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The Effect of Enzymatic Alcoholysis on the Physicochemical Properties of Commercial Cocoa Butter Substitutes

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Abstract: The need for low-calorie fats has increased because of recent changes in consumers' concerns about leading healthier lifestyles. Three types of commercial Cocoa Butter Substitutes (CBSs) were studied for use as substrates in the production of low-calorie structured lipids. Mixtures of commercial CBSs, ethanol and medium-chain fatty acids in a molar ratio of 1:2:1 were incubated in a water bath at 50°C for 24 h at 250 rpm, using 10% immobilized lipase as a catalyst. The following physicochemical properties were determined before and after enzymatic alcoholysis: the Solid Fat Content (SFC), the slip melting point, the iodine value, the free fatty acid content, the thermal behavior and the fatty acid composition. Hisomel CBSs was chosen as a substrate for further analysis because of its sharp melting profile between room temperature (25°C) and body temperature (37.5°C). At room temperature, the SFC of Hisomel was 60.56% and after the reaction with either caprylic or capric acid, it became 12.50% and 14.26%, respectively, which was the highest value among other CBS sample. Furthermore, Hisomel also had samilar fatty acid profile with cocoa butter before the enzymatic alcoholysis, which was 31.13% palmitic acid, 50.05% stearic acid and 18.80% oleic acid.

Key words: Alcoholysis, cocoa butter substitutes, lipase, medium-chain fatty acid, structured lipid

INTRODUCTION

For nearly the past 40 years, various approaches have been taken to modify the chemical and physical composition of fats and oils in order to produce an alternative to Cocoa Butter (CB). Up to this point, Cocoa Butter Alternative (CBA), Cocoa Butter Equivalent (CBE), Cocoa Butter Improver (CBI), Cocoa Butter Substitutes (CBS) and Cocoa Butter Replacer (CBR) have been widely being produced (Mamot, 2009).

The early stage of research involved was to find a replacement for CB in chocolate manufacturing. CB, processed from cocoa beans, is one of the most important ingredients in the chocolate and confectionery industry. Because CB is dominated by palmitoyl-oleoyl-stearoyl-glycerol, distearoyl-oleoyl-glycerol and dipalmitoyl-oleoyl-glycerol (POS, SOS, POP), it gives a unique thermal profiles which shows a sharp melting point. It will change from a solid to a liquid form quickly and it melted just when it reached the body temperature (Lipp and Anklam, 1998).

However, the increases in the market price of CB, the limited supply, the production of low-quality CB and the availability of technological facilities has led to the development of alternatives to CB. According to Lipp and Anklam (1998), most cocoa butter alternatives are manufactured from a mixture of palm oil, palm mid-fraction and shea and illipe butters.

Currently, CB has been mostly replaced by CBSs in chocolate manufacturing. CBSs are a type of fat that have physical characteristics similar to CB but differ in chemical composition. The substitutes must possess certain requirements in physical properties in order to be suitable as CBSs.

Long-chain fatty acids combined with medium- and/or short-chain fatty acids that are esterified into the same molecule of triacylglycerol are known as Structured Lipid (SL) (Akoh, 1995). In this study, the CBSs used as an ingredient in chocolate have been studied as a potential substrate for the enzymatic alcoholysis reaction for the production of low-calorie SL. Therefore, it can be apply in confectionery industry along with the good health effect of a product.

According to De Oliveira and Alves (2000), less attention has been given to the application of lipase in lipid modification, especially the alcoholysis of Triacylglycerol (TAG). Therefore, the alcoholysis reaction was chosen to produce structured lipids from CBSs as it is not widely discovered yet. The alcoholysis of TAG is a continuous reaction. Alcohol breaks up the acyl group in the TAG molecule and as a result, two forms of the product will be formed: diacylglycerol and monoacylglycerol (Akoh, 1995). Alcoholysis followed by esterification could be used to synthesize beneficial SLs such as Medium-Long-Medium (MLM) structure of SLs for health purposes (e.g., a MCFA at the sn-1 position, a glycerol chain at the sn-3 position and long-chain fatty acids at the sn-2 position) (Irimescu *et al*., 2002). Because of its' regiospecificity and stereospecificity features, lipase

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has been found to be an effective catalyst in the synthesis of SLs. It can be used to determine the required composition and distribution of acyl in the glycerol molecule (Mangos *et al*., 1999). Therefore, lipase is suitable for the production of lipids with nutritional value (Lumor and Akoh, 2005). The objective of this study was to determine the physicochemical properties of commercial CBSs before and after the reaction of enzymatic alcoholysis. Through this physicochemical properties, one of the CBSs type will be selected and modified to produce low-calorie structured lipid.

