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OPTIMIZATION OF COCOA BUTTER EQUIVALENT PRODUCTION FROM FORMULATED HARD PALM OIL MID-FRACTION AND CANOLA OIL BLENDS

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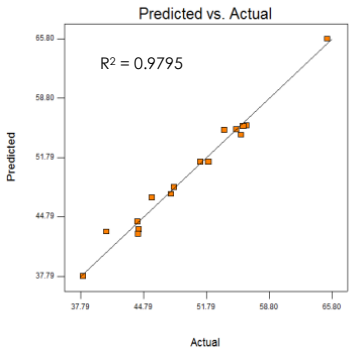
Graphical abstract



Abstract

The study to find cocoa butter equivalent (CBE) as an alternative to cocoa butter (CB) from available and low cost commercial oils or fats has been increased recently. Current study investigates the blending of hard palm oil mid-fraction (PMF) with canola oil to produce high nutritional CBE using immobilized lipase from *Rhizomucor miehei*. The experiments were designed using Response Surface Methodology (RSM) to optimize the percentage of saturated-unsaturated-saturated (StUSt) triacylglycerols (TAGs). The experiment was performed at hard PMF concentration of 50 to 90% (w/w), lipozyme load between 5% and 10% (based on the weight of substrate) with a reaction time between 2 to 14 hours. The best reaction conditions to attain this target was 89.35% (w/w) of hard PMF concentration, 2 hours of reaction time, and 5% (based on the weight of substrate) of lipozyme load, resulting CBE which contains 64.44±1.18% of StUSt. The addition of canola oil improved the nutritional value of CBE which was marked by the higher percentage of linoleic acid (omega-6, 4.53±0.06%) and linolenic acid (omega-3, 0.74±0.14%) in CBE than CB (omega-6, 2.68±0.34%). Enzymatic interesterification was not altering fatty acid content in the CBE, especially linoleic acid (omega-6) and linolenic acid (omega-3) which was characterized by no significant difference ($p > 0.05$) between the fatty acid profile of initial mixture (before interesterification) and CBE (after interesterification).

Keywords: Cocoa butter equivalent; hard palm oil mid-fraction; canola oil; enzymatic interesterification

1.0 INTRODUCTION

Due to the unique composition and its specific characteristic, cocoa butter (CB) becomes highly appreciated and is expensive compared with all other natural vegetable fats and oils. Triacylglycerol (TAG) composition of CB mostly dominated (90%) by three symmetric TAGs (StUSt), namely palmitic-oleic-palmitic (POP), palmitic-oleic-stearic (POS) and stearic-oleic-stearic (SOS) [1]. This characteristic affects melting behavior of CB which is hard and brittle at room temperature while melting completely in the human mouth [2,3]. CB is applied around 36% in chocolate so that the demand for CB can be analyzed by looking at worldwide chocolate consumption [3]. Chocolate product consumption reached more than 6.5 million tons in 2012, means the average of CB used in chocolate products around 2.34 million tons [4]. Meanwhile, world cocoa bean production in 2011/2012 was 4 million tons [5]. If the yield of the CB from cocoa bean around 50%, world CB produced only 2.28 million tons [6]. It means

that about 60 thousand tons of CB are needed to fulfil this demand. Therefore, the production of Cocoa Butter Equivalent (CBE) as an alternative can be useful in filling the high demand of CB. CBE is modified to possess triacylglycerol (TAG) composition similar to CB. It usually prepared from lower value fats and oils, so is able to reduce the selling price.

In the present study, CBE was produced by blending hard PMF with canola oil. Hard PMF is suitable for CBE raw material due to it is rich in StUSt TAGs (80.94%), especially TAG POP (68%) [7]. Meanwhile, canola oil contains low StUSt TAGs (1.56%), but rich in triunsaturated (UUU) TAGs (71.92%), such as TAG oleic-oleic-oleic (OOO) (30%). The present of oleic acid at sn-2 position make canola oil become potential raw material for CBE production. It needs to maintain sharp melting point characteristic of CBE obtained [8].

