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# Lipase-catalyzed production of solid fat stock from fractionated rice bran oil, palm stearin, and conjugated linoleic acid by response surface methodology

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**Abstract**

Solid fat stock was produced from the fractionated rice bran oil (solid phase, S-RBO) and palm stearin (PS) through lipase-catalyzed reaction, in which conjugated linoleic acid (CLA) was intentionally incorporated. For optimizing the reaction, response surface methodology (RSM) was employed with four reaction variables such as water activity, reaction temperature, reaction time, and mole ratio of S-RBO to PS. The predictive model was adequate due to no significant lack of fit and satisfactory level of coefficient of determination ($R^2 = 0.95$). The melting point of solid fat stock was affected by reaction time and substrate mole ratio, whereas water activity and reaction temperature had no significant effect. Based on ridge analysis, the combination of $A_w$ ($X_1$; 0.32), reaction temperature ($X_2$; 65.3 °C), reaction time ($X_3$; 28.9 h), and substrate mole ratio ($X_4$; 1:1.1) was optimized for producing solid fat stock with target melting point of 43.8 °C. The solid fat stock (SFS) contained 39.9% palmitic, 31.3% oleic, 13.2% linoleic acid, and 10.9% CLA isomers. Solid fat contents were 23.4, 10.9, and 2.5% at 20, 30, and 40 °C, respectively. These results suggested that RSM can be used to optimize the lipase-catalyzed production of a solid fat stock.


*Keywords:* Conjugated linoleic acid; Rice bran oil; Lipase-catalyzed reaction; Solid fat stock; Palm stearin; Response surface methodology; Solid fat content

## 1. Introduction

Hydrogenation is implicated in the formation of *trans* fatty acids (TFA) when liquid oils are converted into semi-solid fat stocks, in which some *cis* double bonds are rearranged to result in configuration changes. Since the chemical process of hydrogenation has the disadvantage of forming *trans* isomers as well as reducing essential fatty acids, lipase-catalyzed reaction a non-hydrogenation alternative process is now being explored in order to develop the solid fat stocks (Ming, Ghazali, & Let, 1999). For producing the solid fat stocks through lipase-catalyzed reaction, fractionated rice bran oil (RBO) and palm oil can be used. RBO is considered as an excellent frying oil with high oxidative stability partly due to the presence of several phenolic compounds while palm oil has relatively high saturation compared to other vegetable oils. In addition, palm oil is widely used in food applications, providing various fractions after winterization process in which high melting triacylglycerols are gradually crystallized out at certain temperatures. The palm stearin (PS) is one of the fractions, which is mainly composed of high melting triacylglycerols after crystallization (Zaliha, Chong, Cheow, Norizzah, & Kellens, 2004).

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Recently, conjugated linoleic acid (CLA) is known to have beneficial health effects including the retardation of atherosclerosis (Lee, Kritchevsky, & Pariza, 1994), growth enhancement (Chin, Storkson, Albright, Cook, & Pariza, 1994) and increase in immune response capability (O'Shea, Bassaganya-Riera, & Mohede, 2004) even though some CLA isomers have *trans* configuration.

The purpose of this study was to develop solid fat stock from the fractionated RBO and PS through lipase-catalyzed reaction, in which conjugated linoleic acid (CLA) was intentionally incorporated due to its beneficial health effects. For the reaction, factors (water activity, reaction temperature, reaction time and mole ratio) were optimized to the melting points (as response) using response surface methodology (RSM) analysis. Then, solid fat stock (SFS) was re-produced under the optimized condition to ascertain whether this RSM model was effective to optimize a combination of the factors for producing SFS with specific melting point through lipase-catalyzed reaction. Obtained SFS which can be used for all purpose shortening was further analyzed to determine the fatty acid composition and solid fat content (SFC).

## 2. Materials and methods

### 2.1. Materials

Refined, bleached and deodorized rice bran oil (RBO) was supplied from Searim Company (Daejeon, Korea). Lipozyme TL IM (175 IUN/g catalytic activity with 0.54 g/ml bulk density, 0.3–1.0 mm particle diameter and 5 w/w% water content) was obtained from Novozymes A/C (Bagsvaerd, Denmark). Lipozyme TL IM is a 1,3-specific lipase from *Thermomyces lanuginosus* which is granulated on the silica. Conjugated linoleic acid (CLA) mixtures were provided by Livemax Co. (Sungnam, Korea). The main CLA isomer was *cis*-9, *trans*-11 CLA (36.5%), *trans*-10, *cis*-12 CLA (54.5%), and other CLA isomers (3.3%). Palm stearin (PS) was a gift from CJ Inc. (Seoul, Korea). Acetone, chloroform, 2-propanol, hexane, and acetic acid were obtained from Fisher Scientific (Norcross, GA, USA). All chemicals were of analytical reagent grade. Pancreatic lipase (Triacylglycerol lipase from porcine pancreas) was obtained from Sigma Chemical Co. (St. Louis, MO, USA).

