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# Enzymatic synthesis of low-*trans* blends from fractionated mustard oil and palm stearin with linoleic acid by response surface methodology

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## Abstract

Low-*trans* blend (LTB) was produced from the fractionated mustard oil (solid phase, S-MO) and palm stearin (PS) through lipase-catalyzed reaction, in which linoleic acid (LA) was intentionally incorporated. For optimizing the reaction condition, response surface methodology (RSM) was employed with three reaction variables such as substrate mole ratio of S-MO to PS ($X_1$), reaction temperature ($X_2$) and reaction time ($X_3$). The predictive models were adequate and reproducible due to no significant lack of fit and the *P*-value of the model was very small ω6/ω3 ratio, and satisfactory level of coefficient of determination ($R^2$ = 0.89) for ω6/ω3 ratio. The ω6/ω3 ratio of LTB was affected by substrate mole ratio and reaction temperature but reaction time had no significant effect. For considering the ω6/ω3 ratio, the optimum condition found 1:1.7 substrate mole ratio, 61.42 □ reaction temperature and 25.85 h reaction time.

**Keywords:** Low-*trans* blend, Mustard oil, Palm stearin, Lipase-catalyzed reaction, Response surface methodology, Solid fat content

## Introduction

A trans fatty acid (TFA) is an unsaturated fatty acid whose molecule contains trans configuration at double bonds. Although, TFA is naturally found in some animal-based foods, but the majorities are formed when liquid oils are made into semi-solid fats like shortening and hard margarine through hydrogenation. Before hydrogenation, most naturally occurring unsaturated fatty acids have *cis* configuration at their double bonds. Partial hydrogenation rearranges the double bonds, converting some of them to the *trans* configuration and shifting the double bonds along the carbon chains. However, it is reported that high TFA diet causes adverse changes in the plasma lipoprotein profile with increase in low-density lipoproteins (LDL) cholesterol and decrease in high-density lipoproteins (HDL) cholesterol and increase the risk of coronary heart diseases and atherosclerosis [1,2]. Furthermore, hydrogenation process has a disadvantage of reducing essential fatty acid. Of late, the need of the development of low and/or zero-*trans* solid fats is requested in the food industry due to the negative health effect of TFA. Therefore, lipase-catalyzed reaction, as a non-hydrogenated alternative process, is now being explored to develop the low-*trans* fats [3].

Mustard oil (MO) is commonly used in Bangladesh, and eastern and northern parts of India. MO has a strong smell, hot nutty taste, pungent and sulphury odor, and much used for cooking. This is valuable for human health by reducing the risk of colon tumor incidence and multiplicity as compared to the dietary corn and fish oil [4]. Its major fatty acids are erucic, oleic, linoleic and α-linolenic acid [4,5,6,7]. MO, possibly owing to the presence of omega-3-fatty acids (α-linolenic), may provide protective effects in the patients with acute myocardial infarction [8]. Actually, oils high in erucic acid are useful for the polymer industry and valuable raw material for manufacture of industrial products such as plasticizers, surfactants, detergents, coatings and polyester, but oil enriched with low erucic acid are recommended for food purposes because oils enriched with high erucic acid may causes an accumulation of triacylglycerol in the heart of rats and other species [9]. High consumption of erucic acid may increase the adrenal cholesterol, cause fibrotic changes in myocardium and increase liver weight and cholesterol [10, 11].

Palm oil is widely used in food applications, providing various fractions after winterization process in which palm stearin (PS), one of the fractions, is mainly composed of high melting triacylglycerides [12].

For optimizing the lipase-catalyzed reaction, response surface methodology (RSM) has been successfully applied, considering it as an effective and powerful statistical method [13, 14].

The purpose of this study was to develop low-*trans* blend from the fractionated MO and PS through lipase-catalyzed reaction, in which linoleic acid (LA) was intentionally incorporated due to its beneficial health effects. Linoleic acid is an essential fatty acid that human needs to get it from the foods since our body can not synthesize it. For the reaction, factors (substrate mole ratio, reaction temperature and reaction time) were optimized to the ω6/ω3 ratio (as response) using response surface methodology (RSM) analysis.

