As. J. Food Ag-Ind. **2013**, 6(3), 157-170

OPEN ACCESS

Asian Journal of Food and Agro-Industry

ISSN 1906-3040

Available online at www.ajofai.info

Research Article

Optimization of the production of alpha linolenic acid enrichedstructural lipids and evaluation of oxidative stability underaccelerated storage conditions

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Abstract

Response surface methodology was used to optimizethe production conditions of alpha linolenic acid (ALA) enriched structural lipids (SL) from perilla oil (rich inω3:ALA) and corn oil (rich in ω6). Reaction condition was optimized using central composite design with reaction time (6-24hr, X_1), reaction temperature (50-65ºC,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), 59ºC (X_2) and 1:1ratio (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) ω6:ω3 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 a combination form of antioxidants.

Keywords: Response surface methodology, enzymatic interesterification, ALA, omega-6/omega-3 ratio,TAG species, Bangladesh, Korea.

Introduction

Biological compounds from alpha linolenic acid (ALA) have been proven to prevent blood clotting, cancer,decrease the inflammatory process andbe beneficial to individuals suffering from arthritis[1, 2]. ALA is one major source of plant ω3 (C 18:3) fatty acids which arethe metabolic precursor to C 20:5 ω3 and C 22:6 ω3 PUFA [3].Evidence suggests that ALA, along and other omega 3 fatty acids, has been associated with decreasing levels of blood cholesterol, prevention against cardiovascular disease and management of other chronic disorders[4,5]. Perilla oil (PO) is a rich source of ALA which is also able to decrease plasma histamine levels and increase higher hypo-cholesterolemic ability [6,7].Generally,vegetable oils (such as corn, soybean, safflower, and sunflower oil) contain

high amounts of ω6 (linoleic acid C18:2) fatty acid. Increased consumption of vegetable oils whichare rich in ω6 fatty acids has drastically shifted the dietary ω6/ω3ratio. Various studies indicate that human beings evolved on a diet with a ratio of ω6/ω3 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 ω6/ω3 fatty acids is needed for the prevention and management of chronic diseases, suppressed inflammation in patients with rheumatoid arthritis, prevention of asthma and reduction proteins and hemostatic factors[4,8, 9, 10,11]. One previous study reported that PO might be used to optimize the ratio of ω6/ω3[12], yet few studies were published on modifying other vegetable oils with PO.

Vegetable oils were modified previously by lipase-catalyzed reaction[13,14, 15,16].In the present study, SL by lipase-catalyzed interesterificationfrom perilla and corn oil was attempted in order to produce ALA enriched SL, as well as produce low ω6/ω3 ratio lipids. 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 produced SL was analyzed to obtainitscharacteristics.

Lipid oxidation is a major quality problem in that antioxidants can prevent the oxidative deterioration, to maintain the quality and to extend the shelflife of fats and oils. Some studies showed that antioxidants can prevent 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 activity was observed because it contains phenolic diterpenes such as carnosic acid and carnosol[22].The effectiveness of rosemary extract and catechinas antioxidantswereinvestigated to prevent or delay the oxidation of PO, COand their SL under acceleratedstorage 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). LypozymeRMIM (150 IUN/g of catalytic activity with a bulk density of 350-450 kg/m^3, a partical size of 0.3-0.6 mm and a water content of 2-3 w/w%)from *Rhizomucormiehei*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 ,δtocopherols) 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 (*Xi)* and their levels are shown in Table 1.

In enzymatic transesterification reaction, reaction occurrs in TAG-TAG bond. Consequently, the difference is observed in TAG species in product from the substrate after the reaction. Since ALA is a 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. Thusas a 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.

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]X_1=time (hr); X_2=reaction temperature (°C); X_3= substrate mole ratio; Y_1 =Area % of LnLnL; Y_2 =Area % of LnLnO; Y_3 =Area % of LnLnLn.