MATERIALS AND METHODS

Samples and reagents: Three types of commercial CBSs - Isfat Hisomel, Isfat H2100G and Isfat 369 used in this study were sponsored by KL-Kepong Cocoa Products Sdn Bhd, Port Klang, Selangor. The capric and caprylic acid was obtained from Sigma-Aldrich Co. Immobilized lipase from *Thermomyces lanuginosus* (Lipozyme TLIM) was purchased from Novozyme Co. All chemicals were of analytical grade.

Saponification value: The saponification value was determined by the Association of Analytical Communities (AOAC) method (1990). Determination of the molecular weight for this commercial CBSs was used to calculate the molar ratio of substrate in the reaction.

Enzymatic alcoholysis: Commercial CBSs were go through enzymatic alcoholysis reaction. The analyses were carried out in screw-capped test tubes. With a ratio of 1:2:1 molar according to their molecular weight between the CBS (Hisomel, H2100G or 369), the ethanol and the MCFAs (caprylic acid or capric acid); the mixtures were run at 50°C for 24 h at a rate of rotation of 250 rpm. A total of 10% TLIM (*Thermomyces lanuginosus*) enzyme was used as a catalyst. After the analysis, the reactants were separated from the enzyme and transferred to another test tube. The TLIM enzymes used in this study were based on selectivity to the sn-1 and sn-3 in the TAG (Hayes, 2004).

Free fatty acid: The free fatty acid content were determined before and after the reaction according to PORIM method (1995).

Alkali refining: After determined the FFA content, it was further processed by removing the FFA and other impurities using John *et al*. (2002) method that have been modified.

Chemical analysis: The iodine value (g I_2/100 g) in the samples before and after enzymatic alcoholysis were determined by PORIM method (1995).

Solid fat content: The Solid Fat Content (SFC) in each sample was determined by pulse Nuclear Magnetic Resonance (pNMR) (Model Bruker Minispec No.120, Rheinstetten, Germany) using the PORIM method (1995).

Slip melting point: The Slip Melting Point (SMP) of each sample was determined by the PORIM method (1995).

Thermal behavior: The thermal behavior of each sample was referenced to the AOAC standard (1990) using Mettler Toledo Differential Scanning Calorimetry 822 (DSC) (SW Stare 9:01).

Fatty acid composition determination: The methyl ester fatty acid composition was analyzed by Gas Chromatography (GC). The method was modified from (Md Ali and Dimick, 1994) using a polar silica capillary column HP-5 (0.32 mm i.d. x 30 mm, 0.25 μm film thickness) with a Flame Ionization Detector (FID) system.

Statistical analysis: One-way ANOVA was used to analyze the data experiments.

RESULTS AND DISCUSSION

The SFC can be interpreted as the ratio of solid fat to liquid fat at a certain temperature; it determines the texture of the fat. The temperature and mixture ratio affects the rate of change of the SFC (Noor Lida *et al*., 2001). The SFC value at room temperature for all of the samples before the enzymatic alcoholysis reaction (Fig. 1) were more higher compare with the samples measured after the reaction (Fig. 2 and 3). The formation of the mid-fraction and the low-melting fraction after alcoholysis caused the restructuring of the fatty acids in TAG and lowered the SFC percentage at room temperature (Norizzah *et al*., 2004). The presence of SCFAs and MCFAs also reduced the lipid density. The addition of alcohol as solvent also reduced the percentage of SFC after the enzymatic alcoholysis. Overall, the SFC approached 0% at 37.5°C which showed that the fat was completely melted at body temperature. This proves that interesterification affected the SFC profile and the hardness level of the fat. The SFC percentage seemed to inversely proportional with temperature and the fat melted before reaching the body temperature (37.5°C). Normally, with these properties, the oil or fat is more suitable for use in margarine and shortening manufacturing and in confectionery fats, cream and related products. The gentle melting characteristics create a pleasant mouth feel that is suitable for related products (Nazaruddin and Suriah, 2005). Among the three types of CBSs, Hisomel showed a higher percentage of SFC at room temperature (25°C) compared with the other CBSs, whether the enzymatic