## Materials and methods

Mustard oil (MO) was supplied from Agricultural Marketing Co. Ltd (Dhaka, Bangladesh). Lipozyme TL IM was obtained from Novozymes A/C (Bagsvaerd, Denmark). 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) is a 1, 3-specific lipase from *Thermomyces lanuginosus* which is granulated on the silica. Linoleic acid was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Acetone, n-hexane, choloroform, 2-propanol, heptadecanoic acid, and acetic acid were purchased from Fisher Scientific (Norcross, GA, USA). The standard α- tocopherol and pancreatic lipase was obtained from Sigma Chemical Co. All chemicals were of analytical reagent grade.

### Winterization

Mustard oil (300 g) and acetone (1500 ml) were mixed (1:5, w/v) together in 3-L conical flask [15] and placed in a freezer (–16$^0$C for 24 h). After winterization, the liquid phase was separated from the solid phase by decanting quickly, and acetone was completely evaporated from solid phase using a rotary vacuum evaporator and a stream of nitrogen with moderate heating (40$^0$C). The fractionated solid phase of mustard oil (S-MO) was obtained and further used as a substrate for lipase catalyzed reaction.

### Experimental design for RSM analysis

A three-factor and three-level face-centered cube design was chosen to evaluate the combined effect of three independent variables such as substrate mole ratio ($X_1$), reaction temperature ($X_2$) and reaction time ($X_3$). In this study, 17 individual run points were taken for analysis in which 8, 6 and 3 replicates were considered as a factorial, axial and center point respectively [16,17].

Using the experimental data, the polynomial equation for the yield of melting point and ω6/ω3 ratio was shown as below:

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

Where, $\beta_o$, $\beta_i$, $\beta_{ii}$, $\beta_{ij}$ were the regression coefficients for interception, linear, quadratic and interaction terms respectively, and $X_i$, and $X_j$ were the independent variables. The actual experiments and the independent variables ($X_i$) with their levels are shown in Table 1. All data were analyzed by the response surface regression (RSREG) procedure of the Statistical Analysis System (SAS) [18] and verified to the polynomial equation after logarithmic transformation [19]. The ridge analysis of RSREG of SAS was used to determine the estimated ridge of maximum or minimum response when the result was a saddle point. Response contour plot and predicted plot were generated using Modde version 5.0 software (Umetrics, Umeå, Sweden).

### Lipase-catalyzed reaction

The S-MO and PS were mixed together as a blend in a screw-capped test tube in proportions of 1:1 (S-MO: PS, w/w), 1:1.5, and 1:2, respectively. Then, 30% linoleic acid (LA) of the S-MO and PS mixture and lipase (10% of the total weight of the substrate) was added. Immediately, the mixtures were incubated in an orbital-shaking water bath at 180 rpm following the reaction conditions mentioned in Table 1.