### 2.2. Water activity

For controlling as well as equilibrating water activity to the desired level prior to reaction, 100 g of lipase was stored in a closed chamber containing three sets of saturated salt (LiCl, $MgCl_2$, and $Mg(NO_3)_2$) solutions with water activity values on 0.16, 0.32, and 0.51 at 24 °C, respectively. AquaLab (Decagon Devices Inc., Pullman, WA, USA) was used for measuring water activity.

### 2.3. Winterization

RBO (300 ml) and acetone (1500 ml) were mixed (1:5, v/v) together in a 3-L conical flask (Yu et al., 2006). The conical flask with RBO and acetone mixture was placed in a freezer (−16 °C for 24 h). After winterization, the liquid phase was separated from the solid phase by decanting, and the acetone used as a solvent was completely evaporated from solid phase using a rotary vacuum evaporator and a stream of nitrogen with moderate heating. Then, fractionated rice barn oil (S-RBO) was obtained and further used as a substrate for lipase-catalyzed reaction.

### 2.4. Experimental design for RSM analysis

A four-factor ($X_1$, $X_2$, $X_3$, and $X_4$) and three-level (−1, 0, and +1) central composite rotational design was considered in this study. To avoid bias, 29 runs were performed in a random order in which 16 factorial points, 8 axial points and 5 center points ($16 + 8 + 5 = 29$) were considered. Table 1 shows the independent variables ($X_i$) with their levels and the actual experiments that were carried out for developing the model.

The second order polynomial equation used for optimization the reaction conditions was

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_{ii} X_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} \beta_{ij} X_i X_j$$

where $Y$ was response variable (melting point), and $\beta_0$, $\beta_i$, $\beta_{ii}$, and $\beta_{ij}$ were the regression coefficients for interception, linear, quadratic, and cross product terms, respectively. All data were analyzed by response surface regression (RSREG) procedure of the Statistical Analysis System (SAS, 2000) and fitted to the second order polynomial equation after logarithmic transformation (Thompson, 1982). The ridge analysis of RSREG of SAS was used to determine the estimated ridge of maximum or minimum response when the results expressed a saddle point in response surfaces. Response contour plot and predicted plot were generated using Modde version 5.0 software (Umetrics, Umeå, Sweden).

### 2.5. Lipase-catalyzed reaction

The S-RBO and PS were pre-mixed together as a blend in a screw-capped test tube in proportions of 1:1, 1:2, and 1:3 mole ratios, respectively. Then, CLA was added into each reaction mixture at a mole ratio of 1:1, and this mole ratio was constantly kept because the supplementation of CLA for the purpose of this study was satisfactory at this mole ratio (Cho & Lee, 2003). Therefore, actual weights of S-RBO and PS mixture were 1.2 g with 372 mg of CLA. Lipozyme TL IM (10% of the total weight of the substrate) was also added of which the water activities were

Table 1
Three-level and four-factor central composite rotational design arrangements and responses[a]

