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Synthesis of esterified solid fat from fractionated rapeseed oil and palm stearin modeling by response surface methodology

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Abstract

The main purpose of the study was to produce esterified solid fat (ESF) from fractionated rapeseed oil and palm stearin through lipase-catalyzed reaction. The response surface methodology (RSM) was applied for optimization of three reaction factors such as substrate mole ratio, enzyme percent, and reaction time. The design was adequate and reproducible due to satisfactory levels of coefficient of determination (R^2 , 0.98) for both cases and coefficient variation (CV, 4.06 for SFC at 10 °C and 7.95 for SFC at 30 °C, respectively). The substrate mole ratio was the significant term for affecting the response of SFC ($P < 0.05$) rather than reaction time and enzyme percent. Based on ridge analysis, the production of ESF with SFC 51.48 ± 0.94 % at 10 °C would be predicted by the combinations of optimized 24.07 h reaction time, 10.66 % enzyme and 1: 1.52 substrate mole ratios. On the other hand, the SFC of 21.44 ± 0.83 at 30 °C would be predicted by the combinations of optimized 24.37 h reaction time, 10.23 % enzyme and 1: 1.5 substrate mole ratios. The ESF contained mainly of palmitic (45.1%), oleic (40.8%), linoleic (5.6%) and stearic (4.5%) acids, respectively. The total sterol and tocopherol contents of ESF were 243.27 mg/100g and 19.26 mg/100 g, respectively. Therefore, these results in this study suggested that RSM can be used to optimize the lipase-catalyzed synthesis of ESF with suitable physical characteristics.

Keywords: Fractionation, Solid fat content, Esterification, Response surface methodology; Lipase-catalyzed reaction

Introduction

During hydrogenation, the liquid oils are converted into semi-solid fat stocks such as shortening and margarine in which some *cis* configuration are rearranged to *trans* configuration. Many scientists have suggested that *trans* fatty acid (TFA) has direct relation which increased the risk of coronary heart disease (Gebauer *et al.*, 2007; Mozaffari *et al.*, 2006; Khatoon & Reddy, 2005). A high TFA food causes adverse change on plasma lipoprotein by decreasing high-density lipoprotein (HDL) good cholesterol and increasing low-density lipoprotein (LDL) bad cholesterol, resulting also in increased the risk of coronary heart disease and atherosclerosis (Parcerisa, *et al.*, 1999; Pretch & Molkentin, 2000). However, TFA intakes should be as low as possible. Since the hydrogenation process has disadvantage of forming *trans* double bonds isomers, lipase-catalyzed reaction is an alternative process to develop the solid fat stocks without TFA (Mayamol *et al.*, 2009). Enzymatic esterification is a reaction which can be catalyzed by enzymes or chemical catalysts and is a good way to produce structured lipids by changing the fatty acid composition to improve the nutritional properties of TAG molecule (Senanayake & Shahidi, 2001). Fractionated rapeseed oil and palm stearin can be used for producing esterified solid fat (ESF) through lipase-catalyzed reaction. Rapeseed oil (RSO) is the most useful of all cooking oils, because it contains a significant amount of omega-3 and omega-6 fatty acids while palm stearin (PS) has relatively high saturation compared to other vegetable oils. PS is a fraction of palm oil which is mainly composed of high melting saturated triacylglycerols (TAG) after crystallization (Zaliha *et al.*, 2004). Among all vegetable oils, PS is a good substitute for animal fats like lard, tallow and butter for the production of shortenings and/or margarines because of their good solid fat content (SFC) and ability to promote β crystals (Khatoon & Reddy, 2005; Norizzah *et al.*, 2004; Reddy & Jayarani, 2001).

Response surface methodology (RSM) is an effective statistical method for optimizing experimental conditions by reducing the number of experimental trails (Myers & Montgomery, 2002). RSM also is a faster and more economical method for gathering optimizing research results than one-variable-at-a-time. RSM has been used to optimize enzymatic esterification reactions in organic solvent (Uosukainen *et al.*, 1999) as well as to optimize biochemical and biotechnological processes to food system (Cacace & Mizza, 2003b; Senanayake & Shahidi, 2002; Telez-Luis *et al.*, 2003).

The main purpose of this study was to produce ESF from fractionated solid part of rapeseed oil (S-RSO) and PS through lipase-catalyzed reaction. The response surface methodology (RSM) is applied for optimization. For the reaction, three factors (substrate mole ratio, enzyme percent, and reaction time) were optimized to the solid fat content (SFC) as response using RSM analysis. The ESF was then scale-up under optimized conditions to compare with the optimum response. The produced ESF was further investigated for determining the fatty acids composition, sterols and tocopherols content.