**Table 1. Three-levels and three-factors experimental design arrangements and responses[a]**

| Experiment No. | Factors | | | Response ($Y_1$, ω6/ω3) |
|---|---|---|---|---|
| | $X_1$ | $X_2$ | $X_3$ | |
| 1 | 1:1(-1) | 55(-1) | 12(-1) | 8.3 |
| 2 | 1:1(-1) | 55(-1) | 36(+1) | 10.1 |
| 3 | 1:1(-1) | 75(+1) | 12(-1) | 5.3 |
| 4 | 1:1(-1) | 75(+1) | 36(+1) | 5.3 |
| 5 | 1:2(+1) | 55(-1) | 12(-1) | 12.1 |
| 6 | 1:2(+1) | 55(-1) | 36(+1) | 15.8 |
| 7 | 1:2(+1) | 75(+1) | 12(-1) | 8.9 |
| 8 | 1:2(+1) | 75(+1) | 36(+1) | 8.0 |
| 9 | 1:1(-1) | 65(0) | 24(0) | 7.7 |
| 10 | 1:2(+1) | 65(0) | 24(0) | 10.6 |
| 11 | 1:1.5(0) | 55(-1) | 24(0) | 9.5 |
| 12 | 1:1.5(0) | 75(+1) | 24(0) | 9.3 |
| 13 | 1:1.5(0) | 65(0) | 12(-1) | 7.7 |
| 14 | 1:1.5(0) | 65(0) | 36(+1) | 10.3 |
| 15 | 1:1.5(0) | 65(0) | 24(0) | 9.2 |
| 16 | 1:1.5(0) | 65(0) | 24(0) | 9.7 |
| 17 | 1:1.5(0) | 65(0) | 24(0) | 9.2 |

[a] $X_1$ = substrate mole ratio (S-MO to PS); $X_2$ = reaction temperature (°C); $X_3$ = reaction time (h); $Y_1$ = ω6/ω3 fatty acids ratio

After each reaction, the lipases were removed from the reactant by passing through a 5 ml disposable syringe 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 also added. The mixture was titrated with a 0.5 N KOH solution in 20% ethanol until pink color was appeared. Moderate heating was provided to prevent solidification of reactant during the de-acidification. The upper phase was passed through an anhydrous sodium sulfate column and dried with nitrogen putting in the heating module, and then the lipase-catalyzed low-*trans* blend (LTB) was obtained.

**Fatty acid composition**

The produced LTB were separated by thin-layer chromatography (TLC) on a silica gel 60 $F_{254}$ plate (20 x 20 cm, Merck KGaA, Germany) developed with hexane/diethyl ether/acetic acid (50/50/1, v/v/v). After drying, the bands were displayed by viewing under short wavelengths (254 nm). The visualized triacylglycerol (TAG) band was scraped off into a test tube and methylated with 3 ml of 6% $H_2SO_4$ in methanol for 1 h at 70 $^0$C. Fifty micro liters of heptadecanoic acid (C17:0, 1 mg/ml in hexane) as an internal standard was also added in the tube. After putting on ice immediately**,** 2 ml hexane was added and vortexed for 30 sec. The upper layer was passed through an anhydrous sodium sulfate column**,** and solvent was evaporated with nitrogen. Gas chromatography (GC, Agilent, HP 6890 Series, Avondale, PA), accompanied with auto injector and flame-ionization detector was used for fatty acid analysis. A fused-silica capillary column (SP-WAX, 60 m x 0.25 mm id, Supelco, Bellefonte, PA) was used for separation. The column temperature was held at 100 °C for 5 min and increased to 220 °C at the rate 4 °C/min, then held for 30 min. 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 temperature of injector and detector were 250 and 260 °C, respectively.

**Positional fatty acid analysis**

S-MO and LTB (each 7 mg) were taken in a test tube. Seven milliliters 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 sec. Other steps were followed as

described by Lee and Akoh [20]. The hydrolytic products were separated on TLC plate by developing solvent containing of hexane/ diethyl ether/ acetic acid (50/50/1, v/v/v). The band of monoacylglycerol was scrapped off for methylation and analyzed by GC. The percent of fatty acid at sn-1, 3 position was calculated by the formula: sn-1, 3 (%) = (3T- sn-2)/2 where T is the total fatty acid contents found in S-MO and LTB.