| Experiment number | Factors | | | | Response ($Y$, °C) | CLA incorporation |
|---|---|---|---|---|---|---|
| | $X_1$ | $X_2$ | $X_3$ | $X_4$ | | |
| 1 | 0.16(−1) | 55(−1) | 12(−1) | 1:1(−1) | 46.0 | 5.8 ± 0.2 |
| 2 | 0.51(+1) | 55(−1) | 12(−1) | 1:1(−1) | 46.5 | 4.6 ± 0.5 |
| 3 | 0.16(−1) | 55(−1) | 36(+1) | 1:1(−1) | 43.0 | 8.1 ± 0.3 |
| 4 | 0.51(+1) | 55(−1) | 36(+1) | 1:1(−1) | 44.0 | 6.4 ± 0.9 |
| 5 | 0.16(−1) | 75(+1) | 12(−1) | 1:1(−1) | 46.5 | 7.6 ± 0.1 |
| 6 | 0.51(+1) | 75(+1) | 12(−1) | 1:1(−1) | 45.5 | 5.4 ± 0.3 |
| 7 | 0.16(−1) | 75(+1) | 36(+1) | 1:1(−1) | 42.5 | 9.8 ± 0.1 |
| 8 | 0.51(+1) | 75(+1) | 36(+1) | 1:1(−1) | 44.0 | 8.4 ± 0.5 |
| 9 | 0.16(−1) | 55(−1) | 12(−1) | 1:3(+1) | 49.5 | 4.0 ± 0.5 |
| 10 | 0.51(+1) | 55(−1) | 12(−1) | 1:3(+1) | 49.0 | 5.1 ± 0.9 |
| 11 | 0.16(−1) | 55(−1) | 36(+1) | 1:3(+1) | 47.5 | 9.2 ± 0.3 |
| 12 | 0.51(+1) | 55(−1) | 36(+1) | 1:3(+1) | 47.5 | 7.0 ± 0.5 |
| 13 | 0.16(−1) | 75(+1) | 12(−1) | 1:3(+1) | 49.0 | 6.5 ± 0.4 |
| 14 | 0.51(+1) | 75(+1) | 12(−1) | 1:3(+1) | 49.0 | 4.1 ± 0.6 |
| 15 | 0.16(−1) | 75(+1) | 36(+1) | 1:3(+1) | 46.5 | 8.5 ± 0.6 |
| 16 | 0.51(+1) | 75(+1) | 36(+1) | 1:3(+1) | 47.5 | 6.4 ± 0.5 |
| 17 | 0.16(−1) | 65(0) | 24(0) | 1:2(0) | 46.5 | 8.2 ± 0.3 |
| 18 | 0.51(+1) | 65(0) | 24(0) | 1:2(0) | 45.5 | 5.6 ± 0.5 |
| 19 | 0.32(0) | 65(0) | 12(−1) | 1:2(0) | 48.5 | 4.1 ± 0.2 |
| 20 | 0.32(0) | 65(0) | 36(+1) | 1:2(0) | 44.5 | 8.5 ± 0.4 |
| 21 | 0.32(0) | 55(−1) | 24(0) | 1:2(0) | 47.5 | 8.8 ± 0.2 |
| 22 | 0.32(0) | 75(+1) | 24(0) | 1:2(0) | 47.0 | 5.1 ± 0.2 |
| 23 | 0.32(0) | 65(0) | 24(0) | 1:1(−1) | 43.5 | 8.0 ± 0.4 |
| 24 | 0.32(0) | 65(0) | 24(0) | 1:3(+1) | 48.0 | 8.4 ± 0.5 |
| 25 | 0.32(0) | 65(0) | 24(0) | 1:2(0) | 46.5 | 7.9 ± 0.1 |
| 26 | 0.32(0) | 65(0) | 24(0) | 1:2(0) | 46.5 | 7.5 ± 0.9 |
| 27 | 0.32(0) | 65(0) | 24(0) | 1:2(0) | 47.5 | 9.7 ± 0.1 |
| 28 | 0.32(0) | 65(0) | 24(0) | 1:2(0) | 46.5 | 7.9 ± 0.1 |
| 29 | 0.32(0) | 65(0) | 24(0) | 1:2(0) | 46.5 | 8.2 ± 0.7 |

[a] $X_1$ = water activity; $X_2$ = reaction temperature (°C); $X_3$ = reaction time (h); $X_4$ = substrate mole ratio (S-RBO to PS); $Y$ = melting point of product.

previously controlled. Immediately, the mixtures were incubated in an orbital-shaking water bath following the reaction conditions in Table 1, respectively.

After each reaction, lipases were removed from the reactant by passing through a 5 ml disposable syringe (Norm-Ject Zentrisch, Daejon, Korea) with PTFE filter (0.5 μm). One gram of reactant was weighed and dissolved in 5 ml of hexane. For removing free fatty acids in each reactant, 2 ml ethanol and 3–4 drops of phenolphthalein solution were added, and then the mixture was titrated with a 0.5 N KOH solution in 20% ethanol until pink colour appeared. Moderate heating was provided to prevent solidification of reactant during the de-acidification. The upper phase was passed through an anhydrous sodium sulphate column and solvent removed with nitrogen in the heating module, and then the lipase-catalyzed solid fat stock (SFS) was obtained.

### 2.6. Melting point

The sample after complete melting was introduced into an open glass capillary tube (1 cm from the bottom), and solidified (−24 °C) in a freezer for 16 h. Then, the tubes with solidified samples were gradually heated in a water bath at a rate of 1 °C/min. The temperature at which the tube was completely clear throughout was considered as the melting point (AOCS, 1990).

### 2.7. Fatty acid composition

The sample was methylated in a screw-capped tube with 3 ml of 6% $H_2SO_4$ in methanol. Heptadecanoic acid (C17:0; 50 μL of 1 mg/ml in hexane) as an internal standard was also added. The tubes were placed in an oven for 1 h at 70 °C, and then the tubes were immediately placed in ice. Hexane (2 ml) was added and vortexed for 30 s. Thereafter, the upper layer was passed through an anhydrous sodium sulphate column. Gas chromatography (GC; Hewlett-Packard, HP 6890 Series, Avondale, PA, USA), accompanied with autoinjection and flame-ionization detection was used for analyzing fatty acid composition. A fused-silica capillary column (SP-wax, 60 m × 0.25 mm i.d., Supelco, Bellefonte, PA, USA) was used for separation. The column was held at 100 °C for 5 min and increased to 220 °C at a rate of 4 °C/min, holding for 30 min at the final temperature. The carrier gas was nitrogen, and the total gas flow rate in inlet was 52 ml/min (constant flow rate) with split mode (50:1). The temperatures of injector and detector were 250 and 260 °C, respectively.