Materials and Methods

Oil, enzyme and chemicals

The palm stearin (hard) and the rapeseed oil (RSO) were acquired from AAK (AarhusKarlshamn AB, Malmö, Sweden). The lipozyme RM IM (1,3-specific, produced from *Mucor miehei*, with a specific activity of 30 U/g, and containing micro-porous ion-exchange resin), heptadecanoic acid, acetic acid and pancreatic lipase from porcine pancreas (Type II) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). The standard for sterols, 5 α -cholestane, was obtained from Steraloids (Newport, RI, USA), the reference samples of tocopherols were obtained from Merck (Darmstadt, Germany) and the Tri-Sil reagent came from Pierce Chemical Co. (Rockford, IL, USA). The standard mixture of fatty acid methyl esters (FAME), F-07, was acquired from Larodan Fine Chemicals AB (Malmö, Sweden). All the other chemicals and solvents came from VWR (Stockholm, Sweden), unless otherwise stated.

Fractionation of RSO

RSO and acetone were mixed together at a ratio of 1:5 (v/v) in a conical flask. The conical flask was placed in a deep freezer at a temperature of -20°C for fractionation. The liquid part of RSO (L-RSO) was separated from the solid part of RSO (S-RSO) by decanting after 24 h, and the acetone was evaporated completely from both parts by a rotary vacuum evaporator by a stream of nitrogen (Yu *et al.*, 2006). Then the S-RSO was taken for further use in this study.

Experimental design for RSM

In the study, a three-factor (X_1 , X_2 and X_3) and three-level (-1, 0 and +1) faced-centered cube design was considered. Seventeen runs were performed for analysis in which 8 factorial points, 6 axial points and 3 center points (Wanasundara & Shahidi, 1999). Table 1 shows the experimental settings of the reaction factors and responses. The used second-order polynomial equation for optimization of the reaction conditions is as follows:

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

Where, β_0 , β_i , β_{ii} , β_{ij} are the regression coefficients for interception, linear, quadratic and interaction terms, respectively, and X_i , and X_j are the independent variables. All data were analyzed by response surface regression (RSREG) procedure of Statistical Analysis System (SAS, 2000) and verified to the second order polynomial equation after logarithmic transformation. The ridge analysis of RSREG was employed to determine the estimated ridge of maximum or minimum response when the results expressed a saddle point in response surfaces.

Lipase-catalyzed esterification reaction

The S-RSO and the PS were mixed together in proportions of 1:1 (881mg: 835mg), 1:2 (881mg: 1670mg), and 1:3 (881mg: 2505mg), mole ratios, respectively in a test tube and 3 mL hexane was added in all reaction mixtures. Lipozyme RM IM was added according to the experimental design. The mixtures were incubated immediately in a continuous shaking water bath following the experimental reaction conditions in Table 1. In this case, the reaction temperature and continuous shaking were kept constant at 65° C and 110 rpm, respectively. After lipase-catalyzed esterification reaction, lipases were removed from the reactant by passing through a 5 mL disposable syringe (REF 300911, BD, Franklin Lakes, NJ, USA) with Whatman PTFE filter (0.45 μ m). 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 2-3 drops of phenolphthalein solution were

added, and then the mixture was titrated with a 0.5 N KOH solution in 20% ethanol until pink color was appeared. The upper phase was separated and blown up by nitrogen in the heating module (Alim *et al.*, 2008), and then ESF was obtained.

Table 1. Three-factors and three-levels face-centered cube design and their response for RSM

Run no.	Factors			Response (Y, SFC %)	
	X ₁	X ₂	X ₃	10° C	30° C
1	12(-1)	5(-1)	1:1(-1)	46.40±0.88	15.33±0.24
2	12(-1)	5(-1)	1:3(+1)	58.40±0.29	30.94±3.08
3	12(-1)	15(+1)	1:1(-1)	31.68±1.24	8.94±0.14
4	12(-1)	15(+1)	1:3(+1)	55.34±2.02	28.28±3.99
5	36(+1)	5(-1)	1:1(-1)	44.45±1.14	11.88±0.56
6	36(+1)	5(-1)	1:3(+1)	60.07±1.58	32.07±0.52
7	36(+1)	15(+1)	1:1(-1)	30.77±2.54	7.71±1.06
8	36(+1)	15(+1)	1:3(+1)	61.25±1.52	32.38±0.15
9	12(-1)	10(0)	1:2(0)	55.16±0.75	25.97±0.16
10	36(+1)	10(0)	1:2(0)	54.43±2.01	23.33±1.93
11	24(0)	5(-1)	1:2(0)	58.94±0.33	26.71±1.53
12	24(0)	15(+1)	1:2(0)	59.27±1.85	29.46±0.08
13	24(0)	10(0)	1:1(-1)	42.14±3.93	11.40±1.47
14	24(0)	10(0)	1:3(+1)	63.80±0.11	32.83±1.05
15	24(0)	10(0)	1:2(0)	57.54±0.50	28.23±0.00
16	24(0)	10(0)	1:2(0)	57.18±2.04	27.61±1.83
17	24(0)	10(0)	1:2(0)	58.85±0.66	29.95±0.05

