

Research Paper

Optimization of the production of alpha linolenic acid enriched structural lipids and evaluation of their oxidative stability under accelerated storage conditions

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Abstract

Response surface methodology was used to optimize the production conditions for alpha linolenic acid (ALA) enriched structural lipid (SL) from Perilla oil (rich in ω 3: ALA) and Corn oil (rich in ω 6). Reaction condition was optimized using central composite design with reaction time (6-24hr, X_1), reaction temperature (50-65°C, X_2) and substrate mole ratio (1-3, X_3) as three variables. Contents of area % of TAG species like LnLnL (Y_1), LnLnO (Y_2) and LnLnLn (Y_3) were selected as responses. Based on ridge analysis, the combination of 16 h (X_1), 59°C (X_2), and 1:1 ratio (X_3) was optimized for producing SL. On basis of the model, synthesized SL contained 32.39 area % of ALA in fatty acid profile with low (1:1) ω 6: ω 3 ratio. The effect of antioxidants such as catechin, rosemary extract and their combinations on the oxidative stability in SL was investigated. Among all antioxidants, the highest stability was obtained from combination form of antioxidants.

Keywords: Response surface methodology, enzymatic interesterification, Perilla oil, omega-6/omega-3 ratio, Corn oil, TAG species.

Introduction

Biological compounds from Alpha linolenic acid (ALA) have been proven to prevent blood clotting, cancer, decrease the inflammatory process and beneficial to individual suffering from arthritics [1, 2]. ALA is one of a major source of plant ω 3 (C 18:3) fatty acids which is the metabolic precursor to C 20:5 ω 3 and C 22:6 ω 3 PUFA [3]. Evidence suggests that ALA along and other omega 3 fatty acids have been associated with decreasing levels of blood cholesterol, preventing against cardiovascular disease and management of chronic disorders[4, 5]. Perilla oil (PO) is a rich source of ALA which is also able to decrease plasma histamine level and increase higher hypo cholesterolemic ability [6, 7].

Generally vegetable oils (such as corn, soybean, safflower, and sunflower oil) contain high amount of $\omega 6$ (linoleic acid C18:2) fatty acid. Increased consumption of vegetable oils which are rich in $\omega 6$ fatty acids has drastically shifted the dietary $\omega 6/\omega 3$ ratio. Various studies indicate that human being evolved on a diet with a ratio of $\omega 6/\omega 3$ essential fatty acid of 1 whereas in western diets the ratio is relatively high 15/1 to 16.7/1 [8, 9]. A lower ratio of $\omega 6/\omega 3$ fatty acids is needed for the prevention and management of chronic diseases, suppressed inflammation in patients with rheumatoid arthritis, prevent asthma, reduce plasma lipoproteins and haemostatic factors [4, 8, 9, 10, 11]. Previous study reported that PO might be used to optimize the ratio of $\omega 6/\omega 3$ [12], yet few studies were published on modifying other vegetable oil with PO.

Vegetable oils were modified previously by lipase-catalyzed reaction [13, 14, 15, 16]. In the present study, SL by lipase-catalyzed interesterification from perilla and corn oil was attempted in order to produce ALA enrich SL as well as produce low $\omega 6/\omega 3$ ratio lipid. Reaction was optimized by response surface methodology (RSM) designed on 3 parameters (time, temperature and substrate mole ratio). On basis of the model PO and CO were further isolated to SL. Newly produce SL was analyzed to obtain its characteristics.

Lipid oxidation is a major quality problem in the processing and storage of fats, oils and fat containing food [17]. Some study showed that antioxidant can prevent the oxidative deterioration, to maintain the quality and to extend the shelf life of fats and oils [18, 19]. Catechin is one phenolic compound found in green tea extract, known as antioxidant. According to some studies, catechin can reduce the risk of coronary heart disease and cancer [20, 21]. In rosemary extract, noticeable antioxidant active was observed because of having phenolic diterpenes such as carnosic acid and carnosol [22]. The effectiveness of rosemary extract and catechin as antioxidants were investigate to prevent or delay the oxidation of PO, CO and their SL under accelerate storage condition.

Materials and Methods

Materials

Refined, bleached and deodorized corn oil and perilla oil from roasted perilla seeds were supplied by C.J. Co. (Seoul, Korea). Lipozyme RM IM (150 IUN/g of catalytic activity with a bulk density of 350-450 kg/m³, a partical size of 0.3-0.6 mm, and a water content of 2-3 w/w%) from *Rhizomucor miehei* was purchased from Novozymes A/S (Bagsvaerd DK-2880, Denmark). All solvents were HPLC grade and obtained from Fisher Scientific (Norcross, GA). Standards of Tocopherol (α , γ , δ tocopherols) and phytosterols were purchased from Sigma Chemical Co. (St. Louis, Mo., USA).

Experimental design for RSM analysis

A three-factor (reaction time; X_1 , reaction temperature; X_2 , and substrate mole ratio; X_3) and three-level (-1, 0, and 1) face-centered cube design was employed in this study, and 17 individual run points were taken for analysis. The independent variables (X_i) and their levels are shown in Table 1.

In enzymatic transesterification reaction, reaction is occurred in TAG-TAG bond. Consequently, the difference is observed in TAG species in product from the substrate after the reaction. Since, ALA is highly oxidation-susceptible molecule. Therefore, it could be suspected that the interesterification reaction can stabilize the ALA component in the TAG molecules by distributing it within the TAG species. Therefore as response of the interesterification of the substrate were observed as increasing (LnLnL ; Y_1 , LnLnO ; Y_2) and decreasing (LnLnLn ; Y_3) of linolenic acid TAG molecule.