Canola oil contains a balanced ratio (2:1) of linoleic acid (omega-6) and linolenic acid (omega-3), thus it has a unique health benefit than many other vegetable oils [9]. This benefit could be used in enhancing the nutritional value of the product, since there has been an increasing of healthy lifestyle awareness recently [10]. Hence, the addition of canola oil in the formulation could be used to enhance the nutritional value of CBE.

To keep the nutrition of the CBE remain unchanged in the final product, a proper fat or oil modifying technique is needed. Enzymatic interesterification is one of the fat or oil modification techniques which is worked by modifying acyl-arrangement on the TAG backbone, but is not altering the fatty acid composition of fat or oil [10]. Mild reaction conditions and easy process control make enzymatic interesterification preferable in producing enriched lipids [2,11]. Moreover, it offers more raw materials that can be used, especially in CBE production [2,12-15].

CBE production by enzymatic interesterification is affected by many factors such as raw material composition, lipozyme load, and reaction time. Thus, a statistical tool is needed to optimize the process condition. Response surface methodology (RSM) was used to optimize the enzymatic interesterification conditions, namely hard PMF concentration (%w/w), reaction time (h), and lipozyme load (% based on the substrate). The main objective of this study is to find out the condition to optimize the percentage of StUSt TAGs in producing CBE as well as to enhance the nutritional value of CBE by adding canola oil.

2.0 METHODOLOGY

2.1 Materials

Hard PMF (IV 35.3) was obtained from Sime Darby Kempas Sdn. Bhd. (Johor Bahru, Malaysia). Canola oil was purchased from Lam Soon Edible Oils Sdn. Bhd. (Selangor, Malaysia). Lipozyme, a sn-1,3 specific immobilized lipase from Rhizomucor miehei (RML, EC.3.1.1.3) was purchased from Sigma-Aldrich (German). Stearic acid was provided by Fluka Chemie GmbH (Switzerland).

2.2 Enzymatic Interesterification

The lipase catalyzed interesterification was conducted based on the method by Soekopitojo [13] and Naessens [14] with modification. Various concentrations of hard PMF (%w/w) were blended with canola oil. The total reaction mixture was 10 grams. The mixture was blended with stearic acid at a ratio of 5:4 (w/w). Various dosages of lipozyme were added to the mixture. The reaction was carried out at various times with temperature of 68±2˚C and mechanical agitation of 200 rpm (Pol-Eko aparatura, EU). After selected range times (2, 8, and 14 hours), the stirrer was turned off for 1 minute in order to let the enzyme particles sediment. Samples were taken from the top of the oil and stored in the freezer at -18°C. The samples produced were analysed for its TAG composition.

2.3 Analysis of Triacylglycerol (TAG) Composition

Triacylglycerol (TAG) was determined by GC (Agilent 6890 N, Minnesota, USA) in accordance to Nielsen [16]. The GC was equipped with flame ionization detector and column size was 30 m x 0.25 mm x 1.0 µm. Inlet temperature was programmed at 336˚C with detector flow 2 ml/min and carrier pressure 23.8 psig. For the injector temperature, it was set to be at 360˚C. Hydrogen was used as the carrier gas with nitrogen and compress air as make up gas.

2.4 Experimental Design, Statistical Analysis, and Model Verification

The experiment was designed by using software called Design Expert statistical software (Version 6.0.4, State-Ease Inc., Minneapolis, USA). Central composite design (CCD) was set up to optimize the process variables and to investigate the interactions between parameter. The coded and uncoded levels of the independent variables used in the RSM design are listed in Table 1 below. Three factors design with three levels was used in this study, resulting 16 experimental runs, with one replicate of factorial point, one star point and two replicates of centre points. StUSt was chosen as the response to optimize the reaction.