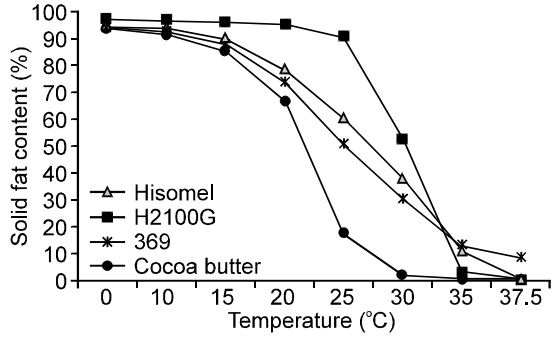

Fig. 1: The percentage of solid fat content for cocoa butter and commercial cocoa butter substitutes before enzymatic alcoholysis

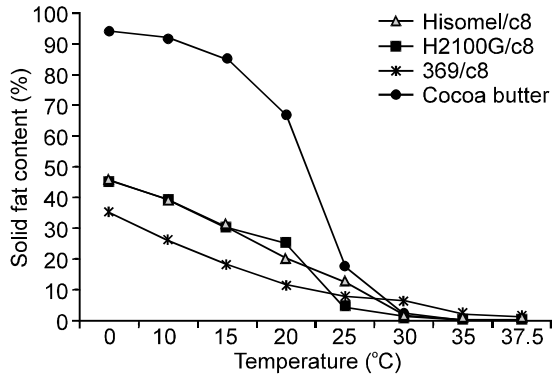

Fig. 2: The percentage of solid fat content for unmodified cocoa butter as reference and commercial cocoa butter substitutes after enzymatic alcoholysis in the presence of caprylic acid

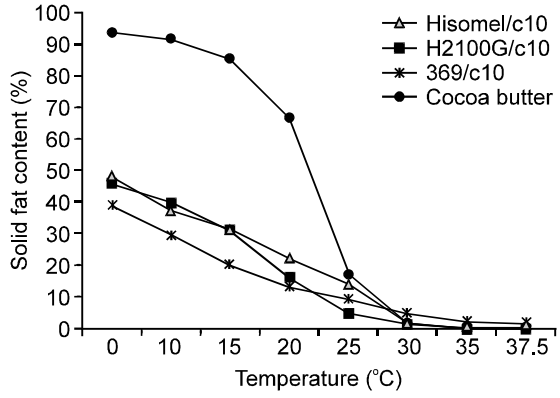

Fig. 3: The percentage of solid fat content for unmodified cocoa butter as reference and commercial cocoa butter substitutes after enzymatic alcoholysis in the presence of capric acid

alcoholysis was performed with capric or caprylic acid. A higher percentage of SFC is necessary to give the end product its exceptional quality of relative hardness at

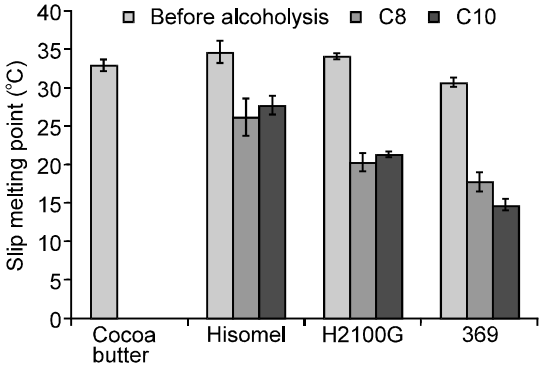

Fig. 4: The slip melting point of cocoa butter and commercial cocoa butter substitutes before and after enzymatic alcoholysis in the presence of caprylic or capric acid

room temperature, yet with complete melting at body temperature. Therefore, Hisomel was selected for the next phase of the study because it possessed a narrower melting range for SFC percentage between the room temperature (25°C) until body temperature (37.5°C) rather than H2100G and 369.