Table 1. Three level and four factor central composite rotational design arrangements and responses^a

Experiment number	Factors			Response		
	X ₁	X ₂	X ₃	Y ₁	Y ₂	Y ₃
1	6(-1)	55(-1)	1(-1)	9.40±0.02	18.52±0.01	1.78±0.04
2	24(1)	55(-1)	1(-1)	10.49±0.01	19.84±0.01	1.43±0.02
3	6(-1)	65(1)	1(-1)	8.16±0.03	17.64±0.03	0.91±0.01
4	24(1)	65(1)	1(-1)	8.93±0.01	18.74±0.01	0.53±0.04
5	6(-1)	55(-1)	3(1)	1.83±0.01	10.19±0.02	0.31±0.01
6	24(1)	55(-1)	3(1)	2.70±0.02	10.91±0.01	0.03±0.01
7	6(-1)	65(1)	3(1)	1.74±0.01	10.10±0.01	0.28±0.01
8	24(1)	65(1)	3(1)	2.52±0.02	10.97±0.02	0.02±0.01
9	6(-1)	60(0)	2(0)	3.77±0.03	14.69±0.01	0.45±0.05
10	24(1)	60(0)	2(0)	4.31±0.01	15.38±0.03	0.29±0.01
11	15(0)	55(-1)	2(0)	3.72±0.01	14.22±0.02	0.51±0.01
12	15(0)	65(1)	2(0)	3.47±0.02	14.10±0.01	0.11±0.01
13	15(0)	60(0)	1(-1)	8.90±0.01	18.39±0.04	1.33±0.04
14	15(0)	60(0)	3(1)	1.50±0.03	10.05±0.02	0.07±0.01
15	15(0)	60(0)	2(0)	3.48±0.01	14.11±0.02	0.21±0.01
16	15(0)	60(0)	2(0)	3.30±0.02	14.01±0.04	0.25±0.01
17	15(0)	60(0)	2(0)	3.72±0.04	14.41±0.02	0.27±0.02

^a X₁=time (hr); X₂=reaction temperature (°C); X₃= substrate mole ratio; Y₁=Area % of LnLnL; Y₂=Area % of LnLnO; Y₃=Area % of LnLnLn.

The second-order polynomial equation used 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 fitted to the second-order polynomial equation after logarithmic transformation. The ridge analysis of RSREG of SAS system was used to determine the estimated ridge of maximum 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, Umea, Sweden).

Synthesis of structured lipids

According to the experimental design, corn oil and perilla oil were mixed (1:1 mole ratio), together for synthesis of SL. Lipozyme TL IM from *Thermomyces lanuginosea* (10% by weight of total substrates) was added to the mixture. No additional solvent was added into reaction system. The combined mixtures were incubated in an orbital-shaking water bath for 16 h at 59°C. After incubation, the reaction product was passed through 0.50 µm disposable syringe filter unit to remove lipase.

Fatty acid composition

The sample was separated by thin-layer chromatography (TLC) on a silica gel 60 F₂₅₄ plate (20×20 cm, Merck K GaA, Germany) developed with hexane/diethyl ether/acetic acid (50/50/1, v/v/v). After drying the plate, the visualized triacylglyceride (TG) band was separated and analyzed by GC followed by methylation, as previously described [18]. Briefly, oils (25 mg) were placed in a tube (15 mL) with screw cap and mixed with 0.5 N methanolic NaOH (1.5 mL). The mixtures were heated at 100 °C for 5 min and cooled at room temperature. BF₃ methanol (2 mL) was added and heated at 100 °C for 3 min. The mixture was cooled and the fatty acid methyl esters were extracted with 3 mL iso-octene, followed by adding saturated NaCl (1 mL). The aliquots (1 µL) of the extracts were injected into a gas chromatography (Hewlett-Packard 6890 series, USA) equipped with an auto injector and a flame-ionization detector (Agilent Technologies, Little Falls, DE, USA) using a fused-silica capillary column (Supelco SP-2560, 100 m × 0.25 mm I.d.; Supelco, Bellefonte, PA, USA). The temperatures of the injector and detector were set at 250 °C, and 260 °C, respectively. The column heated to 150 °C and held for 5 min. Then, increased to 200 °C at the rate of 4 °C /min, and held for another 30 min at the final temperature. Nitrogen was used as carrier gas. Fatty acids compositions were identified by comparison with relative retention times of standard mixtures. Duplicate analyses were performed.

Sn-2 Positional distribution

Fatty acid composition at *sn*-2 position was determined by pancreatic lipase analysis as described previously [16, 23]. Sample (7mg) was placed in a test tube. 7mL Tris-HCL buffer (1 M, pH 7.6), 1.75 mL of bile salt (0.05 % in distilled water (w/v)), 0.7 mL of CaCl₂ (2.2 % in distilled water w/v) and 7 mg pancreatic lipase were mixed for hydrolysis. The hydrolytic products were separated on a thin-layer chromatography (TLC, silica gel G) plate by developing solvent of hexane-diethyl ether-acetic acid (50:50:1 v/v/v). The band corresponding to *sn*-2 monoacylglycerol was isolated and methylated for GC analysis.