The second-order polynomial equation used for optimization of the reaction conditions is as follows:

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

where β_o, 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 [24] 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 are expressed as 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 (1:1mole ratio), together for synthesis of SL. LipozymeTLIM from *Thermomyceslanuginosea* (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 0.50μm disposable syringe filter unit to remove lipase.

Fatty acid composition

The sample was separated by thin-layer chromatography (TLC) on a silica gel 60 F_{254} 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 triacylglyceried (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 methanolicNaOH (1.5 mL). The mixtureswere heated at 100˚C for 5 min and cooled at room temperature. BF_3 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 chromatograph (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 was heated to 150˚C and held for 5 min. Then, heat was 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 $CaCl_2$(2.2% indistilled 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 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 µLof 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 Rancimatanalyzer (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 [24]was used to perform statistical analysis. Duncan's multiple range tests were performed to determine significance of difference at $\rho < 0.05$.

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 threevariables ($X_{1,}$ 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).

The least squares technique was applied for determining multiple regression coefficients to predict polynomial models for $Y_{1,}$ $Y_{2,}$ andY_3. The values of regression coefficient were β_o= 35.646370(P = 0.09), β_1= - 064219(P = 0.5780), β_2= -0.416493 (P = 0.5171), β_3= -13.930813(P = <0.0001), β_{11}= 0.006113(P = 0.0059),β_{22}= 0.002006(P = 0.7046), β_{33}= 1.655141(P = <0.0001), β_{12}= -0.001139(P = 0.5079), β_{13}= -0.002917(P = 0.0.7314) and β_{23}= 0.063250(P = 0.0035) for area % of LnLnL, and β_o= 3.085164(P = 0.8658), β_1= -0.165533(P = 0.1654), β_2= -0.790704 (P = 0.2240), β_3= -6.309844(P = 0.0004), β_{11}= 0.008416(P = 0.0009),β_{22}= -0.007732(P = 0.1607), β_{33}= -0.133310(P = 0.3152), β_{12}= -0.000194(P = 0.9058), β_{13}= -0.011528(P = 0.1890) and β_{23}= 0.048750(P = 0.0112) for area % of LnLnO, and β_o= 7.462592(P = 0.3785), β_1= -0.032515(P = 0.5217), β_2= -0.010959 (P = 0.9684), β_3= -4.622147(P = 0.0004), β_{11}= 0.00434(P = 0.5473),β_{22}= -0.000994(P = 6682), β_{33}= 0.365141(P = 0.0003), β_{12}= -0.000027778(P = 0.9701), β_{13}= 0.002639(P = 0.4843) and β_{23}= 0.043250(P = 0.0003) for area % of LnLnLnrespectively. 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).

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

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

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

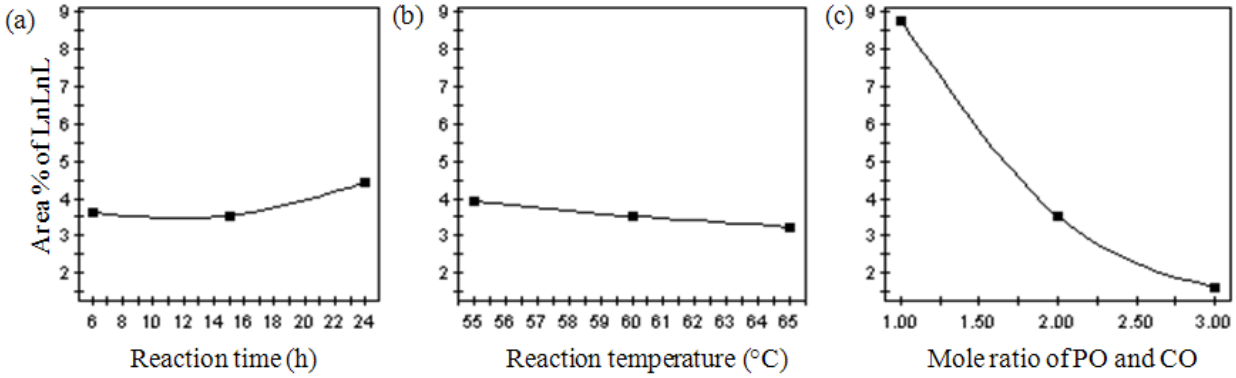
Table 2.Analysis of variance (ANOVA) of the independent variables for response surface model.