After alcoholysis, each sample had a low Slip Melting Point (SMP). The SMP was measured to determine the temperature at which the fat changed from a solid fat to a liquid oil phase. According to Rousseau *et al.* (1996), a fat crystal network reduces a fat's ability to hold the fat crystals in the matrix and it slowly melts until it suddenly rises up in the capillary column. Before alcoholysis, all of the CBSs samples had an SMP above 30°C. As seen in Fig. 4, the SMP of CB is not significantly different ($p>0.05$) than other CBSs samples before enzymatic alcoholysis. However, the SMP decreased for all of the samples after alcoholysis because of the addition of MCFAs and ethanol solvent, which caused the SMP to decrease. It is also possible that the ester bonds failed to reattach to the glycerol backbone; thus, the amount of high-melting point TAG may have been reduced, lowering the melting temperature. The CBSs samples were not significantly different ($p>0.05$) from each other, neither before nor after the reaction. Hisomel CBS was melted at 34.63±1.44°C before the reaction, at 26.19±2.46°C with the addition of caprylic acid and at 27.66±1.25°C with the addition of capric acid. The SMP of H2100G CBS was 34.06±0.40°C before alcoholysis, 20.33±1.26°C with the addition of caprylic acid and 21.32±3.30°C with the addition of capric acid. Meanwhile, the 369 CBS was melted at 30.66±0.61°C before the reaction and at 17.76±1.25°C and 14.76±0.75°C after the addition of caprylic and capric acid, respectively. The 369 sample changed from a solid to a liquid phase at a lower temperature than most of the other samples. The 369 sample had the lowest percentage of SFC at low temperatures (0-20°C) after the enzymatic alcoholysis reaction.

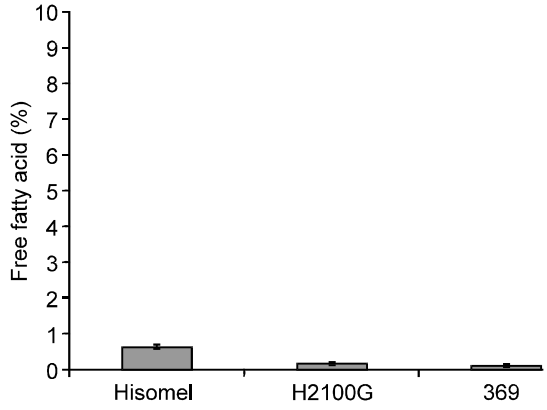

Fig. 5: The percentage of Free Fatty Acids (% FFAs) for commercial cocoa butter substitutes before enzymatic alcoholysis

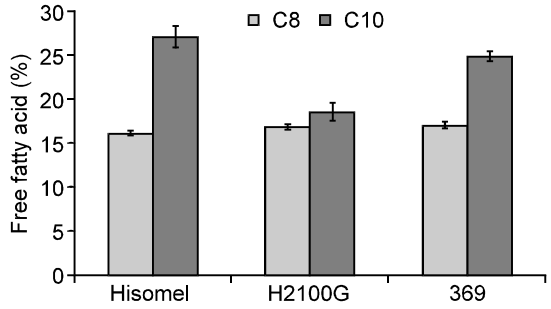

Fig. 6: The percentage of Free Fatty Acids (% FFA) for commercial cocoa butter substitutes after enzymatic alcoholysis in the presence of caprylic or capric acid

The degree of fat hydrolysis was measured by the Free Fatty Acid (FFA) value (Hamilton and Bhati, 1980). The FFA value is also one of the criteria used to determine the quality of the oil (Aftab *et al*., 2008). The percentage of FFAs in the commercial CBSs before the reaction is shown in Fig. 5. Hisomel and 369 show similarities or has significant differences ($p<0.05$) between samples, with 0.12±0.04% and 0.62±0.05% of FFAs, respectively. The H2100G sample with 0.16±0.06% was significantly different. After enzymatic alcoholysis, all CBSs showed an increase in the FFA percentage (Fig. 6). The FFA value of Hisomel was higher with the addition of capric acid than with the addition of caprylic acid. Because of this, caprylic acid was chosen as one of the substrates to be used in further studies involving the production of SLs that have capric acid added to ensure product quality. The inability of fatty acids that were produced from the breakdown of esters to form a new TAG also caused a high FFA content (Chen *et al*., 2007). Chen *et al*. (2007) also stated that a possible cause of the high content of FFAs was the lipase that tends to hydrolyze TAG and which formed Diacylglycerol (DAG) and