X₁ = Reaction time (h); X₂ = Enzyme (%); X₃ = Substrate mole ratio (S-RSO to PS);
Y = solid fat content (SFC, %) at 10 and 30° C

Solid fat content (SFC, %) determination by DSC

The solid fat content (SFC, %) was determined with a Perkin-Elmer Pyris Diamond differential scanning calorimetry (DSC) (Norwalk, CT). Purge gas was liquid nitrogen and each sample was weighed 10±1.0 mg. The base line was obtained with an empty aluminum pan. The DSC 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. 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 (%) (Alim *et al.*, 2008).

Positional fatty acid composition

The S-RSO and ESF (8 mg) were taken in a test tube. Eight mL of Tris-HCl buffer (pH 8.0), 2 mL of 0.05% bile salt in distilled water (w/v), 0.8 mL of 2.2% CaCl₂ in distilled water (w/v) and 20 mg of pancreatic lipase (porcine pancreatic lipase, crude type II) were mixed for hydrolysis and vortexed for 30 sec. Other steps were followed as described by Sahin *et al.* (2005). The mixture was incubated in a water bath at 40°C for 3 min, and 1 mL of diethyl ether was added. The diethyl ether was evaporated under nitrogen gas. The hydrolytic products were separated on a TLC plate (silica gel 60, 20x20 cm, 0.25 mm thickness, Merck, Eurolab AB, Stockholm, Sweden) by developing a chromatogram with the solvent system hexane/diethyl ether/acetic acid (50/50/1, v/v/v). The band of monoacylglycerol was scraped off for methylation and analyzed by GC as described below. After that, the percentage of fatty acid at the sn-1,3 position was calculated by the following formula: Sn-1,3 (%) = (3 T – sn-2)/2, where T is the total fatty acid contents of the S-RSO and the TSF, respectively. All the analyses were conducted in duplicate.

Fatty acid composition

The S-RSO, PS and the optimized ESF were separated by thin-layer chromatography (TLC) plate (silica gel 60, 20x20 cm, 0.25 mm thickness, Merck, Eurolab AB, Stockholm, Sweden) developed with hexane/diethyl ether/ acetic acid (85/15/1, v/v/v). The visualized band corresponding to TAG molecule was scraped off into a screw-capped test tube and dissolved in 0.5 mL hexane as well as 3 mL 0.01 M NaOH in dry methanol was added. Fifty μ L heptadecanoic acid (C17:0, 1 mg/mL in hexane) as an internal standard was also added in the test tube. The test tube was closed with a stopper and vortexed for proper mixing. The sample was incubated in water bath at 50 °C for 30 minutes under continuous shaking. Two milliliters (50% NaHSO₄: 25% NaCl in water) solution were added and cool under running water. Three milliliters water and 1 mL hexane were added and vortexed vigorously (Azardmard-Damirchi & Dutta, 2008). The hexane layer was separated and solvent was evaporated with nitrogen. Gas chromatography (GC, Chrompack CP 9001, Middelburg, The Netherlands), accompanied with auto-injection and flame-ionization detection was used for fatty acid composition analysis. A 50 m X 0.22 mm, 0.25 μ m film thickness fused-silica capillary column BPX70 (SGE, Austin, TX, USA) was used for separation. The injector and detector temperatures were 240 and 280 °C, respectively. Oven conditions were 160 °C increased to 220°C at a rate of 2°C/min and maintained for 5 min. The carrier gas was helium and nitrogen as a make-up gas at a flow rate of 30 mL/min. Fatty acid methyl esters (FAME) were identified by comparison of their retention time with standard FAME. The peak areas were integrated by maestro version 2.4 (Chrompack Middelburg, The Netherlands) and reported as a percentage of the total fatty acids (Appelqvist, 1968). All the analyses were conducted in duplicate.