### 2.8. Pancreatic lipase hydrolysis for positional fatty acids composition

Each S-RBO and SFS (7 mg) was taken in a test tube. Seven millilitres of Tris–HCL buffer (pH 7.6), 1.75 ml of 0.05% bile salt in distilled water (w/v), 0.7 ml of 2.2% $CaCl_2$ in distilled water (w/v) and 7 mg of pancreatic lipase were mixed for hydrolysis and vortexed for 30 s. Other steps were followed as described by Lee and Akoh (1996). The hydrolytic products were separated on a silica gel 60 $F_{254}$ plate with a developing solvent of hexane/diethyl ether/acetic acid (50/50/1, v/v/v). The band of monoacylglycerol (sn-2) was scrapped off for methylation and analyzed by GC. After that, the percent fatty acid at sn-1,3 position was calculated by the formula: Sn-1,3 (%) = $(3T - \text{sn-2})/2$, where $T$ is the total fatty acid contents found in S-RBO and solid fat stock (SFS).

### 2.9. α-Tocopherol analysis

Quantitative analysis of α-tocopherol in PS, S-RBO and SFS was performed by HPLC (AOCS, 1990). The HPLC system consisted of a Yonglin SP930D dual pump (Yonglin, Anayang, Korea) and Yonglin UV830 detector set a 295 nm. The column was Chromsep Cartridge, LiChrosorb Diol (5 μm, 3 × 100 mm, Chromapack, Rartian, NJ, USA). The mobile phase was a mixture of hexane fortified with 0.1% acetic acid (1000:1, v/v) at a flow rate of 0.5 ml/min. The area of each peak was integrated by Autochro-2000 software (Yonglin, Anayang, Korea).

### 2.10. Rancimat test for oxidative stability

The induction period, measuring the increase in the volatile by-products released from the oxidizing oil of RBO, S-RBO, and SFS was determined by the rancimat method (Rancimat 743, Metrohm, Switzerland) at 100 °C with an air flow rate of 20 l/h. The conductivity was measured for estimating the concentration of the degradation products and longer induction periods showed higher oxidative stability. The protection factor (PF), which is calculated by dividing the induction period either of the S-RBO or SFS by that of RBO (induction time, 5.16 h) was obtained as the relative activity (Schwarz & Ernst, 1996).

### 2.11. Differential scanning calorimetry (DSC)

The DSC 2010 Differential Scanning Calorimeter (TA Instruments Inc., New Castle, DE, USA) was used for obtaining the thermograms of S-RBO, PS, and the SFS. The base line was obtained with an empty aluminum pan. Purge gas was nitrogen and each sample was weighed 9 mg, accurately (0.1 mg). The instrument temperature was increased to 80 °C and held there for 10 min. Thereafter, the temperature was decreased at 10 °C/min to −60 °C. After holding for 10 min at −60 °C, the melting curve was obtained by heating to 80 °C at 5 °C/min. The solid fat content (SFC, %) was obtained from the obtained thermograms by Universal Analysis 2000 (TA Instruments Inc., New Castle, DE, USA). Each DSC thermogram was divided at different temperature (−10, 0, 5, 10, 15, 20, 25, 30, 35 and 40 °C) and the total crystallization energy (J/g) was calculated into percentage at each temperature for SFC (%).

## 3. Results and discussion

### 3.1. Response surface methodology (RSM)

RSM is an experimental strategy for developing a new process and optimizing its performance. For example, the optimum processing factors such as level of primary ingredients and condition of procedure (temperature and time) could be determined by using a minimum number of experiments by a suitable experimental design.

Using RSM, the effects of four variables namely water activity ($A_w$: 0.16, 0.32, and 0.51), reaction temperature (55, 65, and 75 °C), reaction time (12, 24, and 36 h) and mole ratio of S-RBO to PS (1:1, 1:2, and 1:3) on melting point of SFS were considered in this study. The natural variables are transformed into coded variables (Table 1) that have been defined as dimensionless with a mean zero and the same standard deviation (Myers & Montgomery, 2002). A four-factor and three-level central composite rotational design arrangement and responses are summarized in Table 1. The desirable melting point ($Y$) ranged from 40 to 50 °C in the present study. In other words, SFS having this range of melting point could be used as the building block for different general and specialized fat products. The analysis of variance (ANOVA) of response surface for melting point showed no significant lack of fit ($P = 0.2789$, data not shown), and satisfactory levels of coefficient of determination ($R^2$, 0.95) and coefficient of variation (CV, 1.23, data not shown). These values indicated that this experimental model was adequate and reproducible. In addition, linear terms showed $R^2 = 0.899$ and $F = 64.86$ ($P < 0.0001$) while quadratic ($R^2 = 0.028$ and $F = 2.03$, $P < 0.1451$) and cross product ($R^2 = 0.024$ and $F = 1.17$, $P < 0.3745$) were not considered as significant terms (data not shown).

