the melting point of cocoa butter required in tempering to form V crystals (~34°C), that assured best chocolate appearance and texture.

Keywords: Melting and crystallization profile; palm mid fraction; rice bran oil; solid fat content; pulsed nuclear magnetic resonance

ABSTRAK

Profil peleburan dan penghabluran bagi formulasi gantian lemak koko yang terdiri daripada fraksi pertengahan sawit dan minyak dedak padi ditentukan menggunakan Resonans Nuklear Magnet Denyut (pNMR) dan Kalorimeter Pengimbasan Pembezaan (DSC). Gantian lemak koko dibangunkan melalui adunan binari lemak fraksi pertengahan sawit (PMF) dan minyak dedak padi (RBO). Adunan binari ini disediakan berdasarkan peratusan tertentu yang diterbitkan melalui aplikasi Mixture Design, Design Expert versi 6.0. Adunan ini dipilih untuk pengubahsuaian sifat fizikokimia secara interesterifikasi berenzim (EIE) menggunakan enzim lipase iaitu Novozyme® 435 dengan parameter yang dikaji iaitu suhu (40-60°C), peratus enzim (2-10%), dan masa (4-48 jam). Hasil analisis menunjukkan bahawa adunan lemak 75% fraksi pertengahan sawit dan 25% minyak dedak padi sebelum dan selepas EIE mempunyai potensi untuk dibangunkan sebagai gantian lemak koko kerana melebur sepenuhnya pada julat 32.48 hingga 38.28°C. Walau bagaimanapun, adunan lemak hasil EIE menggunakan parameter 60°C, 10% lipase dan 48 jam tempoh tindak balas (adunan N) boleh membentuk hablur β paling stabil semasa proses penyepuhan dan kerana mempunyai puncak suhu penghabluran pada 34.19°C, iaitu suhu yang paling hampir bagi lemak koko untuk membentuk hablur bentuk V (~34°C), yang memberikan tekstur dan rupa coklat yang terbaik.

Kata kunci: Fraksi pertengahan sawit; kandungan lemak pepejal; minyak dedak padi; profil peleburan dan penghabluran

INTRODUCTION

Oil blends consisting of palm mid fraction and rice bran oil were studied for the formulation of cocoa butter substitute. Selection of oils or fat for this functionality is from the basis that some vegetable fats may have characteristics and melting profiles that is very similar to cocoa butter. In this study, palm mid fraction was chosen because it has similar triglycerides compounds to the one in cocoa butter which are POS (2-oleo palmitostearin), SOS (2-oleo distearin) and contain 57% POP (2-oleo dipalmitin) (Gunstone 2004). This makes it suitable to be used in developing cocoa butter substitute (CBS). Some vegetable fats/oils have different melting profiles compared to cocoa butter, thereby providing greater shelf life. In this case, the composition of rice bran oil which contains palmitic acid, lieolenic acid, linoleic acid, oleic

acid, stearic acid, tocopherol and squalene (Chow 1992: Saito 1994; Hyung-Jin Kim 1998) has the potential to preserve the shelf life of this blend.

Investigating crystallization profile of the oil blends is crucial to determine which type of crystals are form during tempering process. Basically, there are three types of vegetable fat polymorphs of which are α crystals that are unstable and waxy, β' crystals that are ideal for baking applications, giving smooth creamy texture with small crystal form obtained from the diversity of fatty acids chain length and triacylglycerol diversity. The most stable crystal form required in tempering is β crystals which are also recognized as polymorph forms V and VI that have melting points of 34°C and 36°C. However, making a good chocolate is about forming as many type V crystals as possible that promise the glossy look and firm texture as it creates the most stable crystals so that the texture and appearance will not degrade over time with best snap, melts near body temperature (37°C). Since the studied oil blends had involved lipase-catalayzed interesterification with controlled parameters time-temperature-enzyme concentration, the yields isothermal profile might differ within a small range as slight variation in these conditions would affects the kinetics of polymorphic transformations, and consequently on the melting/crystallization temperature profile and texture of the final product (Kawuamura 1979; Meltin & Hartel 1990; Toro-Vazquez et al. 2002; Zhang et al. 2004). Hence, appropriate measurement of the parameters that distinguish the melting and crystallization thermodynamics of the binary blend studied must be investigated. Thus, isothermal of oil blends was examined using differential scanning calorimeter (DSC) to determine the type of crystal polymorph formed. The solid fat content (SFC) from crystallized form to melting point were appropriately measured using pulse Nuclear Magnetic Resonance (pNMR) (Wright et al. 2000; Miskandar et al. 2004).