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

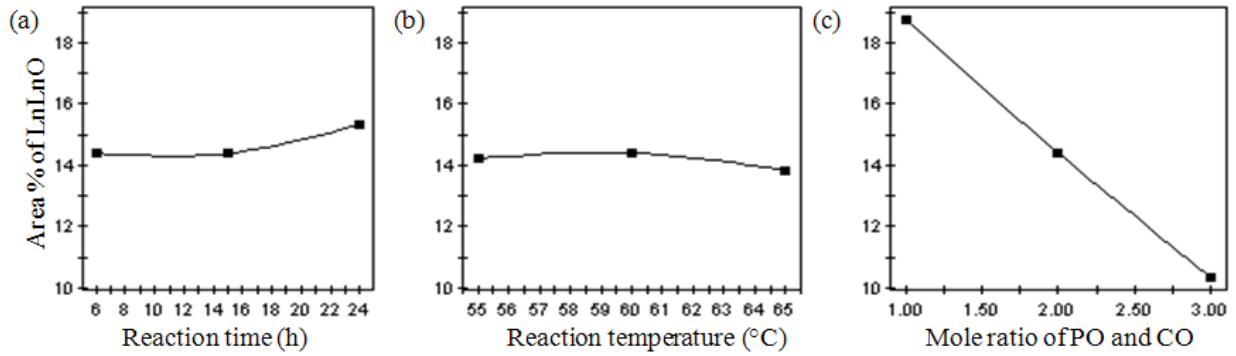
[a]Independent variables; $X_{1=}$ Reaction time (h), $X_{2=}$ Reaction temperature (°C); X_3 = Substrate molar ratio (PO:CO, in which PO is perilla oil and CO is corn oil)
[b]CV=Coefficient of Variation
[c] DF= Degree of freedom
[d] Lack of fit value.

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. 1Ashows 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 LnLnLhad negative relation substrate molar ratio.Area % of LnLnLdecreased8.8%to 0.89%when molar ratio increased 1:1 to 1:3. Forarea % of LnLnO, the factor X_1,X_2andX_2were significant terms for affecting the response ($P< 0.05$), (Table 2). The area % of LnLnOincreased linearly with the increased time and also increased slowly with increase of reaction temperature up to 60°C. Whereasthe area % of LnLnO decreased with increased substrate molar ratio (Fig. 1B). Table 2 also shows that all the three factors (X_1,X_2 andX_2) were significantly effective ($P< 0.05$) for the response of decreasing area % of LnLnLn. Figure 1Cdemonstrates that all the three factors had negative influence on area % of LnLnLn.

(A)



(B)



(C)