### Grinnard reaction

Fatty acid composition of PS at sn-2 position was determined by Grinnard reaction. A sample (25 to 35 mg) was dissolved in diethyl ether and 300 µl ethyl magnesium bromide mixed together. It was vortexed vigorously for 5 min. After that 3 ml boric-HCl buffer (0.27 M HCl in 0.4 m boric acid) and 1 ml diethyl ether were added to the mixture. Again vortexed for 1 min. The upper phase was washed with 3 ml of 0.4 N boric acid solutions at least two times. The upper phase was passed through sodium sulfate column and diethyl ether was blown up by $N_2$ gas. The sample was separated on TLC plate which was impregnated by 0.4% boric acid solution and developed in hexane/diethyl ether/ acetic acid (50/50/1, v/v/v). After spraying with o.2% 2,7-dichlorofluorescein in methanol, a band corresponding to sn-2 monoacylglycerol was scratched and taken into a test tube for methylation and analyzed by GC as described above.

### The tocopherol content analysis

The high performance liquid chromatography (HPLC) was used for quantitative analysis of α-tocopherol of PS and S-MO [21]. The HPLC consisted of a Yonglin SP930D dual pump (Yonglin, Anayang, Korea) and Yonglin UV830 detector set a 295 nm. The used column was a Chromsep Cartridge, LiChrosorb Diol (5 µm, 3 X 100 mm, Chromapack, Rartian, NJ, USA) and the mobile phase was a mixture of hexane fortified with 0.1% acetic acid (1000: 1, v/v). The flow rate was 0.5 ml/min. The area of each peak was integrated by Autochro-2000 software (Yonglin, Anayang, Korea).

### Differential scanning calorimetry (DSC)

The solidifying thermograms of S-MO, PS and LTB were determined with the DSC 2010 differential scanning calorimeter (TA Instruments Inc, New Castle, DE, USA). The base line was obtained with an empty aluminum pan. Purge gas was nitrogen and each analysis required 5 to 9 mg sample. The temperature was increased to 80□ and held for 10 min, and then the temperature decreased to –60 $^0$C at 10 $^0$C/min. After holding for 10 min, the melting behavior was obtained by heating to 80 $^0$C at 5 $^0$C /min. The solid fat content (SFC, %) was calculated by Universal Analysis 2000 (TA Instruments Inc, New Castle, DE, USA). Each solidifying thermogram was divided at different temperature (-10, 0, 5, 10, 15, 20, 25, 30, 35 and 40 $^0$C) and the total crystallization energy (J/g) was calculated into percentage at each temperature for SFC.

## Results and Discussion

### Response surface methodology (RSM)

The optimum processing factors could be determined by using a minimum number of experiments through the suitable experimental design of RSM. The effects of substrate mole ratio of S-MO to PS (1:1, 1:1.5 and 1:2), reaction temperature (55, 65 and 75 °C) and reaction time (12, 24 and 36 h) on melting point and ω6/ω3 ratio of LTB were considered in this study by using RSM. The uncoded variables changed to coded variables (Table 1) with zero mean and standard deviation that had been defined dimensionless [22]. The smaller the *P*-values are the bigger the significance of the corresponding coefficient. In Table 2, analysis of variance (ANOVA) of response surface showed that this experimental model was adequate and reproducible because the *P*-value of the model was very small (0.0132) for ω6/ω3 ratio and significant lack of fit (*P* = 0.0389) did not exceed the tabulated value 25.00 (5, 2 d.f.). The satisfactory levels of $R^2$ (0.89) for ω6/ω3 ratio were found. The CV (13.32) for ω6/ω3 ratio also was found. Generally, high value of CV indicates that variation in the mean value is high and does not develop an adequate experimental design satisfactorily [23]. Further, the observed ω6/ω3 ratio was well correlated with the predicted values showing linear distribution ($R^2$ = 0.89 for ω6/ω3 ratio). For response ω6/ω3 ratio, linear term showed (*P* = 0.0014) significant effect while quadratic (*P* = 0.9889) and cross product (*P* = 0.3107) were not considered as significant terms.