Table 1 Range and parameters of experimental design

Symbol	Independent variables	Coded levels		
Factor Levels		-1	0	1
A	Hard PMF concentration (%w/w)	10	50	90
B	Reaction time (h)	2	8	14
C	Lipozyme load (%)	5	7.5	10

The second order polynomial regression model was used to express Y as a function of the independent parameters as shown in Equation 1

$$Yi = \beta o + \sum_{i}^{k} \beta iXi + \sum_{i=1}^{k} \beta iiXi^2 + \sum \beta ijXiXj \qquad (1)$$

Where Y_i is StUSt, β_o is constant, β_i, β_{ii} and β_{ij} are the linear, quadratic, and interactive coefficient, respectively. X_i and X_j are the independent parameter levels, an k is the number of independent variables.

The model was evaluated by performing analysis of variance (ANOVA) included in Design Expert 6.0.4 software to test the significance of the model. The effects of the parameters on the StUSt were studied, and the conditions were established which can produce a CBE with optimum StUSt.

The model verification was conducted to confirm the predicted results of the models. The method for model verification was similar to Section 2.2, but hard PMF concentration, lipozyme load, and reaction time used were the best conditions obtained from StUSt models. Then, the observed value of StUSt was compared to the predicted value. T-test was performed to find out the similarity or the difference of the observed value and predicted value.

2.5 Profiling of Fatty Acid

CBE obtains from the best reaction condition in StUSt model was analyzed its fatty acid profile to confirm that enzymatic interesterification was not altering the fatty acid composition of CBE, especially linoleic acid (omega-6) and linolenic acid (omega-3) content. Analysis of fatty acid content was conducted by using GC (Agilent 6890, Minnesota, USA) in accordance to Whitaker [17]. The column used was HP-88 60m x 0.25 mm x 0.20 µm. The detector temperature was programmed at 250˚C with detector flow 40 ml/minutes and carrier pressure 30 psig. For the injector temperature, it was set to be at 110˚C. Hydrogen was used as the carrier gas with nitrogen and compress air as make up gas.

The fatty acid profile of initial mixture (before interesterification) was compared to fatty acid profile of CBE (after interesterification). The difference of fatty acid between initial mixture and CBE) was determined by using statistic (t-test).

3.0 RESULTS AND DISCUSSION

To determine the optimum conditions of CBE production and to find the interaction between the parameters, the RSM was used. Three parameters including hard PMF concentration (%w/w), reaction time (h), and lipozyme load (%, based on the weight of substrate) were selected to optimize the StUSt, which is the sum of three important TAGs in CBE (POP, POS, SOS). Table 2 gives the factor levels for the three reaction parameters and the corresponding response. The result was fitted by multiple regressions. The fit of the model was evaluated by the coefficients of determination (R^2) and analysis of variance (ANOVA).

Table 2 The level of the factors and percentage of StUSt TAGs from the experiments

Hard PMF concentration (w/w%)	Reaction Time (h)	Lipozyme Load (%)	StUSt (%)	
			Observed	Predicted
90.00	2.00	10.00	55.67	54.46
50.00	14.00	5.00	44.24	43.33
70.00	14.00	7.50	47.83	47.49
70.00	2.00	7.50	55.14	55.09
70.00	8.00	5.00	55.91	55.51
50.00	8.00	7.50	40.62	43.05
50.00	2.00	10.00	44.20	42.78
70.00	8.00	7.50	52.03	51.29
90.00	8.00	7.50	53.82	55.03
90.00	14.00	5.00	56.30	55.60
90.00	14.00	10.00	44.10	44.26
70.00	8.00	7.50	51.11	51.29
50.00	2.00	5.00	48.18	48.32
50.00	14.00	10.00	38.02	37.79
90.00	2.00	5.00	65.27	65.80
70.00	8.00	10.00	45.71	47.07

3.1 Model Fitting

Based on three statistical criteria, including the sequential model sum of squares, lack of fit tests, and model R^2, a quadratic model was selected. ANOVA result of StUSt is shown in Table 3. The values of "Prob > F" less than 0.0500 indicates that StUSt models was significant. The model did not show a significant lack of fit. It indicates that the model was reliable to predict the response. The quadratic model of StUSt showed an R^2 of 0.9795, indicates a good agreement between experimental and predicted values (Figure 1). It means that the model was acceptable and can be used to navigate the design space.