Regression coefficients were determined by employing least squares technique to predict quadratic polynomial models for melting point. The values of regression coefficient were $\beta_0 = 80.023481$ ($P < 0.0001$), $\beta_1 = 6.040186$ ($P < 0.5468$), $\beta_2 = -1.121032$ ($P < 0.0309$), $\beta_3 = -0.20488$ ($P < 0.1837$), $\beta_4 = 4.316773$ ($P < 0.0277$), $\beta_{11} = -13.093872$ ($P < 0.2775$), $\beta_{22} = 0.008544$ ($P < 0.0314$), $\beta_{33} = 0.000725$ ($P < 0.7745$), $\beta_{44} = -0.645631$ ($P < 0.0922$), $\beta_{12} = 0.018271$ ($P < 0.8255$), $\beta_{13} = 0.137029$ ($P < 0.0627$), $\beta_{14} = -0.563705$ ($P < 0.4995$), $\beta_{23} = -0.00026$ ($P < 0.8309$), $\beta_{24} = -0.003125$ ($P < 0.8309$), and $\beta_{34} = 0.018229$ ($P < 0.15$), respectively. The fitted predictive response surface model equation containing values of the coefficients of independent variables ($X_1$, water activ-

ity; $X_2$, reaction temperature; $X_3$, reaction time; and $X_4$, mole ratio) determined for the quadratic polynomial model are as follows:

$$\begin{aligned} Y = {} & 80.023481 + 6.040186X_1 - 1.121032X_2 \\ & - 0.20488X_3 + 4.31677X_4 - 13.093872X_1^2 \\ & + 0.008544X_2^2 + 0.000725X_3^2 - 0.645631X_4^2 \\ & + 0.018271X_1X_2 + 0.137029X_1X_3 \\ & - 0.563705X_1X_4 - 0.00026X_2X_3 \\ & - 0.003125X_2X_4 + 0.018229X_3X_4 \end{aligned} \quad (1)$$

In this model, the results clearly indicated that $X_3$ and $X_4$ were the primary factors for affecting the response ($P < 0.05$) and no significant effect was observed for $X_1$ and $X_2$ on the response ($P > 0.05$) (data not shown). Fig. 1 shows that the reaction time was negatively related with the melting point of SFS while the substrate mole ratio of S-RBO to PS was positively related. Otherwise, water activity and reaction temperature did not have an appreciable influence on the melting point. For example, the melting point decreased from 47.4 to 46.5 °C as the reaction temperature increased from 55 to 65 °C, however, after then the melting point increased up to 47.1 °C (Fig. 1).

The observed vs. predicted plot and response contour plot for melting point were generated using Modde software. The observed melting points were well correlated with the predicted values showing linear distribution ($R^2 = 0.95$) (Fig. 2). The response contour plots were presented as a function of substrate mole ratio (1:1–1:3) and reaction time (12–36 h) while keeping the reaction temperature

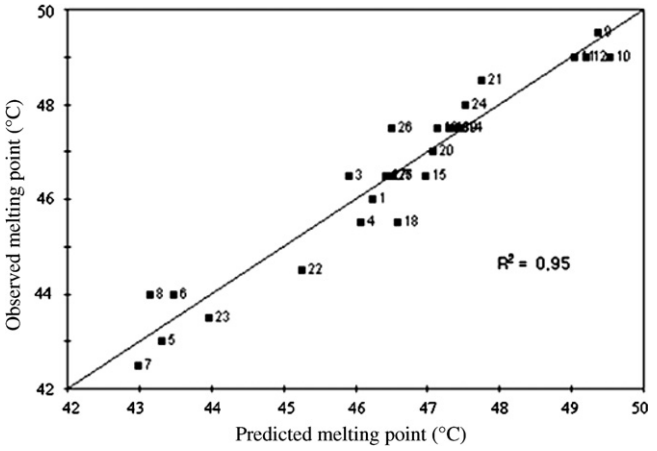


Fig. 2. Plot for relationship between observed and predicted melting point of lipase-catalyzed solid fat stock (SFS). The numbers indicate experiment numbers presented in Table 2.