Materials and Methods

SAMPLE PREPARATION AND EXPERIMENTAL DESIGN

The sample preparation began with direct binary oil blending of palm mid fraction (PMF) and Rice Bran Olein (RBO). The multiple ratio of direct blending was done with the help of Mixture Design, Design Expert version 6.0. with the ratios of 100:0, 75:25, 50:50, 25:75 and 0:100 that were created using Mixture Design, Design Expert version 6.0. Solid fat content (SFC) for all mixtures were analyzed using pulsed Nuclear Magnetic Resonance (pNMR) Bruker Minispec. Selected mixture has been proceeded for compositional modification using biocatalyst immobilized lipase catalyzed interesterification which involved immobilized lipase (Novozyme® 435) through controlled parameters such as temperature (40-60°C), catalyst concentration (2-10%), time (4-48 h) and orbital shaking at 200 rpm.

SOLID FAT CONTENT PROFILE (PULSED NUCLEAR MAGNETIC RESONANCE)

The prepared samples were melted at 50°C, and transferred into sample tubes about 3 cm in height. The pNMR instrument was set and calibrated by measuring the 3 standard samples of 0%, 31.5%, and 75.3% solid, respectively. Each sample tube was placed into 0°C water bath and kept for 90 minutes. Then the other water bath was set for the next measuring temperature begining with 5, 10, 15, 20, 25, 30, 35 and 37.5°C where each chilled sample tubes would be shifted in sequence at the same time interval in batches. Each tube was hold for 30 min at measuring the temperatures. The solid fat content for all samples were measured. The SFC results was displayed without any calculation involved (Lin et al. 1995).

ISOTHERMAL PROFILE AND THERMODYNAMIC (DIFFERENTIAL SCANNING CALORIMETER)

Isothermal analysis was measured through Perkin Elmer Differential Scanning Calorimeter (DSC), where 3-5 mg sample of the PMF-RBO blend was sealed in an aluminum pan at 60°C and held for 20 min with the rate of 5.0°C/min. This was followed by cooling the system at 10°C/min to -60°C for 2 minutes and heating again to 60°C (5.0°C/min) and hold for 1 minutes to prevent recrystallization to create melting profile. Then, the system was cooled to -60°C at 1°C/min and left for 30 min for polymorphism transformation until the crystallization exothermic was completed. Finally, the of melting thermogram was determined by heating at 5°C/min from 0 to 60°C, and after 1 minutes, the melting thermogram was obtained. The melting temperature at the peak of the endothermic in the thermogram was determined (David Pérez-Martínez et al. 2005).

Results and Discussion

THERMAL BEHAVIOUR OF DIRECT BLEND

The new cocoa butter substitute (CBS) was developed through binary mixture of palm mid fraction and rice bran oil with the ratios of (100:0), (75:25), (50:50), (25:75) and (0:100) that were created using Mixture Design, Design Expert version 6.0. (Table 1). Solid fat content (SFC) for all mixtures was analyzed using pulsed Nuclear Magnetic Resonance (pNMR) Bruker Minispec. The steepness of PMF curved from 25 to 30°C represented the sharp melting of TAG for each formulation as demonstrated by the highest peak in melting behaviour profile. Figure 1 shows 100% PMF and blend E (75% PMF 25% RBO) having sharp melting points from 25°C to 30°C, while PMF content for PMF at 25°C is 72.99% and blend E 54.99%. Then, the SFC for both PMF and blend E droped to 4.42% and 2.43%, respectively when the temperature reach 30°C. This illustrates that both samples had melting points exceeding 30°C and began to crystallize below 25°C, and more accurate below 20°C.

TABLE 1. Direct blending ratio between palm mid fraction and rice bran oil

Fat blend sample	Palm Mid Fraction (PMF)	Rice Bran Oil (RBO)
A	100%	0%
B	50%	50%
C	0%	100%
D	25%	75%
E	75%	25%