Table 3 ANOVA result for quadratic model

Source	Degree of freedom (DF)	Sum of squares	Mean square	F-value	P-value
Quadratic	6	730.37	121.73	71.71	< 0.0001 S
Residual	9	15.28	1.70	-	-
Lack of Fit	8	14.85	1.86	4.39	0.3542 NS
Pure Error	1	0.42	0.42	-	-
Total	15	745.65	-	-	-

NS : not significant at p>0.05
S : significant at p<0.05

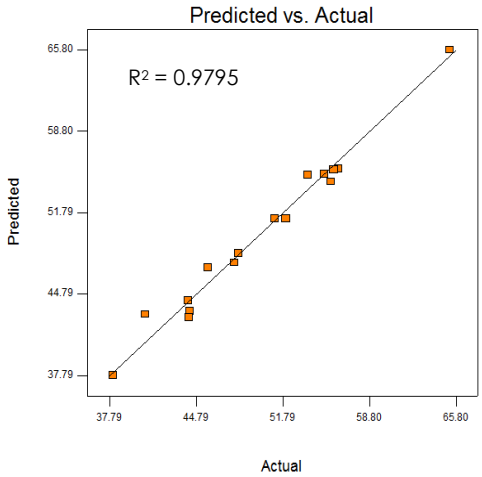


Figure 1 Graphs of predicted versus actual value of StUSt

3.2 Main Effects and Interaction between Parameters

Multiple regression coefficients of each response were summarized in Table 4. It was obtained by using a least squares technique to predict a second-order polynomial model for the CBE production. Table 4 shows that the StUSt TAGs was positively affected by hard PMF concentration, means the percentage of StUSt increased by increasing of hard PMF concentration. Reaction time and lipozyme load had negative effects on StUSt TAGs, indicates that a response was decreased by the increasing of reaction time and lipozyme load. Similarly, the quadratic terms of A (hard PMF concentration), the interaction of hard PMF concentration and reaction time and the interaction of hard PMF and lipozyme load showed negative effects on the response of StUSt. The hard PMF concentration had the greatest effect on StUSt TAG.

Table 4 Regression coefficients and p-values after backward elimination

Factor	StUSt	
	Regression coefficient	P-value
Intercept	51.29	-
A : Hard PMF	5.99	< 0.0001
B : Reaction time	-3.80	< 0.0001
C : Lipozyme load	-4.22	< 0.0001
A^2	-2.25	0.0087
AB	-1.30	0.0198
AC	-1.45	0.0118

The developed regression model for the relationship between percentage of StUSt (Y) and the coded values of independent variables of hard PMF concentration (A), reaction time (B) and lipozyme load (C) and their interaction is shown in the following equation:

$$Y = 51.29 + 5.99A - 3.80B - 4.22C - 2.25A^2 - 1.30AB - 1.45AC \quad (2)$$

The major influence of each parameter in response (StUSt) was shown in the perturbation plot (Figure 2). It was noticed that the effect of hard PMF concentration (A) had positive relation with the StUSt response, while reaction time (B) and lipozyme load had negative relation to StUSt response. The effect of the reaction time (B) and lipozyme load (C) were very similar.

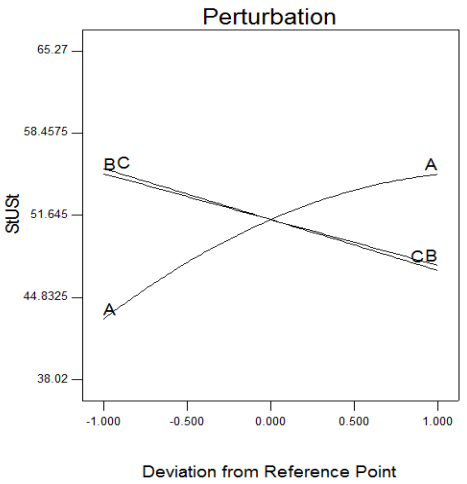


Figure 2 Perturbation plot of StUSt with A: hard PMF ratio (70%); B: reaction time (8 h); and C: lipozyme load (7.5%)