The least squares technique was used for determining multiple regression coefficients to predict polynomial models for ω6/ω3 ratio. The values of regression coefficient were $\beta_o$= 4.01338 ($P$ = 0.8998), $\beta_1$= 9.107606 ($P$ = 0.4273), $\beta_2$= -0.162676 ($P$ = 0.8741), $\beta_3$= 0.530628 ($P$ = 0.1861), $\beta_{11}$= -0.222535 ($P$ = 0.943), $\beta_{22}$= 0.001944 ($P$ = 0.8033), $\beta_{33}$= -0.001428 ($P$ = 0.7922), $\beta_{12}$= -0.08 ($P$ = 0.3882), $\beta_{13}$= 0.020833 ($P$ = 0.782) and $\beta_{23}$= -0.006667 ($P$ = 0.1083) for ω6/ω3 ratio, respectively. The following second order polynomial equation explains the experimental data containing values of the coefficients of independent variables ($X_1$, substrate mole ratio; $X_2$, reaction temperature; $X_3$, reaction time; and $Y_1$, ω6/ω3 ratio).

$$Y_1 = 4.01338 + 9.107606X_1 - 0.162676X_2 + 0.530628X_3 - 0.222535X_1^2 + 0.001944X_2^2 - 0.001428X_3^2 - 0.08X_1X_2 + 0.020833X_1X_3 - 0.006667X_2X_3$$

From Table 2, the results pointed out that the factor $X_1$ and $X_2$ was significant term for affecting the response ($P < 0.05$), and also no significant effect was observed for factor $X_3$ on the response ($P > 0.05$,) for ω6/ω3 ratio. Fig. 1 showed that the ω6/ω3 ratio of LTB increased linearly with increased substrate mole ratio, and also increased slowly with increase reaction time. On the other hand, the ω6/ω3 ratio decreased with reaction temperature. Fig. 2 is presented the response contour plots showing the effect of mole ratio (S-MO to PS) and reaction temperature into LTB at different reaction times: 12, 24 and 36 h. The ω6/ω3 ratio of LTB increased linearly with increased substrate mole ratio while the ratio decreased with increase of reaction temperature. Reason may lie in the fact that the incorporation rate of lenoleic acid slowly decreased with increase of reaction temperature.

**Table 2. Analysis of variance (ANOVA) of the independent variables for response surface model**

| Independent variables[a] | d. f. | Sum of squares | Mean squares | F-value | *P*-value |
|---|---|---|---|---|---|
| $X_1$ | 4 | 36.38 | 9.10 | 6.02 | 0.0202 |
| $X_2$ | 4 | 42.60 | 10.65 | 7.04 | 0.0134 |
| $X_3$ | 4 | 10.54 | 2.64 | 1.74 | 0.2445 |

[a] $X_1$ = substrate mole ratio (S-MO to PS); $X_2$ = reaction temperature (°C); $X_3$ = reaction time (h).

To examine the stationary point and to decide whether it represents a maximum, minimum or saddle point, canonical analysis was performed [24]. The canonical model for the ω6/ω3 ratio of the LTB describing the character of the response surface were:

$$Y_1 = 8.84 + 0.51\ W_1^2 - 0.12\ W_2^2 - 0.46\ W_3^2$$

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 mixed [23]. Therefore, a ridge analysis was used to determine the estimated maximum or minimum response in which combinations of substrate mole ratio, reaction temperature, and time for LTB with target ω6/ω3 ratio.

The World Health Organization and Food & Agriculture Organization recommend a dietary ω6/ω3 fatty acid ratio ranges from 5 to 10 (25, 26). Therefore, the combination with 1:1.7 substrate mole ratio, 61.42 $^oC$ reaction temperature and 25.85 h reaction time would be predicted with estimated response 10.76 $\pm$ 0.52 at coded radius 0.5 from the ridge analysis with maximum responses of ω6/ω3 ratio.