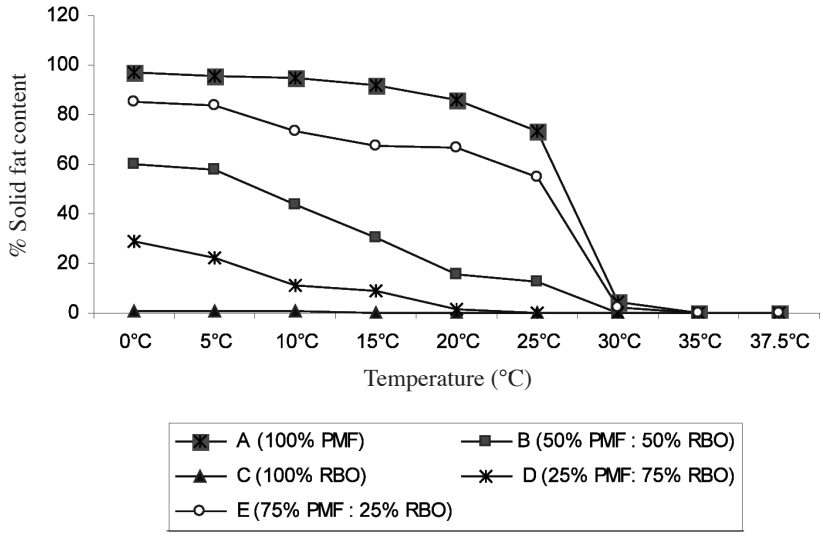


FIGURE 1. Solid fat content of multiple direct blending ratios between palm fraction and rice bran oil

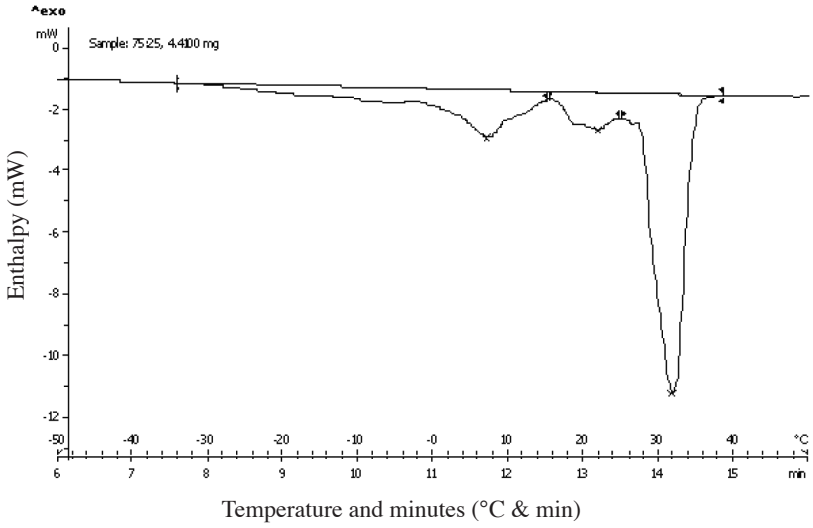


FIGURE 2. Melting behaviour thermogram profile for blend E (PMF 75% : RBO 25%)

It was clarified by melting and crystallization behaviour analysis through differential scanning calorimeter (DSC). The sharp melting points allow the oil blends to attain its most stable crystals form (β crystals) during tempering and retain it at room temperature so that the chocolate texture and appearance will not be degraded over time as well as melt quickly near body temperature as cocoa butter. The result in the Table 3 (DSC thermogram result) shows the melting peak of PMF and blend E are 34.39°C and 31.69°C, while begin to crystallize at 17.64°C and 15.13°C, respectively. Rice bran oil remained in liquid form in the solid fat content profile as it only began to crystallize at -3.27°C and melting peak at -16.77°C, investigated using DSC. The sharp melting

peak for PMF illustrated that higher endothermic energy was required to breakdown the crystal form into liquid form, which PMF required ΔH_f -117.99 compared to other samples with range of ΔH_f -91.45 to -113.04 J/g including direct blending of blend E and other 14 EIE yields. RBO only needs ΔH_f -72.58 since RBO is already in the liquid state at room temperature.

These suggests that the solid fat content profile of binary blends between hard fat (PMF) and soft fat (RBO) simplifies oil blends consisting of PMF content 75-90% with RBO 25-10%, can be a good combination to develop cocoa butter substitute that has PUFA properties. Thus, in this study, sample blend E was selected for modification through enzymatic interesterification whose parameters had been controlled such as temperature (40-60°C), catalyst concentration (2-10%) and time (4-48 h) at 200 rpm orbital shaking. The EIE reaction in this study yieldss 14 formulations labeled from A to N with multiple parameter levels as shown in Table 2.