Analysis of sterols by GC

The S-RSO, PS and the optimized ESF (20 mg) were put into test tubes and 1 mL of 2M KOH in ethanol (95%) was added. The tubes were placed in a boiling water bath for 10 minutes with intermittent shaking and thereafter cooled under running water. Thereafter, 1 mL of water, 2 mL of hexane containing 20 μ g of 5 α -cholestane as an internal standard and 200 μ L of absolute ethanol were added, after which the tubes were shaken vigorously and centrifuged. The hexane layer was transferred to a small test tube and the solvent was evaporated fully under a stream of nitrogen. For derivatization, 100 μ L of Tri-Sil reagents were added and dispersed by brief sonication. Then the sample was incubated at 60°C for 45 minutes and the reagent was dispersed in the ultrasonic bath. The solvent was evaporated under a stream of nitrogen. The TMS-ether derivatives of the sterols were dissolved in 500 μ L of hexane. A sample containing about 1 μ L was injected in the splitless mode into a GC model 6890 with ChemStation software, Rev. B.02.01 (Agilent Technologies, Wilmington, DE, USA), connected with a GC PAL auto sampler (CTC Analytics AG, Zwingen, Switzerland) and a flame ionization detector. A combination of two fused-silica capillary columns, a DB-5MS (length 15 m, i.d. 0.18 mm and film thickness 0.18 μ m) and a DB-35MS (length 10 m, i.d. 0.2 mm, and film thickness 0.33 μ m), was used, and the columns were joined together by a universal press-fit connector. The temperatures of the detector and injector were 310° and 260°C, respectively. The carrier gas was helium and nitrogen was used as a make-up gas at flow rates of 0.7 and 30 ml/min, respectively. The oven temperature was initially 60°C for 1 min, was increased to 290°C at a rate of 50°C per min, and was then maintained at 290°C for 5 min, after which it was increased again by 1°C per min to 305°C for 15 min. All the analyses were conducted in duplicate and the quantification was performed using 5 α -cholestane as an internal standard (Appelqvist, 1968).

Analysis of tocopherols by High-Pressure Liquid Chromatography (HPLC)

The quantitative tocopherols content of S-RSO, PS and optimized ESF were analyzed by HPLC. The HPLC system consisted of a 510 HPLC pump (Waters, Milford, USA) and a Rheodyne injector with a 20 μ L loop, as well as a Varian 9070 fluorescence detector (Walnut Creek, CA, USA). The wavelengths of the detector were set at 294 nm and 320 nm for excitation and emission, respectively. The column was a LiChroCART 250-4 (Merck KGaA, Darmstadt, Germany), packed with Lichrosphere 100 NH₂ (5 μ m particle size) and coupled to a LiChroCART 4-4 guard column (Merck KGaA, Darmstadt, Germany). Approximately 10 mg of each sample were dissolved in 1 mL of n-heptane and 20 μ L were injected manually. The isocratic mobile phase was a mixture of n-heptane:tert-butyl methyl ether:tetrahydrofuran:methanol (79:20:0.98:0.02, v/v/v/v) at a flow rate of 1.0 mL/min. Each tocopherol was quantified using an external standard method and the area of each peak was calculated by an HP 3396A integrator (Hewlett-Packard, Avandale, PA, USA) (Appelqvist, 1968). All the analyses were conducted in duplicate.

Results and Discussion

Response surface methodology (RSM)

The effects of three variables such as reaction time (12, 24 and 36 h), enzyme (5, 10 and 15%) and substrate mole ratio (S-RSO to PS, 1:1, 1:2 and 1:3) on SFC of TSF were considered. Uncoded variables were transferred into coded variables with a zero mean and the same standard deviation that has been defined as dimensionless in the RSM (Myers & Montgomery, 2002). Regression coefficients were determined by employing least squares technique to predict quadratic polynomial models for SFC at different temperature (10 and 30 °C, respectively) is shown in Table 2. Analysis of variance (ANOVA) of response surface for SFC showed that this design was adequate and reproducible due to satisfactory levels of coefficient of determination (R^2 , 0.98) for both cases and coefficient variation (CV, 4.06 for SFC at 10 °C and 7.95 for SFC at 30 °C, respectively). Generally, high value of CV indicates that it does not develop an adequate experimental design. In addition, linear, quadratic and cross product terms were found to be significant ($P < 0.05$) for the observed SFC at 10 °C. On the other hand, linear and quadratic terms showed a significant effect ($P < 0.05$) while cross product terms were not significant ($P > 0.05$) for the observed SFC at 30 °C (data are not shown). However, total model for both cases was significant ($P < 0.05$).