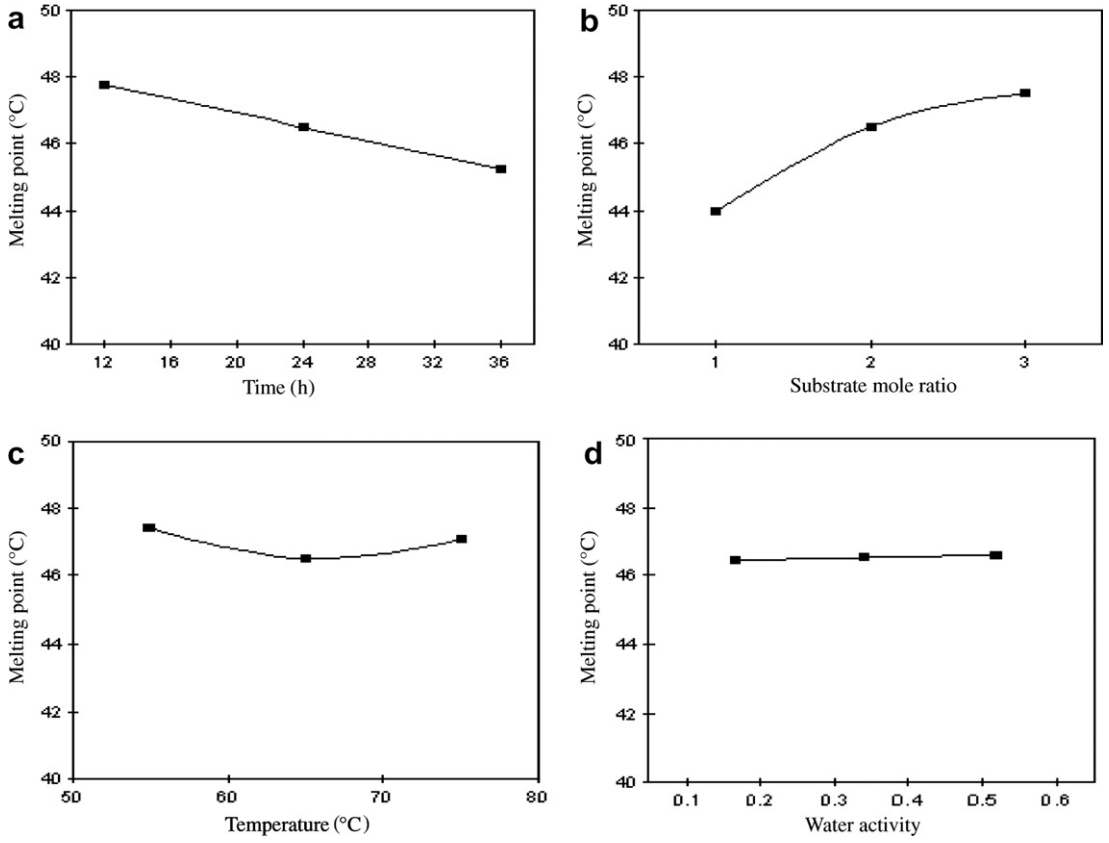


Fig. 1. Prediction plot for melting point of lipase-catalyzed solid fat stock (SFS) by effects of reaction time (a), substrate mole ratio (b), reaction temperature (c), and water activity (d).

constant at 55, 65, and 75 °C, respectively (Fig. 3). The contour plots for melting point shows a steady increase with increasing mole ratio while the melting point decreased with increasing reaction time to a certain level (approximately 36 h).

Canonical analysis is a type of linear statistical analysis used to locate the stationary point and to decide whether it represents a maximum, minimum or saddle point (Montgomery, 1991). It was performed to test the overall shape of the response surface curves and to characterize the nature of the stationary points. The canonical model of the equation describing the character of the response surface was

$$Y = 46.37 + 0.86W_1^2 + 0.15W_2^2 - 0.42W_3^2 - 0.68W_4^2 \qquad (2)$$

where $W_1$, $W_2$, $W_3$, and $W_4$ are the axes of the response surface. The predicted value of the stationary point was a saddle point because eigenvalues were mixed (positive and negative). Thus, a ridge analysis was performed to determine the estimated ridge of maximum or minimum response in which combinations of water activity, reaction temperature, reaction time, and substrate mole ratio were optimized for SFS with specific melting point.

Generally, all purpose shortening for cookie making has about 44 °C as melting point, which is the widely desirable solid fat stock. From the ridge analysis with minimum responses, production of solid fat stocks with melting point 43.8 ± 0.3 °C would be predicted by the combination of optimized reaction condition with $A_w$ 0.32, 65.3 °C, 28.9 h and 1:1.1 mole ratio (S-RBO to PS). To confirm the predicted optimum condition, the SFS was re-produced under these conditions. The melting point of synthesized fat was 43.2 °C ± 0.5 which indicated that the optimum condition was appropriate. Therefore, the RSM used was effective to optimize a combination of the factors for producing SFS with specific melting point through lipase-catalyzed reaction.

The CLA incorporation was determined for all experimental conditions. The results indicated that an increase in reaction time (from 12 to 36 h) seems to result in an increase in CLA incorporation. With 12-h reaction, 4.0–7.6% CLA was incorporated in SFS while 6.4–9.8% CLA was found with 36-h reaction. However, the reaction temperature and water activity did not much influence the CLA incorporation (Table 1).

### 3.2. Fatty acid compositions

The physicochemical properties of the SFS (from the above predicted optimum condition), namely the fatty acid composition, melting properties, and solid fat content (SFC) were determined. Table 2 presents the fatty acid composition of the reaction substrates (S-RBO, PS, CLA mixture) and lipase-catalyzed SFS product. The S-RBO and PS contained 76.2 and 29.8% total unsaturated fatty acids, respectively while the SFS contained 55.6% including 10.9% CLA. As expected, the major fatty acids in PS were palmitic (C16:0) and oleic acid (C18:1) which composed of 63.6 and 24.5%, respectively. In S-RBO, however, 45.9% C18:1, 29.8% linoleic acid (C18:2), and 21.7% C16:0 were mainly found. Presence of a higher level of unsaturated fatty acids in the sn-2 position is important nutritionally since it is absorbed easily in the body (Quinlan & Moore, 1993). In SFS, the major unsaturated fatty acids at sn-2 position were oleic (38.4%), and linoleic acid (15.6%), resulting in 54.0% total unsaturated fatty acids. CLA content at sn-2 position was below the detection limit.