High-performance liquid chromatography

The separation of TAG species from PO, CO and SL were conducted by reversed-phase high performance liquid chromatography (RP-HPLC) as described previously [23]. Sample (15 mg) was prepared by dissolving in chloroform (20 mL). The HPLC system was consisted of Yonglin SP930D dual pump (Yonglin, Anyang, Korea) with evaporative light-scattering detector (ELSD, Yonglin, Anyang, Korea), operated at 55 °C with nitrogen pressure of 1.7 bar. Each sample (10 mg/10 mL) was dissolved in chloroform and vortexed for 1 min. Twenty µL of filtered samples were injected on Nova-Pak C18 column (150 × 3.9 mm, Waters, Milford, MA, USA). Elution solvent consisted of (A) acetonitrile and (B) isopropanol/hexane (2:1, v/v) at a flow rate of 1 mL/min with the following profile: 0-44 min 20% B; 45-50 min, 46% B; 51-58 min, 100% B, and then back to the initial flow rate.

Rancimat test for oxidative stability

The induction period, measuring the increase in the volatile by-products released from the oxidizing oil of PO, CO and SL was evaluated by the Rancimat analyzer (Rancimat 743, Metrohm, Switzerland). The airflow and temperature were set at 20 l/h and 100 °C, respectively, and the results were expressed as induction time (h).

Statistical analysis

Statistical Analysis System software (SAS, 2000) [24] was used to perform statistical analysis. Duncan's multiple range tests were performed to determine significance of difference at $p < 0.05$.

Results and Discussion

Data analysis by RSM

Using RSM, optimum processing factors on the enzymatic interesterification reaction between corn and perilla oil were studied. Table 1 shows the responses Y_1 : LnLnL (area %), Y_2 : LnLnO (area %), Y_3 : LnLnLn (area %), at each of the 17 experimental combinations of the three variables (X_1 , time; X_2 temperature; X_3 mole ratio). The analysis of variance (ANOVA) of response surface for area % of LnLnL (Y_1) experimental model was found to be adequate and reproducible due to no significant lack of fit ($P=0.58$), and satisfactory levels of coefficient of determination (R^2 , 0.99) and coefficient of variation (CV, 4.31) (Table 2). For the model, area % of LnLnO (Y_2) a satisfactory level of R^2 (0.99) and CV (1.39) were found with no significant lack of fit ($P=0.59$). These values indicated that the Y_2 model was adequate and reproducible. The ANOVA of the response surface for the % of LnLnLn (Y_3) also showed no significant lack of fit ($P=0.08$), a satisfactory level of R^2 (0.99) (Table 2).

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

Response	R^2	CV ^b	DF ^c	Sum of squares	Mean squares	F-value	P-value
LnLnL	0.99	4.31	5	0.21	0.04	0.58	0.58 ^d
X_1^a			4	2.32	0.58	13.46	0.0021
X_2^a			4	1.93	0.48	11.18	0.0037
X_3^a			4	134.81	33.70	780.74	<.0001
LnLnO	0.99	1.39	5	0.19	0.04	0.91	0.59 ^d
X_1^a			4	3.54	0.89	21.76	0.0005
X_2^a			4	1.03	0.26	6.33	0.0177
X_3^a			4	167.97	41.99	1032.18	<.0001
LnLnLn	0.99	17.61	5	0.06	0.01	12.01	0.08 ^d
X_1^a			4	0.21	0.05	6.41	0.02
X_2^a			4	0.86	0.22	26.10	0.0003
X_3^a			4	3.51	0.88	106.12	<.0001

^a Independent variables; X_1 =Reaction time (h); X_2 =Reaction temperature ($^{\circ}$ C); X_3 =Substrate molar ratio (PO:CO, in which PO is perilla oil and CO is corn oil)

^b CV= Coefficient of Variation

^c DF= Degree of freedom

^d Lack of fit value.

The least squares technique was applied for determining multiple regression coefficients to predict polynomial models for Y_1 , Y_2 , and Y_3 . The values of regression coefficient were $\beta_0= 35.646370$ ($P = 0.09$), $\beta_1= -0.064219$ ($P = 0.5780$), $\beta_2= -0.416493$ ($P = 0.5171$), $\beta_3= -13.930813$ ($P = <0.0001$), $\beta_{11}= 0.006113$ ($P = 0.0059$), $\beta_{22}= 0.002006$ ($P = 0.7046$), $\beta_{33}= 1.655141$ ($P = <0.0001$), $\beta_{12}= -0.001139$ ($P = 0.5079$), $\beta_{13}= -0.002917$ ($P = 0.07314$) and $\beta_{23}= 0.063250$ ($P = 0.0035$) for area % of LnLnL, and $\beta_0= 3.085164$ ($P = 0.8658$), $\beta_1= -0.165533$ ($P = 0.1654$), $\beta_2= -0.790704$ ($P = 0.2240$), $\beta_3= -6.309844$ ($P = 0.0004$), $\beta_{11}= 0.008416$ ($P = 0.0009$), $\beta_{22}= -0.007732$ ($P = 0.1607$), $\beta_{33}= -0.133310$ ($P = 0.3152$), $\beta_{12}= -0.000194$ ($P = 0.9058$), $\beta_{13}= -0.011528$ ($P = 0.1890$) and $\beta_{23}= 0.048750$ ($P = 0.0112$) for area % of LnLnO, and $\beta_0= 7.462592$ ($P = 0.3785$), $\beta_1= -0.032515$ ($P = 0.5217$), $\beta_2= -0.010959$ ($P = 0.9684$), $\beta_3= -4.622147$ ($P = 0.0004$), $\beta_{11}= 0.00434$ ($P = 0.5473$), $\beta_{22}= -0.000994$ ($P = 0.6682$), $\beta_{33}= 0.365141$ ($P = 0.0003$), $\beta_{12}= -0.000027778$ ($P = 0.9701$), $\beta_{13}= 0.002639$ ($P = 0.4843$) and $\beta_{23}= 0.043250$ ($P = 0.0003$)

for area % of LnLnLn respectively. The following second order polynomial equation explains the experimental data containing values of the coefficients of independent variables (X_1 , reaction time; X_2 , reaction temperature; X_3 , substrate mole ratio; Y_1 , area % of LnLnL, Y_2 , area % of LnLnO and Y_3 , area % of LnLnLn).