Table 2. Regression coefficients of predicted quadratic polynomial models for the response SFC at 10 and 30° C

Coefficient	SFC (10° C)		SFC (30° C)	
β_0	24.913775	$P = 0.0109$	-6.502577	$P = 0.342$
Linear				
β_1	1.069623	$P = 0.059$	0.551779	$P = 0.2284$
β_2	-2.363704	$P = 0.0768$	-1.50013	$P = 0.1785$
β_3	24.768479	$P = 0.0109$	26.339352	$P = 0.0012$
Quadratic				
β_{11}	-0.028414	$P = 0.0034$	-0.018412	$P = 0.0549$
β_{22}	0.008735	$P = 0.8722$	0.031346	$P = 0.5183$
β_{33}	-5.916620	$P = 0.0027$	-5.186338	$P = 0.0028$
Crossproduct				
β_{12}	0.011	$P = 0.4126$	0.010813	$P = 0.3631$
β_{13}	0.10875	$P = 0.1287$	0.103229	$P = 0.1056$
β_{23}	0.663	$P = 0.0033$	0.20525	$P = 0.1677$
R^2	0.98		0.98	
CV	4.06		7.95	

Regression coefficients were determined by employing least squares technique to predict quadratic polynomial models for SFC at 10 and 30 °C, respectively. The following second-order polynomial equations explain the experimental data containing values of the coefficients of independent variables (X_1 , reaction time; X_2 , enzyme %; X_3 , substrate mole ratio; Y_1 , SFC for 10 °C and Y_2 , SFC for 30 °C):

$$Y_1 = 24.913775 + 1.069623X_1 - 2.363704X_2 + 24.768479X_3 - 0.028414X_1^2 + 0.008735X_2^2 - 5.916620X_3^2 + 0.011X_1X_2 + 0.10875X_1X_3 + 0.663X_2X_3$$

$$Y_2 = -6.502577 + 0.551779X_1 - 1.50013X_2 + 26.339352X_3 - 0.018412X_1^2 + 0.031346X_2^2 - 5.186338X_3^2 + 0.010813X_1X_2 + 0.103229X_1X_3 + 0.20525X_2X_3$$

In Table 3, the results showed that X_3 was the primary factor and significant term for affecting the response of SFC ($P < 0.05$) and no significant effect was observed for X_1 and X_2 on the response ($P > 0.05$). The response contour plots were presented as an axis of enzyme percent (1 – 20%) and substrate mole ratio (1: 0.5 – 1: 3.5) as well as substrate mole ratio (1: 0.5 – 1: 3.5) and reaction time (10 – 40 h) for SFC at temperature of 10 and 30 °C, respectively. The SFC increased at any temperature with increased substrate mole ratio but the SFC decreased with increased addition of enzyme percent for both cases (Fig.1 and Fig.3). The SFC also increased with increased reaction time to a certain level approximately 23 h and then decreased (Fig. 2 and Fig. 4) for both cases.

Table 3. Analysis of variance of the factors and the critical values for the response SFC at temperature 10 and 30° C

Independent variables ^a	Analysis of variance					Critical values	
	d.f	Sum of square	Mean square	F-value	P-value	Coded	Uncoded
SFC (% , 10°C)							
X ₁	4	63.555198	15.8888	3.46	0.073	0.183	26.197
X ₂	4	181.226623	45.306656	9.86	0.0053	-0.065	9.67
X ₃	4	1264.898342	316.224586	68.85	<0.0001	0.875	2.875
SFC (% , 30°C)							
X ₁	4	34.91384	8.72846	2.45	0.1417	0.182	26.191
X ₂	4	23.760479	5.94012	1.67	0.2598	-0.075	9.62
X ₃	4	1117.721898	279.430475	78.53	<0.0001	0.99	2.99

^a X₁ = reaction time (h); X₂ = enzyme (%); X₃ = substrate mole ratio (S-RSO: PS)

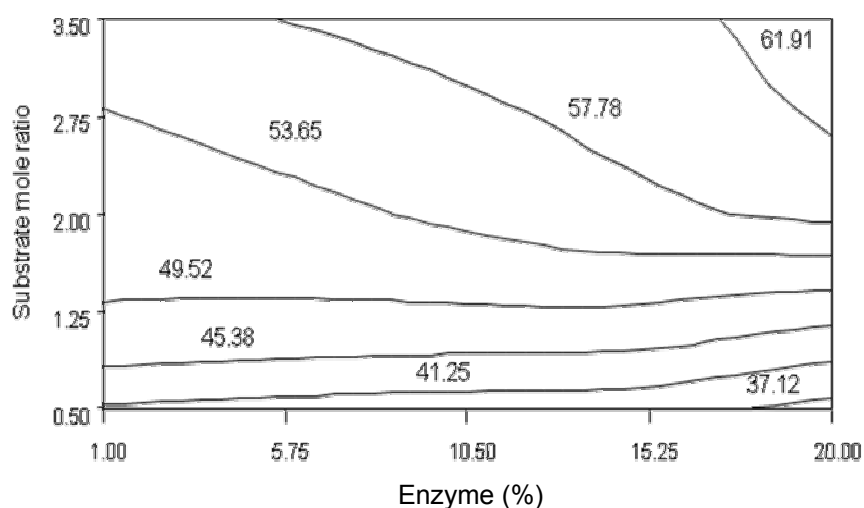


Fig.1. Response contour plot for SFC at 10 °C showing the effect of substrate mole ratio and enzyme (%)