**Fatty acids composition**

Table 3 presents the fatty acid composition of the reaction substrates (S-MO and PS). The S-MO and PS contained 94.3 and 29.8% total unsaturated fatty acids respectively. Many countries have limited the use of erucic acid in the fat component. The European Union recommends 5% level in food (27). The major fatty acids in PS were palmitic and oleic acid which composed of 63.6 and 24.5%, respectively. In S-MO, mainly erucic (45.3%), α-linolenic (17.7%), oleic (16.3%) and linoleic acid (15.0%), respectively were found. The ω6/ω3 ratio also important in oil and oil based products. The recommended dietary allowances of ω6/ω3 fatty acid ratio have been published in several countries

and organization. A joint committee of the World Health Organization and Food & Agriculture Organization recommends a ω6/ω3 dietary ratio between 5 and 10. In Western diets, the ω6/ω3 ratio ranged 15 to 16.7 [25]. By comparison, the ratio for corn oil is 45 and for soybean oil 10. According to nutritional point of view, higher level of unsaturated fatty acids in the sn-2 position is good since it is absorbed easily in the human body [28]. In S-MO, the major fatty acids at *sn*-2 position were linoleic (39.2%) and oleic (36.8%) acid, respectively. Therefore, higher content of total unsaturated fatty acids (98.7%) was found at *sn*-2 position of S-MO, which was further used for transesterification with linoleic acid. PS contained 74.3% total saturated and 25.7% total unsaturated fatty acids, respectively at sn-2 position.

**α-Tocopherol content**

The α-tocopherol content of PS and S-MO are shown in Table 3. The natural antioxidant such as α-tocopherol prevents lipid peroxidation by scavenging radicals in membranes and lipoprotein particles [29]. The PS and S-MO contained 0.4 and 3.7 mg/100g of α-tocopherol, respectively.

**Table 3. Estimated ridge of maximum response**

| Coded radius | Estimated response | Standard error | Uncoded factor values[a] | | |
|---|---|---|---|---|---|
| | | | $X_1$ | $X_2$ | $X_3$ |
| 0.0 | 9.27 | 0.53 | 1.5 | 65.00 | 24.00 |
| 0.1 | 9.55 | 0.53 | 1.5 | 64.31 | 24.33 |
| 0.2 | 9.84 | 0.52 | 1.6 | 63.60 | 24.68 |
| 0.3 | 10.14 | 0.52 | 1.6 | 62.88 | 25.05 |
| 0.4 | 10.45 | 0.52 | 1.6 | 62.15 | 25.44 |
| 0.5 | 10.76 | 0.52 | 1.7 | 61.42 | 25.85 |
| 0.6 | 11.08 | 0.53 | 1.7 | 60.67 | 26.27 |
| 0.7 | 11.42 | 0.54 | 1.7 | 59.92 | 26.70 |
| 0.8 | 11.76 | 0.56 | 1.7 | 59.17 | 27.15 |
| 0.9 | 12.11 | 0.59 | 1.8 | 58.41 | 27.61 |
| 1.0 | 12.47 | 0.63 | 1.8 | 57.64 | 28.08 |

[a] $X_1$ = substrate mole ratio (S-MO to PS); $X_2$ = reaction temperature (°C); $X_3$ = reaction time (h)

**Table 3. Total and positional distribution of fatty acid contents, and α-tocopherol content (mg/100g) of palm stearin (PS) and solid phase of mustard oil (S-MO)**