$$Y_1 = 35.646370 - 0.064219X_1 - 0.416493X_2 - 13.930813X_3 + 0.006113X_1^2 + 0.002006X_2^2 + 1.655141X_3^2 - 0.001139X_1X_2 - 0.002317X_1X_3 + 0.063250X_2X_3$$

$$Y_2 = 3.085164 - 0.165533X_1 + 0.790704X_2 - 6.309844X_3 + 0.008416X_1^2 - 0.007732X_2^2 - 0.133310X_3^2 - 0.000194X_1X_2 - 0.011528X_1X_3 + 0.048750X_2X_3$$

$$Y_3 = 7.462592 - 0.032515X_1 - 0.010959X_2 - 4.622147X_3 + 0.000434X_1^2 - 0.000994X_2^2 + 0.365141X_3^2 - 0.000027778X_1X_2 + 0.002639X_1X_3 + 0.043250X_2X_3$$

In Table 2, the results pointed out that all factors (X_1 , X_2 , X_3) were the primary factor and significant term ($P < 0.05$) for affecting the response of increasing the area % of LnLnL. Fig. 1A presented that area % of LnLnL increased with the increase of time (3.5 % at 16 h and 4.5% at 24h). Area % of LnLnL had negative relation with reaction temperature up to 60°C, after then no relation was shown. Area % of LnLnL had negative relation substrate molar ratio. Area % of LnLnL decreased 8.8 % to 0.89 % when molar ratio increased 1:1 to 1:3. For area % of LnLnO, the factor X_1 , X_2 and X_2 were significant terms for affecting the response ($P < 0.05$), (Table 2). The area % of LnLnO increased linearly with the increased time and also increased slowly with increase of reaction temperature up to 60°C, whereas the area % of LnLnO decreased with increased substrate molar ratio (Fig. 1B). Table 2 also describes that all the three factors (X_1 , X_2 and X_2) were significantly effective ($P < 0.05$) for the response of decreasing area % of LnLnLn. Figure 1C demonstrates that all the three factors had negative influence on area % of LnLnLn.

The response counter plots for area % of LnLnL and LnLnO were presented as a function of substrate mole ratio (1:1-1:3) and reaction time (6-24h) while keeping the reaction temperature constant at 55, 60, and 65°C, respectively (Figure-2 A, B). The counter plots for area % of LnLnL show a steady increase with increasing reaction time (temperature 60°C to 65°C) while for the same temperature the area % of LnLnO decreased. Increasing substrate molar ratio showed low content (area %) of both TAG species. Figure 2C showed the response counter plots of LnLnLn(area %), showing the effect of mole ratio (PO:CO) and reaction temperature on SL reaction times of 6, 15, 24 h. The area % of LnLnLn decreased linearly with increased reaction temperature and decreased substrate molar ratio.

Fatty acid compositions

The fatty acid profile (mol %) determined by gas chromatography (GC) is presented in Table 3. As expected, the most abundant fatty acids in the PO, and CO were linolenic C 18:3 (63.15%), and linoleic C 18:2 (52.23%), respectively. PO contained 91.77% total unsaturated fatty acids (USFA) whereas CO contained 84.78% of USFA. After interesterification, 88.53% USFA was found on the triacylglycerol backbone of the SL. Oleic (22.20%), linoleic acid (34.05%) and linolenic acid (32.39%) were the major USFA in the SL (Table 3) with low ratio of $\omega 6/\omega 3$ (1:1). Whereas, in CO the ratio of $\omega 6/\omega 3$ was very high (84:1). Previous studies [4] reported that $\omega 3$ and $\omega 6$ ratio significantly influence the ratio of the ensuing eicosanoids (hormones) and metabolic function in humans. Healthy ratio of $\omega 3$ and $\omega 6$ was reported from 1:1 to 1:4 previously [8, 9]. In this study, the ratio of $\omega 6/\omega 3$ was around 1:1 in SL product whereas in CO it was ~ 85:1. The $\omega 3/\omega 6$ ratio (~1:1) observed in SL lead to be speculated more beneficial than commonly used edible oil.