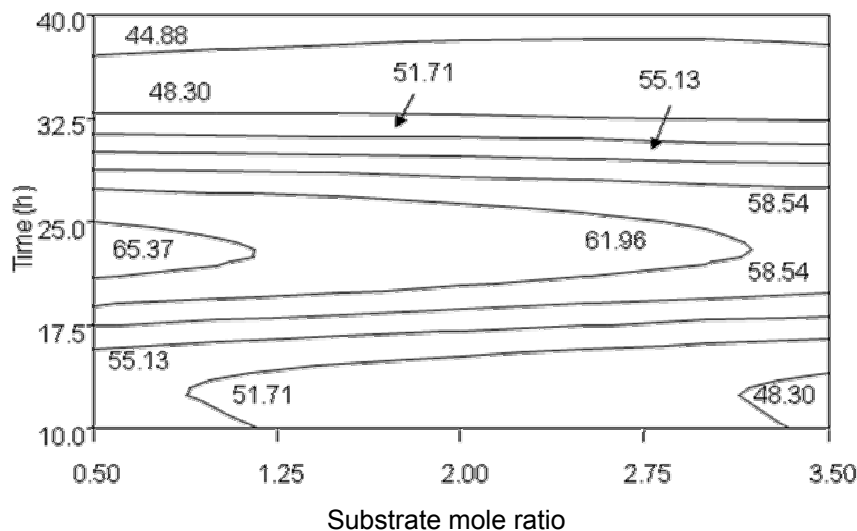


Fig.2. Response contour plot for SFC at 10 °C showing the effect of reaction time (h) and substrate mole ratio

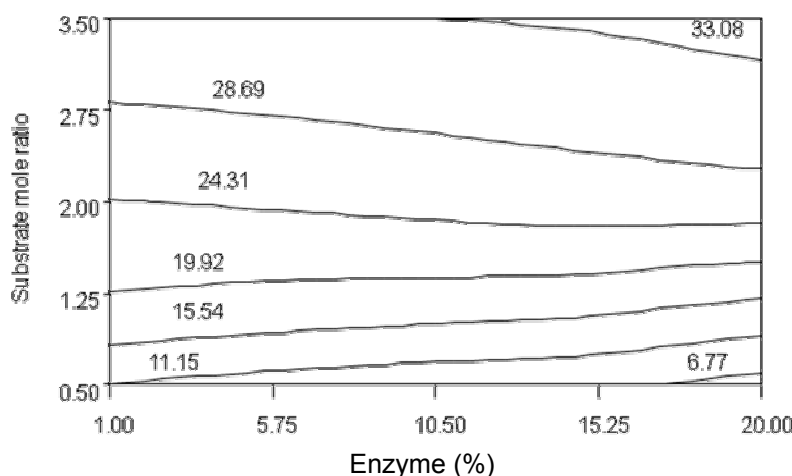


Fig. 3. Response contour plot for SFC at 30 °C showing the effect of substrate mole ratio and enzyme (%)

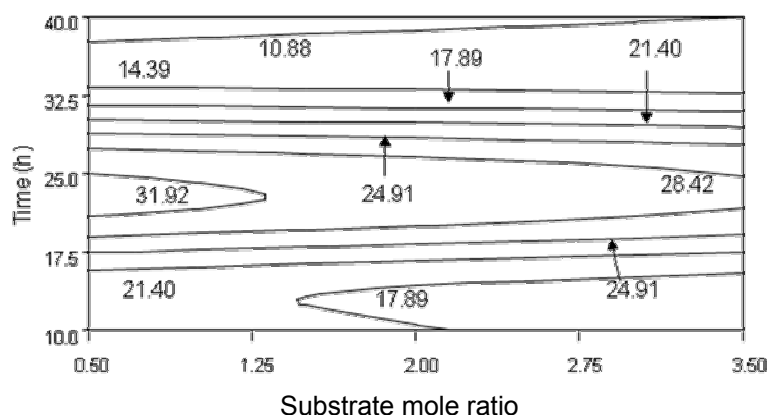


Fig.4. Response contour plot for SFC at 30 °C showing the effect of reaction time (h) and substrate mole ratio