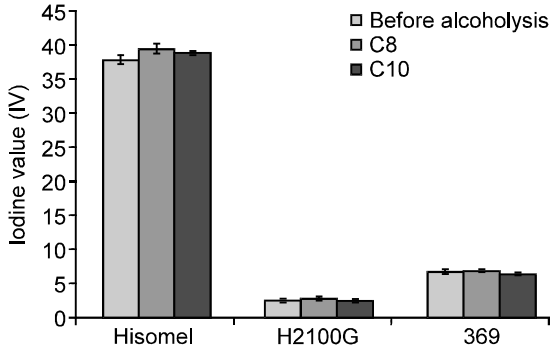

Fig. 7: The iodine value of commercial cocoa butter substitutes before and after enzymatic alcoholysis in the presence of caprylic or capric acid

Monoacylglycerol (MAG) and then produced the FFAs and glycerol. It may also have been caused by the MCFAs that were added into the reaction mixture but did not completely combine with the ester bond at position sn-1, 3 after esterification by lipase.

Figure 7 shows the Iodine Value (IV) for the samples before and after the enzymatic alcoholysis in the presence of MCFAs. The IV is used to measure the degree of unsaturated fatty acids in the fat or oil and it describes the amount of absorbed iodine. Each sample showed a higher IV after the analysis (Fig. 7). The IV is also an appropriate parameter for detecting the presence of olein in the sample (Haryati *et al*., 1998). Hisomel showed the highest IV. This indicates that Hisomel has the highest content of unsaturated fatty acids-such as oleic acid, linoleic acid or linolenic acid-compared with H2100G and 369. This result was proven by an analysis of fatty acid composition using gas chromatography (Table 1). The oleic acid content was highest in Hisomel, followed by 369 and then H2100G. The IV (g I_2/100 g) was 37.82±0.66 for Hisomel, 2.43±0.14 for H2100G and 6.78±0.37 for 369 before the reaction. There was no significant difference in any of the samples either before or after the reaction ($p>0.05$). After the addition of caprylic acid, the value was 39.44±0.77 for Hisomel, 2.76±0.29 for H2100G and 6.92±0.19 for 369. In contrast, with the addition of capric acid, the value for iodine was 38.83±0.24 in Hisomel, 2.55±0.21 for H2100G and 6.40±0.20 for the 369. According to Gunstone (2004), the esterification process does not alter the IV or unsaturated fatty acids contents in fats or oils. Therefore, it is suspected that the esterification reaction did not play a major role in changing the fats.

Table 2 show the melting and crystallization behavior of the samples before and after enzymatic alcoholysis. DSC was used as a technique to observe changes in the chemical and physical characteristics of the sample over a specific temperature range. In contrast with the

Table 1: The composition of fatty acids in commercial cocoa butter substitutes before and after enzymatic alcoholysis

	FAC (wt%)								
Sample	$C_{8:0}$	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Hisomel	-	-	-	-	31.13	50.06	18.80	-	-
Hisomel/$C_{8:0}$	1.44	-	0.73	0.89	27.93	46.07	3.13	1.97	-
Hisomel/$C_{10:0}$	0.42	0.62	0.20	0.66	33.48	49.77	14.19	0.67	-
Hisomel/$C_{8:0}$ refined	2.34	-	0.43	0.86	27.09	44.87	17.93	0.62	-
H2100G	-	-	33.00	23.23	16.57	27.19	-	-	-
H2100G/$C_{8:0}$	0.81	-	34.70	23.12	15.88	25.48	-	-	-
H2100G/$C_{10:0}$	-	1.91	32.48	22.50	16.03	27.07	-	-	-
H2100G/$C_{8:0}$ refined	3.86	1.84	31.16	21.10	11.30	14.17	0.18	-	-
369	0.44	0.75	23.03	11.01	11.92	50.17	2.72	-	-
369/$C_{8:0}$	1.59	0.77	23.79	10.94	11.63	49.01	2.27	-	-
369/$C_{10:0}$	0.45	2.48	23.55	10.75	11.48	49.56	2.18	-	-
369/$C_{8:0}$ refined	8.11	2.67	24.47	11.06	8.29	27.31	2.66	0.02	-