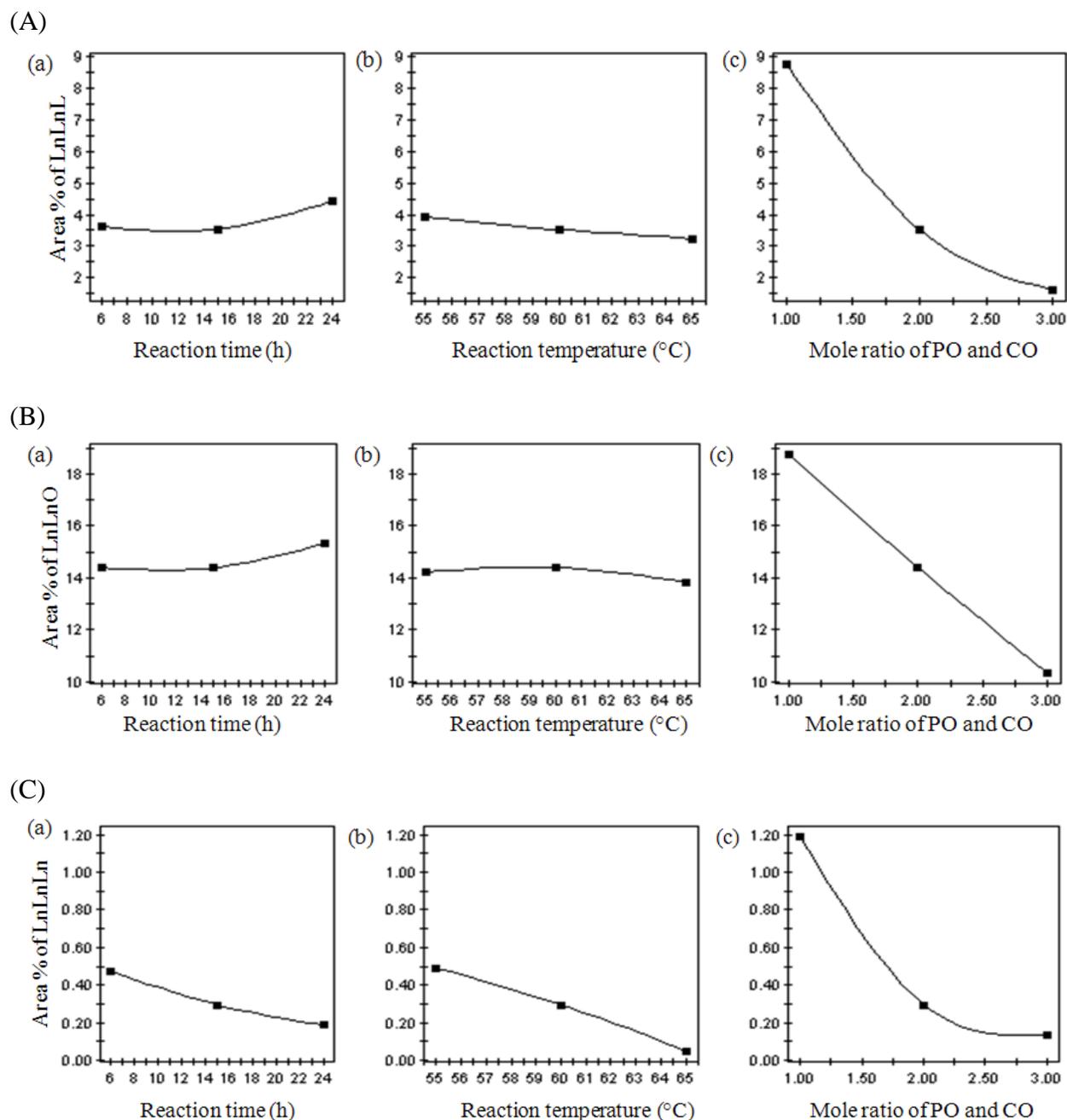


Figure 1. Prediction plot for increasing (A) area % of LnLnL, (B) area % of LnLnO and (C) decreasing area % of LnLnLn of structural lipid by effects of (a) reaction time; (b) reaction temperature; (c) mole ratio of Perilla oil (PO) and corn oil (CO).

The physical properties of oils are greatly influenced not only by the length and the number of double bond of fatty acids but also by the distributions of fatty acids on stereo specific numbering (*sn*) positions of fats and oils. Interestification can be carried out to an equilibrium condition, at which point the fatty acids assume almost random distribution in the *sn* positions of triglycerides [25]. FA at the *sn*-2 position of TAG is known as nutritionally beneficial since they can be conserved during digestion and easily absorbed in the body [23]. Positional fatty acid composition is presented in Table 3. Higher levels of

unsaturated fatty acids (99.24%) were observed at sn-2 position in the SL. This may occur because of sufficient level of oleic (24.78%), linoleic (40.99%) and linolenic (33.47%) acid appeared in the SL at sn-2 position. Content of ALA of SL in sn-2 position was higher than CO. as a results the ratio of $\omega 6/\omega 3$ was lower in SL (~1:1) than the CO (~164:1).

Table 3. Total and positional distribution of fatty acid content of perilla, soybean oil and structural lipids (area%).

Fatty acid	Perilla oil			Corn oil			Structural lipid ^a		
	TG	Sn-2	Sn-1,3 ^c	TG	Sn-2	Sn-1,3	TG	Sn-2	Sn-1,3
C 16:0	6.25±0.01	0.49±0.08	9.16±0.01	13.01±0.03	1.00±0.20	19.02±0.01	9.35±0.02	0.66±0.02	13.55±0.01
C 18:0	1.86±0.10	0.01±0.01	2.94±0.04	2.21±0.01	0.20±0.10	3.21±0.02	2.12±0.02	0.10±0.01	3.28±0.01
C 18:1	14.51±0.02	16.41±0.02	13.62±0.11	31.94±0.05	32.66±1.1	31.54±0.03	21.10±0.02	24.18±0.01	20.72±0.02
C 18:2	14.23±0.02	17.30±0.30	12.46±0.10	52.22±0.02	65.75±0.9	45.51±0.02	35.15±0.04	41.59±0.02	30.60±0.02
C 18:3	63.15±0.10	65.79±0.12	61.82±0.12	0.61±0.01	0.39±0.01	0.72±0.01	32.28±0.01	33.47±0.02	31.85±0.11
ΣSFA	8.11±0.03	0.50±0.11	12.10±0.01	15.22±0.02	1.20±0.01	22.23±0.02	11.27±0.01	0.96±0.01	16.83±0.02
ΣUSFA	91.89±0.12	99.50±0.03	87.90±0.02	84.77±0.01	98.80±0.02	77.77±0.01	88.53±0.02	99.24±0.11	83.17±0.02
$\omega 6/\omega 3^b$	0.23±0.01	0.26±0.08	0.20±0.03	85.60±0.01	168.58±0.03	63.21±0.02	1.05±0.01	1.23±0.02	0.96±0.01

All data are mean values ± standard deviations of duplicate measurements.