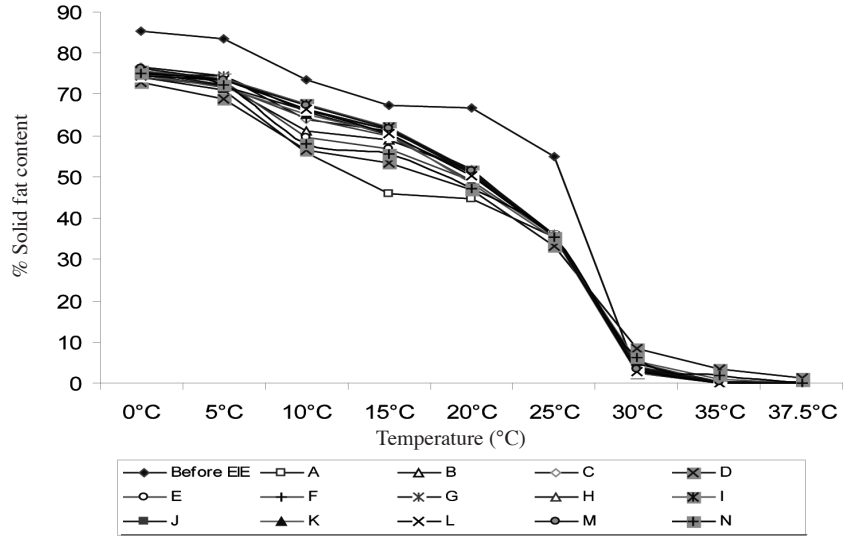
TABLE 2. Experimental design with 3 parameters 5 level for enzymatic Interesterification reaction (EIE)

Sample	Parameters		
Label/code	Temperature (°C)	Enzyme (%)	Time (h)
A	60	10	26
B	60	2	4
C	40	2	4
D	40	2	48
E	60	2	48
F	40	6	26
G	50	6	48
H	50	2	26
I	40	10	44
J	40	10	26
K	50	6	26
L	55	2	4
M	60	10	4
N	60	10	48

THERMAL BEHAVIOUR OF EIE BLENDS

The solid fat content (SFC) and isothermal behaviour for each EIE yields were analyzed using pulsed Nuclear Magnetic Resonance (pNMR) as well as Differential Scanning Calorimeter (DSC) illustrated that the profile for each blend was slightly different as TAG content for these blends were almost similar. The SFC content profile is shown in Figure 3, where the SFC decreased gradually from 0°C to 25°C but the curve became steeper as the temperature reached 30°C. Then the result of SFC by using pNMR was further detailed by the analysis via DSC. Through DSC, the melting profile proved that overall melting peak for all the 14 EIE yields were between 29.94°C and 34.56°C, which represented the sharp melting point with the range of endothermic energy ΔH_f -91.45 J/g to ΔH_f -113.04 J/g. The melting and crystallization behaviour for all samples involved are simplified in Table 3.

Although the SFC profile and melting behaviour between samples are within small range, it is important to determine which sample has closer profile to cocoa butter, as it will characterize the physicochemical and attributes of the chocolate. To determine which sample has this characteristics, samples with melting point around 34°C



Label: A = 60ºC, 10%, 26j. B = 60ºC, 2%, 4j. C = 40ºC, 2%, 4j. D = 40ºC, 2%, 48j. E = 40ºC, 6%, 26j. F = 50ºC, 6%, 48j. G = 50ºC, 2%, 26j. H = 40ºC, 10%, 4j. I = 40ºC, 10%, 48j. J = 50ºC, 6%, 26j. K = 50ºC, 4%, 37j. L = 55ºC, 2%, 4j. M = 60ºC, 10%, 4j. N = 60ºC, 2%, 26j.

FIGURE 3. Solid fat content profile for 14 enzymatic interesterification (EIE) yields

TABLE 3. Isothermal behaviour for each relevant sample based on differential scanning calorimeter (DSC) analyis.

Sample	Melting behaviour					Crystallization behaviour				
	Melting enthalpy (endothermic) (J/g)	Melting peak temperature				Cooling enthalpy (exothermic) (J/g)	Crystallization peak temperature			
		Onset	1st	2nd	Endset		Onset	1st	2nd	Endset
PMF	-117.99	5.21	13.77	34.39	36.86	78.69	17.64	16.15	8.75	4.47
RBO	-72.58	-38.99	-30.05	-16.77	2.24	27.84	-3.27	-5.93	-	-30.62
75:25	-104.60	0.37	21.93	31.69	34.59	59.64	15.13	13.43	5.80	-2.20
A	-107.09	-0.35	18.59	30.41	35.24	63.42	19.43	14.91	6.80	-1.86
B	-109.06	0.45	18.93	31.33	34.52	61.00	15.08	13.28	6.48	-1.45
C	-108.43	-0.97	18.59	30.99	34.13	61.03	15.38	13.44	6.14	-1.86
D	-98.87	1.97	20.26	34.56	38.28	62.48	24.86	23.42	5.28	-4.01
E	-108.03	-0.67	18.42	30.48	33.74	59.36	14.39	12.96	5.81	-2.06
F	-104.45	-0.25	19.61	31.53	34.12	59.15	13.96	12.44	5.80	-2.36
G	-105.44	0.24	18.75	30.83	33.87	61.68	15.05	13.79	6.14	-1.76
H	-108.72	0.26	18.75	30.34	34.13	58.99	15.44	13.28	6.15	-2.03
I	-109.31	-0.34	18.59	30.98	34.01	60.52	15.00	13.80	5.99	-1.79
J	-98.50	2.23	22.75	31.41	34.19	57.06	14.63	13.10	5.78	-2.14
K	-101.71	1.00	19.43	29.94	37.22	63.48	19.76	18.27	5.80	-2.70
L	-112.47	-0.30	18.75	31.00	33.92	63.04	16.21	14.95	6.15	-1.92
M	-113.04	1.23	19.27	30.86	34.27	60.57	16.10	14.43	6.14	-2.08
N	-100.31	2.36	-	34.19	38.27	62.00	21.67	19.91	6.30	-2.89