One of the advantages of using RSM is it could detect the interaction of reaction parameters with each other. Figure 3a and 3b visualized the contour plots of these significant interaction terms. Based on Figure 3a, at a shorter reaction time together with higher hard PMF concentration, the percentage of StUSt is high. A similar trend was found in Figure 3b. Higher lipozyme load together with a concentration of hard PMF increased the percentage of StUSt. Consequently, a percentage of 56.86% (w/w) given by this condition became the best result. Although this percentage is acceptable, an optimization of the reaction is needed to optimize the percentage of StUSt.

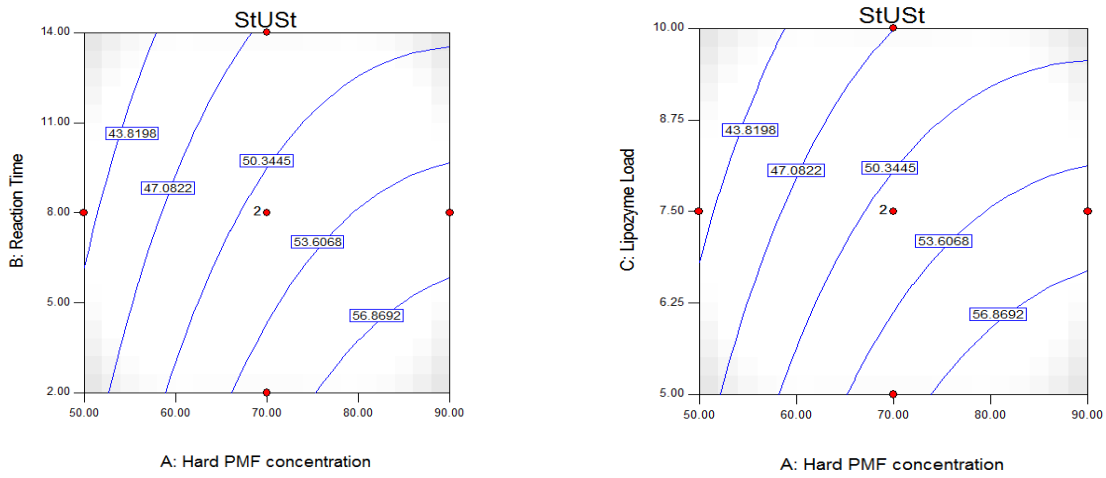


Figure 3 Contour plots of interaction between (a) hard PMF concentration and reaction time and (b) between hard PMF concentration and lipozyme load on % StUSt

3.3 Optimization

Optimum reaction parameters were generated by using Design Expert software. The 3D response surface for StUSt is shown in Figure 4. Each surface plot represents a combination of two test factors, while the third factor was fixed at the constant level. Figure 4a and 4c show the effect of hard PMF concentration on the percentage of StUSt TAGs. The percentage of StUSt gradually increased when hard PMF concentration increased. This is most likely due to hard PMF mainly contains StUSt TAGs (80.94%), especially POP (68%). More concentration of hard PMF would lead to a higher percentage of StUSt TAGs.