| Fatty acids | PS | | | S-MO[a] (90.5%) | | |
|---|---|---|---|---|---|---|
| | Total | Sn-2 | Sn-1,3 | Total | Sn-2 | Sn-1,3 |
| 14:0 | 1.4±0.01 | 2.0±0.0 | 1.1±0.01 | ND[b] | ND | ND |
| 16:0 | 63.6±0.45 | 66.7±0.5 | 62.1±0.42 | 4.0±0.05 | 0.8±0.1 | 5.6±0.02 |
| 18:0 | 4.9±0.02 | 5.6±0.0 | 4.5±0.04 | 1.7±0.0 | 0.5±0.05 | 2.3±0.02 |
| 18:1 | 24.5±0.3 | 18.3±0.4 | 27.6±0.25 | 16.3±0.05 | 36.8±0.02 | 6.0±0.19 |
| 18:2 (ω6) | 5.1±0.02 | 7.4±0.9 | 4.0±0.42 | 15.0±0.1 | 39.2±0.1 | 2.9±0.03 |
| 18:3 (ω3) | 0.2±0.01 | ND | 0.3±0.01 | 17.7±0.2 | 21.7±0.0 | 15.7±0.15 |
| 20:0 | 0.3±0.01 | ND | 0.4±0.01 | ND | ND | ND |
| 22:1 | ND | ND | ND | 45.3±0.3 | 1.0±0.0 | 67.5±0.4 |
| ∑SAF[c] | 70.2±0.49 | 74.3±0.5 | 68.1±0.48 | 5.7±0.05 | 1.3±0.15 | 7.9±0.04 |
| ∑UFA[d] | 29.8±0.33 | 25.7±1.3 | 31.9±0.68 | 94.3±0.65 | 98.7±0.12 | 92.1±0.77 |
| ω6/ω3 ratio | 25.5 | | | 0.85 | | |
| | | PS | | | S-MO | |
| α -tocopherol | | 0.4±0.06 | | | 3.7±0.1 | |

[a] S-MO; fractionated mustard oil (solid phase) after winterization at -16 °C for 24 h. Mustard oil (300 g) and acetone (1500 ml). [b] ND means not detected. [c] Total sum of saturated fatty acid. [d] Total sum of unsaturated fatty acid.

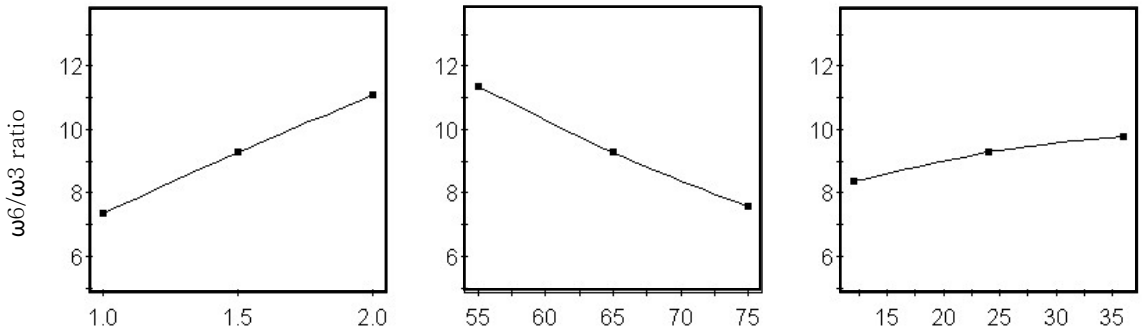


Fig. 1. Prediction plot for ω6/ω3 fatty acids ratio of lipase-catalyzed low-*trans* blend (LTB) by effects of mole ratio of S-MO and PS (A), reaction temperature (B) and reaction time (C).