Of the unsaturated fatty acids, 10.9% CLA isomers were present in the obtained SFS, demonstrating that CLA isomers were successfully incorporated at sn-1,3 positions in SFS through the lipase-catalyzed reaction (Table 2). Such changes of fatty acid composition lead to the different melting properties of SFS compared with S-RBO and PS.

### 3.3. α-Tocopherol content

The results of α-tocopherol content in PS, S-RBO and solid fat stock (SFS) are shown in Table 2. α-Tocopherol is considered as the most important liquid phase natural antioxidant, which prevents lipid peroxidation by scav-

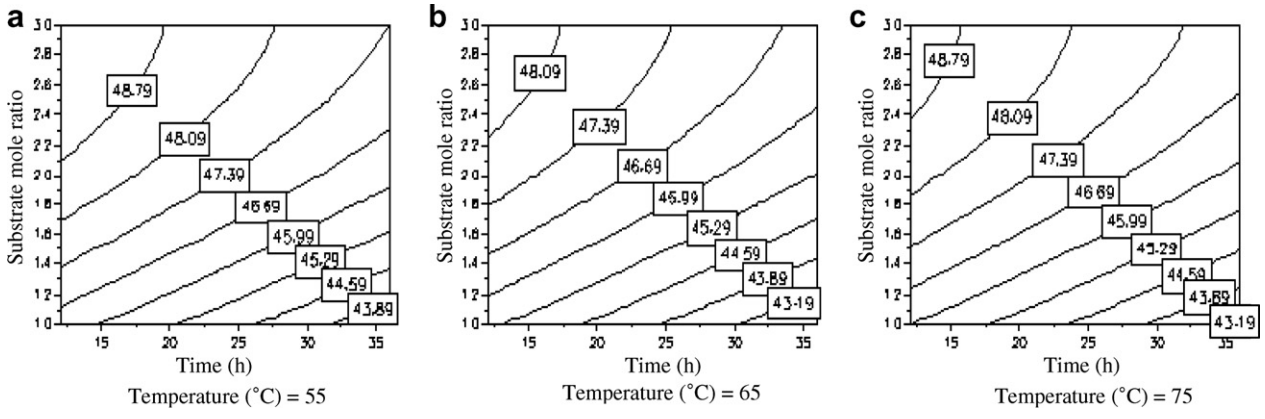


Fig. 3. Contour plots showing the effect of reaction time and substrate mole ratio on melting point of lipase-catalyzed solid fat stock (SFS) at different temperatures of 55 (a), 65 (b), and 75 °C (c).

Table 2
Total and positional distribution of fatty acid content, α-tocopherol content (mg/100 g), and rancimat test results of CLA mixture, palm stearin (PS), solid phase of fractionated rice bran oil (S-RBO) and the lipase-catalyzed solid fat stock (SFS)

| Fatty acids | CLA mixture | PS | S-RBO[a] | | | SFS[b] | | |
|---|---|---|---|---|---|---|---|---|
| | | | Total | Sn-2 | Sn-1,3 | Total | Sn-2 | Sn-1,3 |
| C14:0 | ND[c] | 1.4 ± 0.01 | 0.2 ± 0.01 | ND | 0.3 ± 0.01 | 1.0 ± 0.01 | ND | 1.5 ± 0.01 |
| C16:0 | ND | 63.6 ± 0.45 | 21.7 ± 0.52 | 12.9 ± 0.04 | 26.1 ± 0.76 | 39.9 ± 0.13 | 42.6 ± 0.17 | 38.6 ± 0.11 |
| C18:0 | ND | 4.9 ± 0.02 | 1.9 ± 0.2 | 1.5 ± 0.02 | 2.1 ± 0.29 | 3.0 ± 0.01 | 3.4 ± 0.13 | 2.8 ± 0.05 |
| C18:1 | 3.9 ± 0.01 | 24.5 ± 0.3 | 45.9 ± 0.82 | 47.5 ± 0.41 | 45.1 ± 1.03 | 31.3 ± 0.12 | 38.4 ± 0.14 | 27.8 ± 0.11 |
| C18:2 | 2.0 ± 0.01 | 5.1 ± 0.02 | 29.8 ± 0.3 | 38.1 ± 0.39 | 25.6 ± 0.3 | 13.2 ± 0.02 | 15.6 ± 0.02 | 12.0 ± 0.02 |
| C18:3 | ND | 0.2 ± 0.01 | 0.5 ± 0.01 | ND | 0.8 ± 0.01 | 0.2 ± 0.01 | ND | 0.3 ± 0.01 |
| C20:0 | ND | 0.3 ± 0.01 | ND | ND | ND | 0.5 ± 0.01 | ND | 0.7 ± 0.01 |
| CLA isomers | 94.1 ± 0.37 | ND | ND | ND | ND | 10.9 ± 0.05 | ND | 16.3 ± 0.05 |
| | | | S-RBO | | | SFS | | |
| α-Tocopherol | | 0.5 | 6.2 | | | 0.1 | | |
| Oxidative stability[d] | | –[e] | 1.3 | | | 3.6 | | |