Canonical analysis was performed to examine the stationary point and to decide whether it represents a maximum, minimum or saddle point. The canonical models for SFC at 10 and 30 °C were as follows:

$$Y_1 = 63.11 + 0.69 W_1^2 - 4.01 W_2^2 - 6.47 W_3^2$$

$$Y_2 = 32.89 + 0.87 W_1^2 - 2.56 W_2^2 - 5.36 W_3^2$$

where W_1 , W_2 and W_3 are the axes of the response surface. The predicted values of the stationary point were saddle points for both cases because eigenvalues were positive and negative. Therefore, the ridge analysis was used to determine the estimated ridge of maximum or minimum response in which combinations of reaction time, enzyme percent and substrate mole ratio for SFC at 10 and 30 °C, respectively. From the ridge analysis with minimum responses, production of ESF with SFC 51.48 ± 0.94 % at 10 °C would be predicted by the combinations of optimized 24.07 h reaction time, 10.66 % enzyme and 1: 1.52 substrate mole ratios at coded radius 0.5. On the other hand, the SFC of 21.44 ± 0.83 at 30 °C would be predicted by the combinations of optimized 24.37 h reaction time, 10.23 % enzyme and 1: 1.5 substrate mole ratios at coded radius 1.

The ESF was reproduced under the conditions of 24.07 h reaction time, 10.66 % enzyme and 1: 1.52 substrate mole ratios to confirm the predicted optimum conditions. The SFC of ESF at 10 °C was 48.03 ± 1.5 , which indicated that the optimum conditions were selected. Therefore, this modeling was effective to optimize a combination of the factors for producing ESF with specific SFC through lipase-catalyzed reaction. The produced ESF contained 48.03 ± 1.5 % of SFC at 10 °C and 16.1 ± 3.5 % of SFC at 30 °C,

respectively. The commercial stick margarine has a SFC of 43 – 50.8% at 10 °C and 8.9 – 21.4% at 30 °C, respectively (Petrauskaitė *et al.*, 1998). Therefore, the ESF in the study can be used as stick margarine with suitable physical characteristics.

Fatty acid composition

The PS contained three major fatty acids; these are mainly palmitic (16:0), oleic (18:1) and stearic (18:0) acids which composed of 67.4, 21.8 and 5.6%, respectively (Table 4). The PS also contained higher amounts of total saturated fatty acids (75.0%) than S-RSO (8.5%) and ESF (51.8%). On the other hand, the major fatty acids of S-RSO were oleic (18:1, 63.4%), linoleic (18:2, 18.5%) and linolenic (18:3, 7.6%) acids, respectively. However, the ESF contained mostly of palmitic (45.1%), oleic (40.8%), linoleic (5.6%) and stearic (4.5%) acids, respectively. The ESF contained total sum of unsaturated fatty acids 48.2% and total sum of saturated fatty acids 51.8%, respectively.

Table 4. Fatty acid compositions (%) of palm stearin (PS), solid part of rapeseed oil (S-RSO) and esterified solid fat (ESF)^a

Fatty acids	PS	S-RSO			ESF		
	Total	sn-1,3	sn-2	Total	sn-1,3	sn-2	Total
14:0	1.6±0.1	nd ^b	nd	nd	1.3±0.1	1.2±0.0	1.3±0.1
16:0	67.4±0.3	7.5±0.1	0.1±0.1	5.0±0.0	50.6±0.1	34.2±0.7	45.1±0.3
16:1	0.4±0.1	nd	nd	nd	0.6±0.0	nd	0.4±0.0
18:0	5.6±0.1	3.1±0.0	nd	2.1±0.0	5.1±0.3	3.2±0.5	4.5±0.0
18:1	21.8±0.0	66.8±0.1	56.6±0.2	63.4±0.1	36.7±0.4	49.0±0.2	40.8±0.3
18:2 (ω-6)	2.8±0.0	12.3±0.05	31.0±0.0	18.5±0.0	2.2±0.5	12.4±1.4	5.6±0.1
18:3 (ω-3)	nd	5.2±0.05	12.3±0.2	7.6±0.1	0.9±0.0	nd	0.6±0.0
20:0	0.4±0.0	1.2±0.0	nd	0.8±0.0	0.9±0.0	nd	0.6±0.0
20:1	nd	2.4±0.07	nd	1.6±0.05	0.9±0.1	nd	0.6±0.1
22:0	nd	0.6±0.0	nd	0.4±0.0	0.3±0.1	nd	0.2±0.1
22:1	nd	0.6±0.07	nd	0.4±0.05	0.3±0.0	nd	0.2±0.0
24:0	nd	0.3±0.0	nd	0.2±0.0	0.2±0.0	nd	0.1±0.0
ΣSFA ^c	75.0	12.8	0.1	8.5	58.4	38.6	51.8
ΣUFA ^d	25.0	87.2	99.9	91.5	41.6	61.4	48.2
ω-6/ω-3 ratio	2.8			2.43			9.33

^a Esterified solid fat (ESF) was produced by lipase catalyzed reaction with 24.07 h (reaction time); 10.66% (enzyme)

and 1:1.52 mole ratio S-RSO to PS.