[a]FAC: Fatty Acid Composition; $C_{8:0}$: Caprylic acid; $C_{10:0}$: Capric acid; $C_{12:0}$: Lauric acid; $C_{14:0}$: Myristic acid; $C_{16:0}$: Palmitic acid; $C_{18:0}$: Stearic acid; $C_{18:1}$: Oleic acid; $C_{18:2}$: Linoleic acid

Table 2: The melting and crystallization behavior of cocoa butter and commercial cocoa butter substitutes before and after enzymatic alcoholysis in the presence of caprylic or capric acid

	Melting behavior						Crystallization behavior					
	Melting enthalpy	Melting peaks (°C)					Crystall. enthalpy	Crystallization peaks (°C)				
Sample	(J/g)	Onset	1	2	3	Endset	(J/g)	Onset	1	2	3	Endset
Hisomel	-95.42	-10.83	0.96	11.64	33.13	36.20	87.22	27.49	19.44	-	-	2.87
Hisomel:C_8	-91.22	-31.56	-16.04	22.26	-	28.02	86.18	14.81	12.74	-5.63	-10.80	-38.92
Hisomel:C_{10}	-91.66	-25.14	-5.21	6.45	27.07	31.41	77.39	19.26	12.23	-12.39	-	-14.07
H2100G	-121.77	25.50	31.79	-	-	35.37	119.98	17.39	14.59	-	-	10.94
H2100G:C_8	-118.44	-36.51	-14.72	21.54	-	28.22	117.60	8.50	0.96	-35.95	-	-41.19
H2100G:C_{10}	-126.58	-0.27	8.74	21.86	-	28.60	122.31	-2.68	-5.40	-	-	-8.82
369	-121.72	19.99	31.17	-	-	37.45	110.44	21.20	25.10	10.35	-	3.33
369:C_8	-114.81	-44.03	-22.05	-4.05	19.93	37.42	87.16	11.22	77.07	-	-	-15.00
369:C_{10}	-126.20	-2.96	-0.41	21.58	-	39.23	114.66	19.27	17.24	4.41	-18.40	-21.99

Crystall. = Crystallization

sharp melting point shown by non-esterified substrates, the analytical results obtained after enzymatic alcoholysis with the addition of MCFAs and ethanol showed a broad range of melting peak. Changes in the sn-1, 2 and 1, 3 positions of the fatty acids in DAG resulted in changes in the melting behavior in the fats, which is also associated with polymorphism since the lipase was used in reaction (Siew and Ng, 2000). Almost all of the samples were fully melted at body temperature (37.5°C), except for the 369 sample with MCFAs added. This profile was also shown in the SFC result in Fig. 2 and 3. The ratio of solid to liquid in the 369 samples at 37.5°C was 1.12% and 1.72% with the addition of caprylic or capric acid, respectively, whereas the other samples had already fully melted at this temperature. The presence of more than one thermal thermogram was detected for the substrates after alcoholysis. This may be due to the presence of MAG or DAG that did not react completely (Undurraga *et al*., 2001). The presence of other impurities such as solvent also affected the melting and crystallization behavior of the substrate since it lowered the density of the samples.

Conclusion: For the manufacturing of chocolate and confectioneries, the SFC percentage at room temperature and at body temperature are important. With 12.59% and 14.26% at those temperatures, Hisomel had the highest SFC after the reaction. Therefore, Hisomel has the potential to be used as a substrate for producing low-calorie SLs and could be applied in the manufacture of chocolate and confectioneries. The optimal settings of select parameters will be investigated in future studies.

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