are desirable to be developed as cocoa butter substitute. This is due to its capability to form crystal polymorph type V that form the most stable β crystals during tempering process and potentially to produce chocolate with glossy, firm, best snap, and melts near body temperature (Hernandez & Huertas 2005). Among these 14 EIE yield samples, sample labeled N had the sharp melting point of 34.19°C that requires ΔH_f -100.31 J/g to melt completely at 38.27°C and begins to crystallize at 21.67°C.

Figure 4 demonstrates that SFC profile of sample N is closer to cocoa butter profile when the temperature reached 25°C with solid fat content 35.26% for sample N and 40.47% for cocoa butter. Then, the SFC content for both fats are almost similar at 30°C, while sample N and cocoa butter have solid fat contents of 6.06% and 6%, respectively. However, based on DSC melting behaviour analysis, PMF had sharp melting peak at 34.39°C (closer to desirable melting point to form type V crystal) and melts completely near body temperature which is 36.86°C. Even though PMF would be the best substitute for cocoa butter, blend N is more preferred in this study with the purpose of benefiting the rice bran oil content.

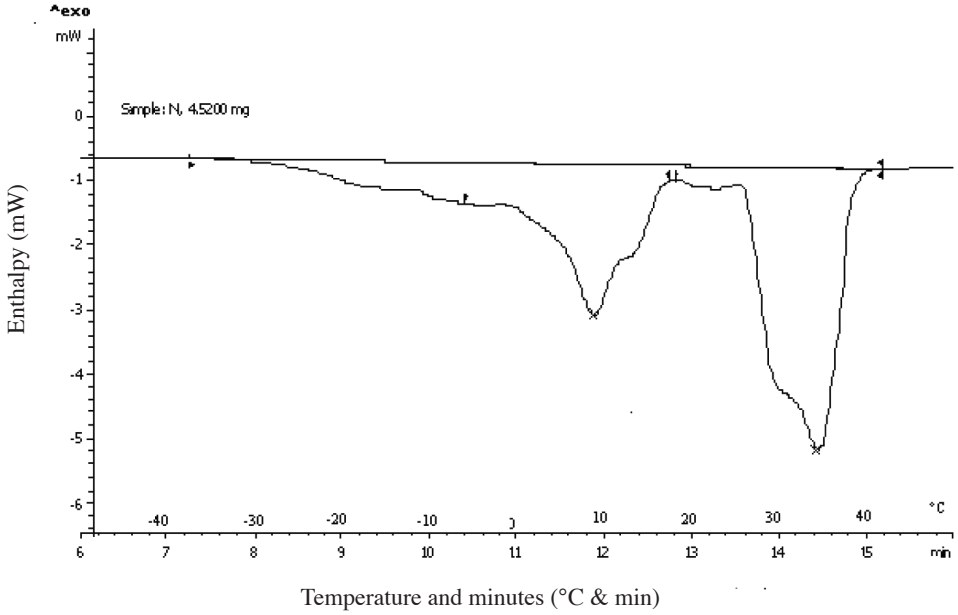


FIGURE 4. Melting behaviour thermogram profile for sample labeled N

CONCLUSION

Analyzing crystallization profile of oil/fat blends as cocoa butter substitute is the key to determine which type of crystal formed during tempering process. 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 requires melting point approximately 34°C similar to cocoa butter polymorphous crystallization. Results from this study revealed that the yields of blend with EIE condition of 60°C, 10% lipase and 48 h incubation time (labeled N) would be able to form stable β crystals during tempering as both have melting peaks at 34.19°C which is closer to melting point of cocoa butter required in tempering to form V crystals (34°C), thus assuring best chocolate appearance, texture and stability.

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