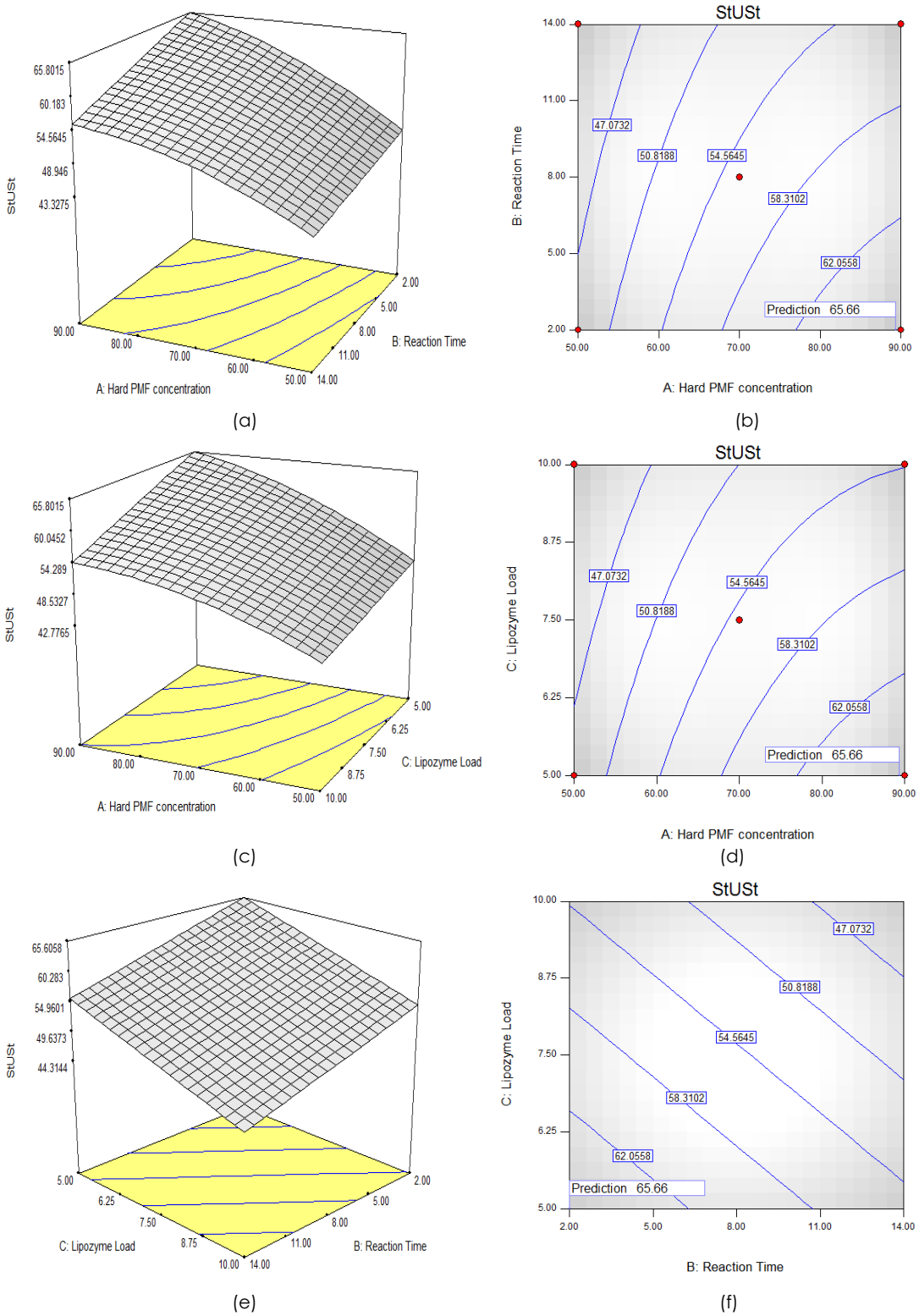


Figure 4 (a) 3D response surface and (b) contour plots showing the effects of hard PMF concentration and reaction time on %StUSt, (c) 3D response surface and (d) contour plots showing the effects of lipozyme load and hard PMF concentration on %StUSt, (e) 3D response surface and (f) contour plots showing the effects of reaction time and lipozyme load on %StUSt

In contrast to the hard PMF concentration, there was a linear decreased of StUSt when reaction time increased (Figure 4a and 4e). The decreasing of StUSt TAGs was likely due to the acyl exchange between StUSt TAGs and others TAGs, such as StStSt (trisaturated) TAGs, StStU (disaturated-unsaturated) TAGs, or UUU (triunsaturated) TAGs, during interesterification. Consequently, desired StUSt TAGs (POP, POS, and SOS) of interesterified blends decreased. Soekopitojo *et al.* [18] came to the same conclusion. Acyl exchange which was occurring during interesterification reaction induced the increasing, decreasing, even synthesizing of several new TAGs.