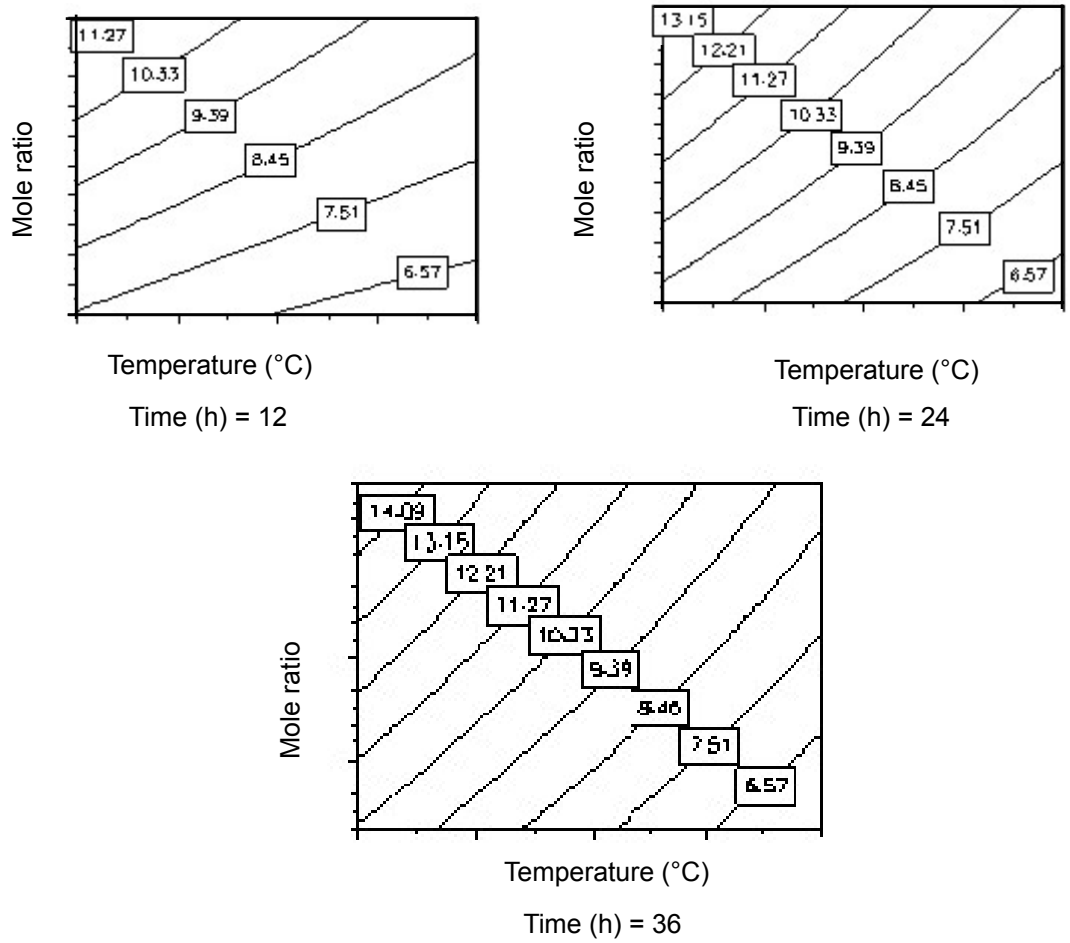


Fig. 2. Response contour plots for ω6/ω3 fatty acids ratio showing the effect of mole ratio (S-MO to PS) and reaction temperature into low-*trans* blend (LTB) at different reaction times: 12 h (A), 24 h (B) and 36 h (C).

**Solid fat contents (SFC)**

The SFC of PS and S-MO are given in Table 4. Melting properties such as the melting point and SFC are considered as important factors for the texture of solid fats. The SFC of solid fat (e.g., margarine and butter) is responsible for physical characteristics [30]. For S-MO, SFC at -10 $^{o}$C was 72.1% while most phases were liquid at 10 $^{o}$C (0.8% SFC). The SFC of PS at -10□ was 99.6%, indicating that most phases were solid. When temperature increased to room temperature ranges from 20 to 25 $^{o}$C, SFC ranged from 71.7 to 73.6%. Even at 40 $^{o}$C, SFC of PS was 43.3%.

**Table 4. Solid fat content (%) of fractionated mustard oil (S-MO) and palm stearin (PS)**

| Lipids | Temperature (°C) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | -10 | 0 | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 |
| S-MO[a] | 72.1 | 43.9 | 18.8 | 0.8 | - | - | - | - | - | - |
| PS | 99.6 | 92.8 | 86.3 | 78.4 | 74.0 | 73.6 | 71.7 | 63.0 | 53.2 | 43.3 |

[a] S-MO; fractionated mustard oil (solid phase) after winterization at -16 °C for 24 h. Mustard oil (300 ml) and acetone (1500 ml) were mixed (1:5, v/v)

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