[a] S-RBO; fractionated rice bran oil (solid phase) after winterization at −16 °C for 24 h. RBO (300 ml) and acetone (1500 ml) were mixed (1:5, v/v).
[b] Solid fat stock (SFS) was produced by lipase-catalyzed reaction with $A_w$ 0.32, 65.3 °C (reaction temperature), 28.9 h (reaction time) and 1:1.1 mole ratio S-RBO to PS.
[c] ND, not detected.
[d] Values expressed as protection factor (PF). PF was calculated by dividing the induction period either of the S-RBO or solid fat stocks by that of RBO (induction time, 5.2 h).
[e] Within 20 h for rancimat test, induction time was not observed.

enging radicals in membranes and lipoprotein particles (Esterbauer, Dieber-Rotheneder, Striegl, & Waeg, 1991). The PS, S-RBO, and SFS contained 0.5, 6.2, and 0.1 mg/100 g of α-tocopherol, respectively. The removal of antioxidants could take place during the transesterification reaction or purification step (i.e., de-acidification), which was previously observed in enzymatically transesterified lipid (Senanayake & Shahidi, 2002).

### 3.4. Rancimat test

The oxidative stability is an important parameter in ascertaining the quality of oils and fats, as it gives a good estimation of their susceptibility to oxidative degradation (Aparicio, Roda, Albi, & Gutierrez, 1999). The results of rancimat test are presented in Table 2. A higher protection factor (3.6) for SFS was observed compared to that of S-RBO (1.3) while induction time of PS was not observed within 20 h for rancimat test. Actually, higher protection factor suggests stronger oxidative stability. The differences in protection factor of SFS and S-RBO were mainly due to the different levels of total saturated fatty acid content rather than α-tocopherol content. SFS contained higher levels of total saturated fatty acid content (44.4%) than that of S-RBO (23.8%), resulting in higher protection factor of SFS than that of S-RBO. Induction time for PS could not be obtained in this study because of its high saturated fatty acid content (70.2%), indicating that it was quite stable to oxidation.

### 3.5. Solid fat contents (SFC)

The melting point and SFC are indications of the melting characteristics of a fat. Thus, it is considered as important defining factors for the texture of solid fats. Generally, the SFC of solid fat (e.g., margarine and butter) is responsible for physical characteristics such as appearance, spreadability and oil exudation, or organoleptic properties (Laia, Ghazalia, Cho, & Chong, 2000). DSC melting thermograms of S-RBO, PS, and the solid fat stock are presented in Fig. 4. In PS, a distinct melting peak was observed at 55.4 °C, representing high melting triacylglycerol. In S-RBO, however, two broadened peaks were shown at −16.5 and −5.6 °C. The melting thermogram of SFS showed small sharp melting peaks from −20 to 5 °C and broadened peak between 15 and 40 °C. The altered melting profiles could result from lipase-catalyzed reaction which produced various types of TAG molecules.

At −10 °C, SFC of PS was 99.6% (data not shown), indicating that most phases were solid. When temperature increased to room temperature range (20–25 °C), SFC of

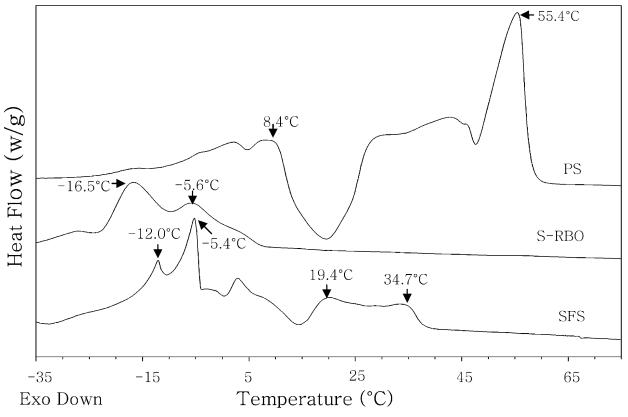


Fig. 4. DSC melting thermograms of palm stearin (PS), solid phase of fractionated rice bran oil (S-RBO), and the lipase-catalyzed solid fat stock (SFS).

PS ranged from 71.7 to 73.6%. Even at 40 °C, SFC was 43.3%. In case of S-RBO, SFC at −10 °C was 44.7% while most phases were liquid at 5 °C (0.2% SFC). The SFC of SFS was 23.4% at 20 °C and 10.9% at 30 °C (data not shown). Generally, the SFC at 20 °C determines the tendency towards oil exudation, and SFC of more than 10% was essential to prevent oiling off (Laia et al., 2000). Therefore, SFC of the obtained SFS suggests that it has suitable physical characteristic as a potential solid fat stock.

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