Effect of lipozyme load on the percentage of StUSt TAGs is shown in Figure 4c and 4e. As presented in Figure 4c and 4e, the percentage of StUSt decreased when lipozyme load increased. The addition of lipozyme load above 5% does not increase the amount of StUSt. Higher concentration of lipozyme load accelerated the reaction rates and improves the acyl exchange activity [2]. This condition would lead to the StUSt reduction.

Figure 4b, 4d and 4f shows the contour plots of the optimal conditions for CBE production from formulated hard PMF and canola oil. The optimal conditions were 89.35% of hard PMF concentration, 2 hours of reaction time, and 5% (based on the weight of substrate) of lipozyme load. The predicted value of StUSt TAGs was 65.66%.

3.4 Model Verification

The experiment was done using optimum conditions (given by RSM) to confirm the predicted results of this model. The observed percentage of StUSt obtained from these conditions was 64.44±1.18%. This value was nearly similar to the predicted value (65.66%) after optimization by RSM. Based on statistics, the response did not show significant differences ($p > 0.05$) between the observed value and predicted value. In conclusion, the predicted results can be accepted.

3.5 Fatty Acid Profile

Table 5 showed that there was no significant difference ($p > 0.05$) between fatty acid in the initial mixture (before interesterification) and CBE (after interesterification), especially the value of linoleic acid (omega-6) and linolenic acid (omega-3). Li *et al*. [19] came with the same finding. They used enzymatic interesterification to produce zero trans shortening fats from high oleic sunflower oil and fully hydrogenated soybean oil. There was no significant statistical difference between the fatty acid composition of the blends (before interesterification) and interesterified products. It indicates that enzymatic interesterification was not altering the fatty acid composition during the reaction.

CB contains considerable amounts of linoleic acid (omega-6) [1]. Current CBE contains higher and significantly different ($p < 0.05$) percentage of linoleic acid (omega-6) and linolenic acid (omega-3) than CB. It indicates that the additions of canola oil succeed to improve the nutritional value of CBEs and enzymatic interesterification was an ideal method for producing nutritional fats. Willis and Marangoni [11] came with the same conclusion. Enzymatic interesterification is an ideal method to produce structured lipids in medical foods or to enrich lipids with specific fatty acids to improve the nutritional properties of fats and oils.

Table 5 Fatty acid profiles of initial mixture and CBE

Fatty acid (%w/w)	Initial mixture (before interesterification)	CBE (after interesterification)	CB*
Palmitic acid	47.07±0.52[a]	46.59±0.03[a]	26.23±0.38
Stearic acid	12.76±0.74[a]	10.79±0.04[a]	35.76±0.87
Oleic acid	32.80±1.11[a]	31.41±0.08[a]	33.60±0.76
Linoleic acid (Omega-6)	4.91±0.56[a]	4.53±0.06[a]	2.68±0.34[b]
Linolenic acid (Omega-3)	1.03±0.02[a]	0.74±0.14[a]	-

*Source : Lipp *et al*. [1]

Note : similar code shows that the value is not different ($p > 0.05$)
different code shows that the value is significantly different ($p < 0.05$)

4.0 CONCLUSION

The optimization was carried out using the response surface methodology (RSM). Parameters which optimized the percentage of StUSt was 89.71% (w/w) of hard PMF concentration, 2 hours of reaction time, and 5% (based on the weight of substrate) of lipozyme load. The optimum response (given by RSM) was 64.44±1.18%. The obtained results showed that the second-order polynomial model was sufficient to predict the significance of each coefficient and also to indicate the strength of interaction between each independent variable.

The additions of canola oil succeed to improve the nutritional value of CBE, which was marked by the higher percentage of linoleic (omega-6) and linolenic acid (omega-3) in CBE than CB, and this difference was significant ($p < 0.05$). Moreover, enzymatic interesterification was an ideal method for producing high nutritional CBE.

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