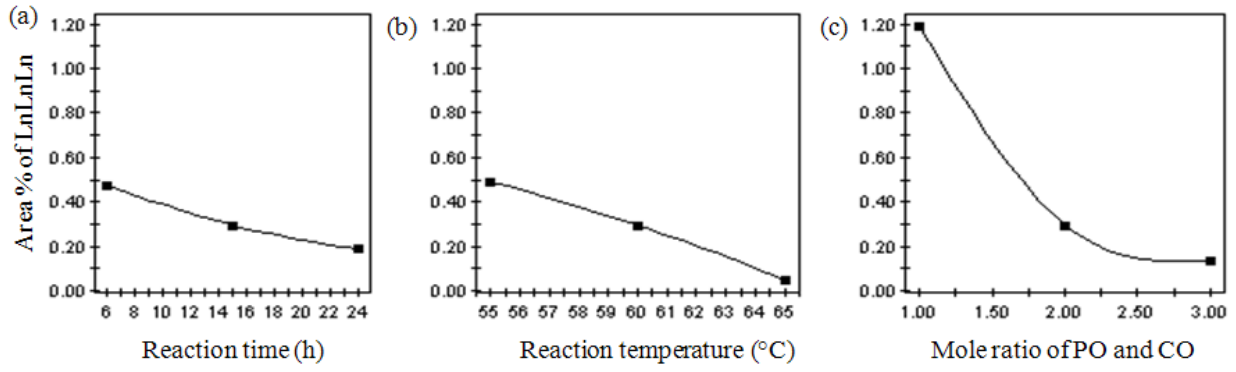
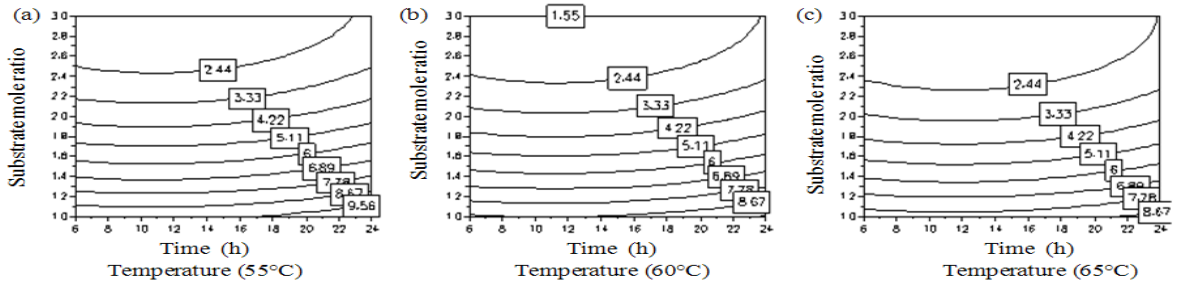


Figure 1. Prediction plot for increasing (A) area % of LnLnL, (B) area % of LnLnOand (C) decreasing area % of LnLnLnof structural lipids by effects of (a) reaction time; (b) reaction temperature; (c) mole ratio of perilla oil (PO) and corn oil (CO).

(A)



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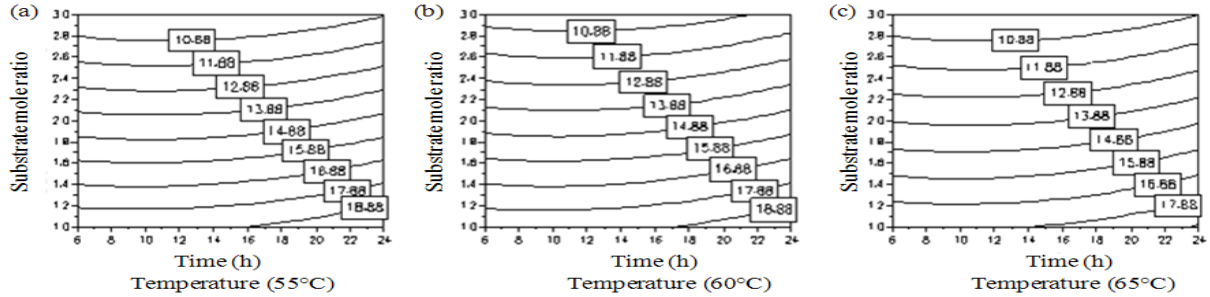

(C)