^aStructural lipid was synthesized with the molar ratio of PO: SO (1:1) at 59 °C for 16h. Lipozyme TL IM (10% of the total substrate)

was used for the reaction.

^b $\omega 6$; C18:2 linoleic acid. $\omega 3$; C18:3 α -linolenic acid (ALA)

^cSn 1, 3 (area %) = $(3T-Sn_2)/2$, Where T is total fatty acid.

TAG analysis

TAG species predetermined composition or different proportional distribution is one of the most important properties of any enzymatic reaction [24]. The principle of TAG separation based on their equivalent carbon no (ECN, i.e. molecular weight and degree of unsaturation). The higher molecular

Table 4. Triacylglycerol (TAG) Composition (area %) of perilla oil, corn oil and structural lipid.

ECN ^a Number	Proposed TAG species ^b	Perilla oil	Corn oil	Structural Lipid ^c
36	LnLnLn	39.46±0.01	ND	1.59±0.02
38	LnLnL	13.56±0.02	ND	9.99±0.01
40	LnLnO	24.20±0.02	ND	20.31±0.02
40	LnLnP	5.56±0.01	ND	1.43±0.01
42	LLL	ND ^d	16.22±0.01	16.16±0.01
42	OLnL	6.10±0.01	ND	6.57±0.01
44	LLO	ND	29.69±0.01	10.63±0.02
44	PLL	2.28±0.01	14.48±0.01	11.65±0.01
46	LOO	ND	13.28±0.02	4.31±0.01
46	PLO	ND	15.20±0.02	5.71±0.01
46	PLP	ND	2.24±0.01	1.25±0.01
48	OOO	ND	3.33±0.02	0.58±0.02
48	POO	ND	5.56±0.01	1.59±0.02
	Others ^e	8.84±0.04	ND	8.23±0.02

^aEquivalent carbon number (ECN) = CN-2DB, where CN is carbon number of TAG and DB is total number of double bonds in TAG.

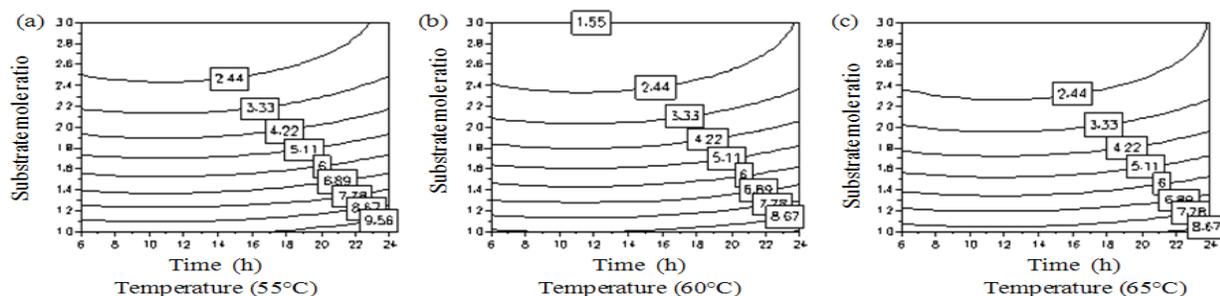
^bLn=Linolenic acid; L=Linoleic acid; O=Oleic acid; P=Palmitic acid; S=Stearic acid.

^cStructural lipid was synthesized with the molar ratio of PO: SO (1:1) at 59 °C for 16h. Lipozyme TL IM (10% of the total substrate) was used for the reaction.

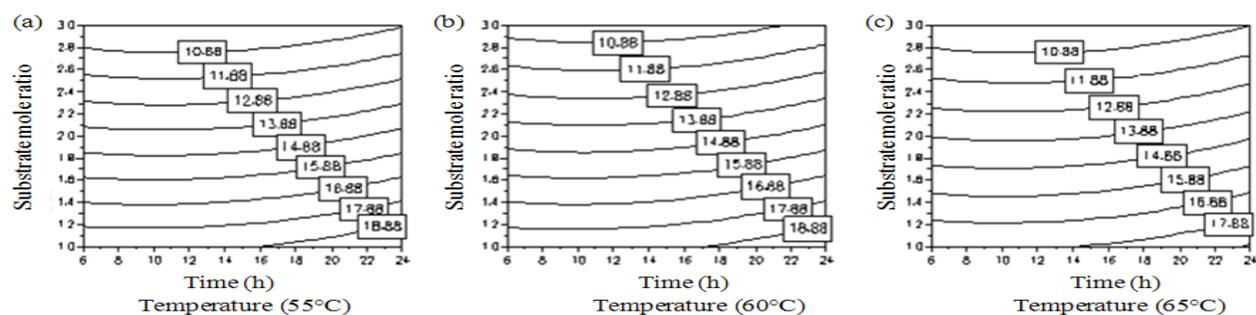
^dND= not detected

^e Unidentified peaks.