^b nd, not detected

^c Total sum of saturated fatty acid

^d Total sum of unsaturated fatty acid

The S-RSO contained higher levels of oleic acid (56.6%), linoleic (31.0%) and linolenic (12.3%) acids, respectively at sn-2 position whereas the ESF contained higher levels of oleic (49.0%), palmitic (34.2%) and linoleic (12.4%) acids, respectively. Generally at sn-2 position, the presence of higher amount of unsaturated fatty acids is important because it is easily absorbed in the body (Quinlan and Moore, 1993).

The fatty acid contents of S-RSO at sn-1,3 position were oleic (66.8%), linoleic (12.3%), palmitic (7.5%) and linolenic (5.2%), respectively. In case of ESF, the major fatty acid contents at sn-1,3 position were palmitic (50.6%), oleic (36.7%), stearic (5.1%) acids. The changes of fatty acid compositions of fats and oils at different position lead to different physical properties such as melting point, crystallization behaviour etc. The ω-6/ω-3 ratio of PS, S-RSO and TSF were 2.8, 2.43 and 9.33, respectively which is important in oil and oil-based products. By comparison with other plant oils, corn and soybean oil have ratios of 45 and 10, respectively. The balance of ω-6/ω-3 ratio is important in decreasing the risk for coronary heart disease (Simopoulos, 2008). The Food and Agriculture Organization (FAO)/World Health Organization (WHO) recommend a ratio of 5:1 to 10:1 omega-6 to omega-3. While a ratio between 1:1 and 4:1 is considered as being optimal (FAO/WHO, 1998).

Sterols and tocopherols content

The results of sterol and tocopherol contents (mg/100g) in PS, S-RSO and ESF are presented in Table 5. The PS contained sitosterol (17.23 mg/100g), campesterol (6.46 mg/100g), stigmasterol (3.85 mg/100g) and cholesterol (1.86 mg/100g), respectively. The S-RSO contained higher amount of total content of sterols (680.20 mg/100g) than the ESF (249.27 mg/100g) and the PS (29.40 mg/100g), respectively. The major sterol in ESF was sitosterol (123.71 mg/100g), followed by campesterol (90.31 mg/100g), brassicasterol (20.93 mg/100g), $\Delta 5$ -avenasterol (5.57 mg/100g), stigmasterol (5.47 mg/100g) and cholesterol (3.28 mg/100g). Vegetable oils are the good sources of tocopherols, which are consider as the most important natural antioxidants for oxidative stability of fats and oils. The PS contained a higher amount of total tocopherols (24.09 mg/100 g) than the S-RSO (19.92 mg/100g) and the ESF (19.26 mg/100 g). The content of δ -tocopherol and α -tocopherol (8.92 and 6.19 mg/100 g, respectively) in PS were higher rather than the S-RSO (1.43 and 3.64 mg/100 g, respectively) and the ESF (4.48 and 4.64 mg/100 g, respectively). On the other hand, the content of γ -tocopherol (14.85 mg/100 g) in S-RSO was higher than the PS (5.55 mg/100 g) and the ESF (8.73 mg/100 g). The ESF contained γ -tocopherol (8.73 mg/100 g), α -tocopherol (4.64 mg/100 g), δ -tocopherol (4.48 mg/100 g) and β -tocopherol (1.41 mg/100 g), respectively. During the enzymatic esterification reaction and de-acidification step, the loss of sterols and tocopherols content in ESF could have taken place but further study is required to elucidate this.

Table 5. Sterols and tocopherols content (mg/100g) of palm stearin (PS), fractionated solid part of rapeseed oil (S-RSO) and esterified solid fat (ESF)^a

Sterols and Tocopherols	PS	S-RSO	ESF
Cholesterol	1.86±0.21	6.57±0.87	3.28±0.26
Brassicasterol	nd ^b	57.61±1.66	20.93±1.34
Campesterol	6.46±0.36	263.15±9.56	90.31±5.41
Stigmasterol	3.85±0.38	nd	5.47±2.86
Sitosterol	17.23±1.56	351.01±13.12	123.71±7.8
$\Delta 5$ -avenasterol	nd	1.86±0.13	5.57±0.52
Total	29.40	680.20	249.27
α -tocopherol	6.19±0.06	3.64±0.04	4.64±0.27
β -tocopherol	3.43±0.13	nd	1.41±0.06
γ -tocopherol	5.55±0.07	14.85±1.59	8.73±0.24
δ -tocopherol	8.92±0.15	1.43±0.03	4.48±0.34
Total	24.09	19.92	19.26

^a Esterified solid fat (ESF) was produced by lipase catalyzed reaction with 24.07 h (reaction time); 10.66% (enzyme) and 1:1.52 mole ratio S-RSO to PS.

^b nd means not detected.

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