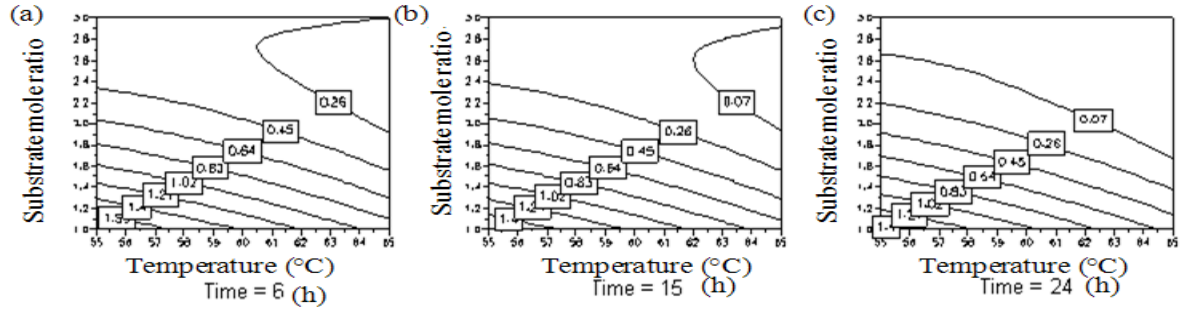

Figure 2. Response contour plot for increasing (A) LnLnL (area %), (B)LnLnO (area %) showing the effect of mole ratio (PO:CO, in which PO is perilla oil and CO is corn oil) and reactiontime to structural lipids at different reaction temperatures: (a) 55°C; (b) 60°C; (c) 65°C. (C) Response contour plot for decreasing LnLnLn (area %) showing the effect of mole ratio (PO:CO) and reaction temperature to structural lipids at different times: (a) 6 h; (b) 15h; (c) 24h.

The response counter plots for area % of LnLnL and LnLnOwere presented as a function of substratemole ratio (1:1-1:3) and reaction time (6-24h) while keeping the reaction temperature constant at 55, 60, and 65°C, respectively (Figure2 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 shows 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 composition

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 COwerelinolenicC 18:3 (63.15%), and linoleicC 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 ofω6/ω3 (1:1). Whereas, in CO the ratio of ω6/ω3 was very high (84:1).Previous studies[4] reported that ω3 and ω6 ratio significantly influence the ratio of the ensuing eicosanoids (hormones) and metabolic function in humans. Healthy ratio of ω3 and ω6 was reported from 1:1 to 1:4 previously [8, 9]. In this study, the ratio of ω6/ ω3 was around 1:1 in SL product whereas in CO it was ~ 85:1. The ω3/ω6 ratio (~1:1) observed in SL lead to be speculated morebeneficial than commonly used edible oil.

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
∑SFA	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
∑USFA	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
ω6/ω3[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.

[a]Structural 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]ω6; C18:2 linoleic acid. ω3; C18:3 α-linolenic acid (ALA)

[c]*Sn* 1, 3 (area %) = (3T-Sn_2)/2, Where T is total fatty acid.

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 distribution 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 compositionis 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 result the ratio of ω6/ω3 was lower in SL (~1:1) than the CO (~164:1).

TAG analysis

TAG speciespredetermined composition or different proportional distribution is one of the most important properties of any enzymatic reaction[24].The principle of TAG separationis based on their equivalent carbon number (ECN, i.e. molecular weight and degree of unsaturation). The higher molecular 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

mostabundant 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%) (Table4, Fig.3).The 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%) arose as the major peaks of SL.

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

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

[a]Equivalent carbon number (ECN) = CN-2DB, where CN is carbon number of TAG and DB is total number of double bonds in TAG.
[b]Ln=Linolenic acid; L=Linoleic acid; O=Oleic acid; P=Palmatic acid; S=Stearic acid.
[c]Structural 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.
[d] ND= not detected
[e]Unidentified peaks.

In this study, reverse-phase HPLC result showed that SL content is a 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 interesterificationreaction can stabilize 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 compared to control SL. It could occurbecause it contains a lot of PUFA, mostly ALA, therefore it is easily oxidized. As SL contained more than 32% of ALA and 88% PUFA it could be oxidizedeasily.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 occur because SL contained high amount of PUFA. When different antioxidantswere 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 inRancimat test. This indicates that rosemary extract, catechinand their mixer form were effective to prolong the incubation time of SL.