(A)



(B)



(C)

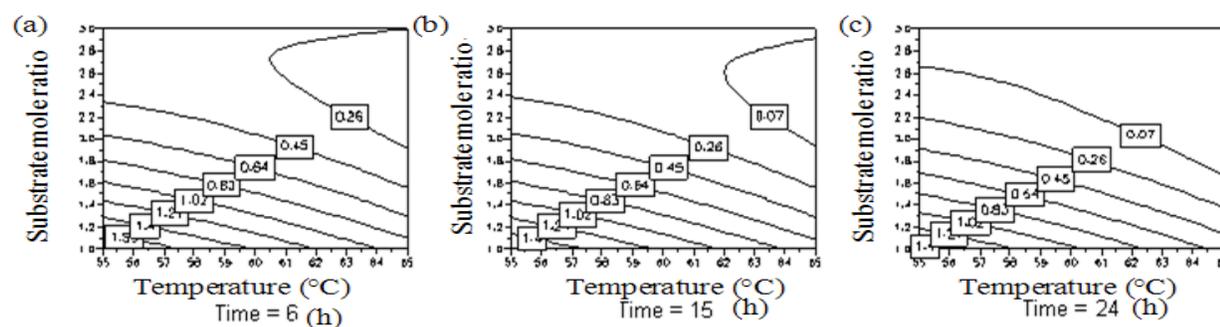


Figure 2. Response contour plot for increasing (A) LnLnL (area %), (B) LnLnO (area %) showing the effect of mole ratio (PO: CO, in which PO is Perilla oil and CO is corn oil) and reaction time to structural lipid at different reaction temperatures.

(a) 55°C; (b) 60°C; (c) 65°C. (C) Response contour plot for decreasing LnLnLn (area %) showing the effect of mole ratio (PO: CO, in which PO is perilla oil and CO is corn oil) and reaction temperature to structural lipid at different times: (a) 6 h; (b) 15h; (c) 24h

weight of a TAG, the longer was the time needed before it could be eluted. However, the presence of unsaturation in a TAG increased its polarity, thus decreasing its retention time [26]. The most abundant TAG species in the PO were LnLnLn (39.45%), LnLnL (13.55%) and LnLnO (24.19%) whereas major TAG species in the CO were LLL (16.12%), LLO (29.59%) and PLO (15.50%). (Table 4, Fig. 3). Our results showed substantial changes in the TAG species in SL comparing physical blend (PB) (Table 4) due to interesterification reaction. The variation in concentrations of several TAG species in the SL were observed (Fig. 3). The major TAG species of PO (LnLnLn, LnLnL) and CO (LLO, LOO, PLO) were

decreased in SL. LnLnO (20.41%), LLL (16.06%), LLO (10.66 %), PLL (11.69%) were arise as the major peaks of SL.

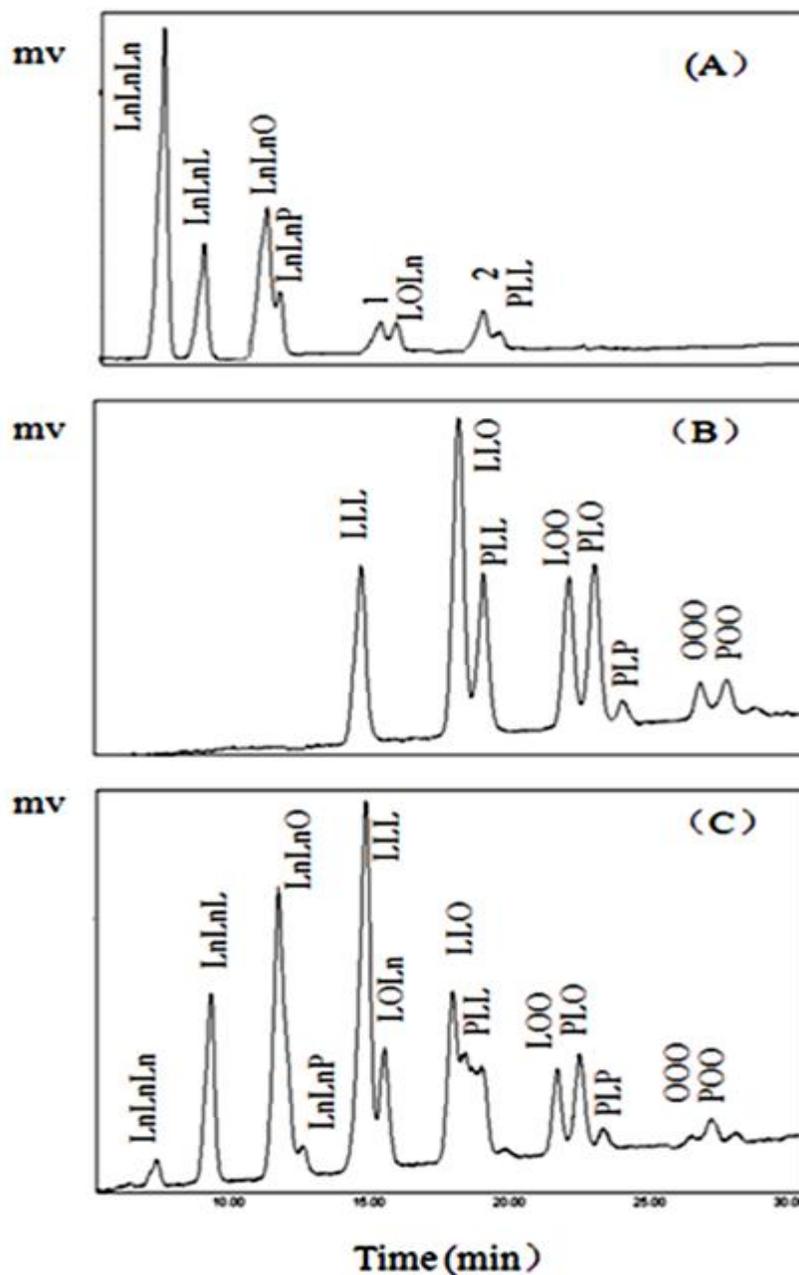


Figure 3. TAG chromatogram of Perilla oil (A), Corn oil (B), and structural lipid (C). Structural lipid was synthesized with the molar ratio of PO: CO (1:1) at 59°C for 16h. Lipozyme TL IM (10% of the total substrate) was used for the reaction.

In this study, reverse-phase HPLC result showed that SL content very low % of LnLnLn (1.49%) species in TAG. However, SL contained 32.39 % of ALA in fatty acid composition. These two results indicate that the interesterification reaction can be stabilized the ALA component in the TAG molecules by distributing it within the TAG species.

Interesterification effect on the Rancimat test

The susceptibility of any oil or fat can be verified by estimating oxidative degradation as well as rancidity test. The result of Rancimat test is presented in Figure 4. Higher protection factor suggests stronger oxidative stability [16]. PO showed lowest oxidative stability comparing control SL. It could be occurred because it contains lots of PUFA, mostly ALA therefore it easily oxidized. As SL contained more than 32 % of ALA and 88% PUFA it could be oxidized easily. However, the SL (12.23 h) is more oxidized stable than PO (9.24 h), as it contains balance linoleic, ALA and saturated fatty acid. Incubation period for CO was observed 15.12 h. SL (control) showed low induction time than CO. This could be happened because SL contained high amount of PUFA. When different antioxidants added with SL, higher incubation period (h) was observed than the substrates and control SL. Highest incubation period (29.88 h) was observed in SL-3 in rancimat test. This indicates that rosemary extract, catechin and their mixer form were effective to prolong the incubation time of SL.

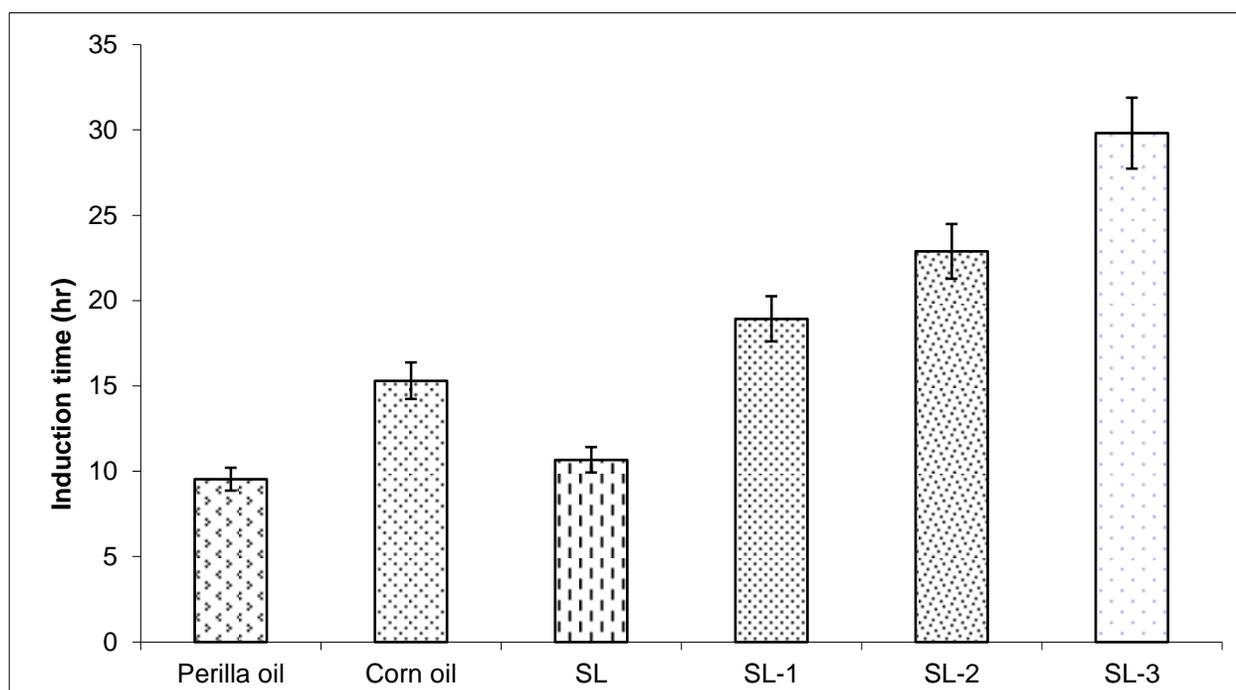


Figure 4. Induction period for perilla oil (PO), corn oil (CO), structural lipid (SL) and SL with different antioxidants by the Rancimat test.

Structural lipid was synthesized with the molar ratio of PO: CO (1:1) at 59 °C for 16h. Lipozyme TL IM (10% of the total substrate) was used for the reaction. SL: without antioxidant, SL-1: SL+ rosemary 200 ppm, SL-2: SL+ BHT 200 ppm, SL-3: SL + catechin 200 ppm.

Conclusion

In this study, the RSM predicted model of the lipase-catalyzed interesterification of PO and SO was attempted. ALA has beneficial physiological effects. Increasing the content of ALA in lipid is desirable as it helps to decrease the $\omega 6/\omega 3$ ratio. Having low $\omega 6/\omega 3$ ratio of oils/lipids is also desirable for preventing heart diseases and blood LDL level. The synthesized SL showed a good source of ALA as well as low $\omega 6/\omega 3$ ratio lipid. SL also shows higher oxidative stability than PO, since it contains a

balance of saturated and unsaturated fatty acid. This study also shows the effectiveness of its use as an antioxidant to prevent the oxidation of SL.

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