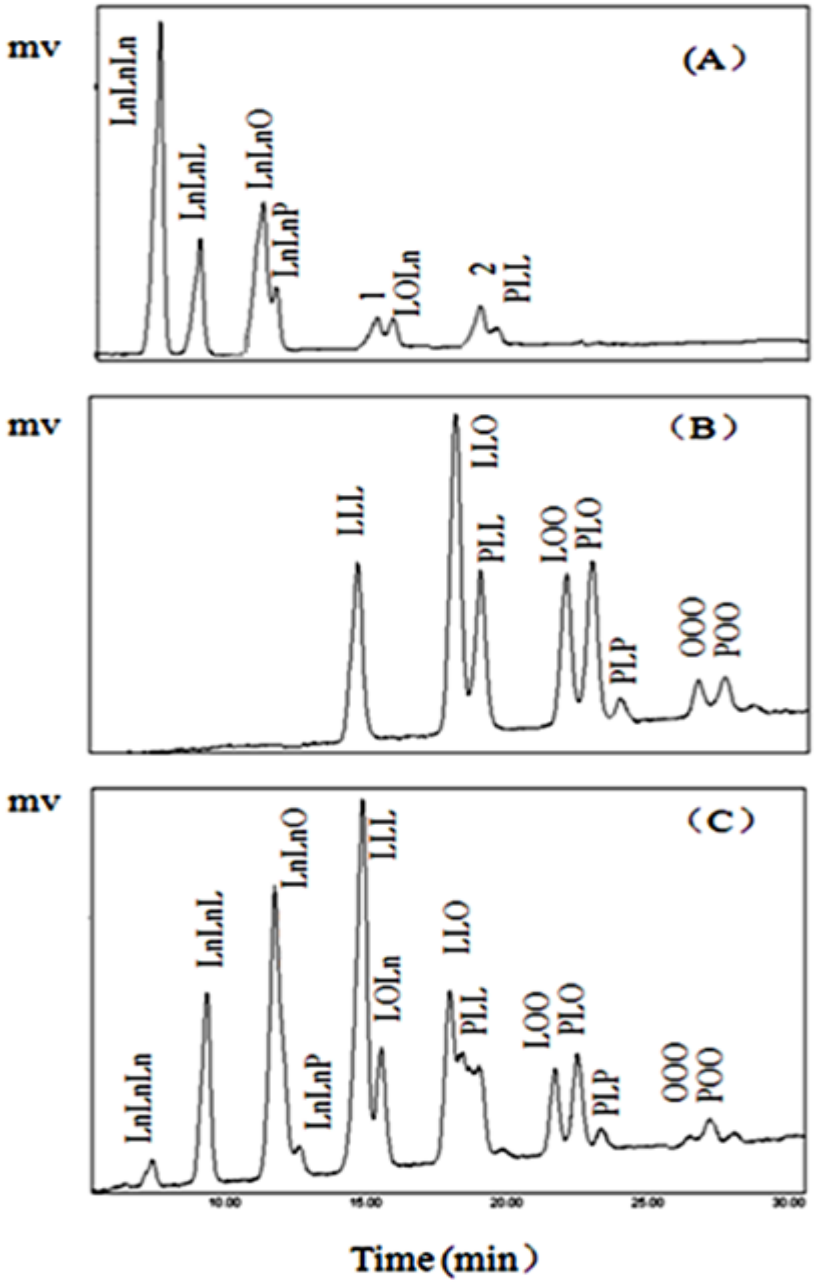

Figure3.TAG chromatogram ofPerilla oil (A), Corn oil (B), and structural lipid (C). Structural lipid was synthesized with the molar ratio of PO:CO (1:1) at 59°C for 16h.Lipozyme TL IM (10% of the total substrate) was used for the reaction.

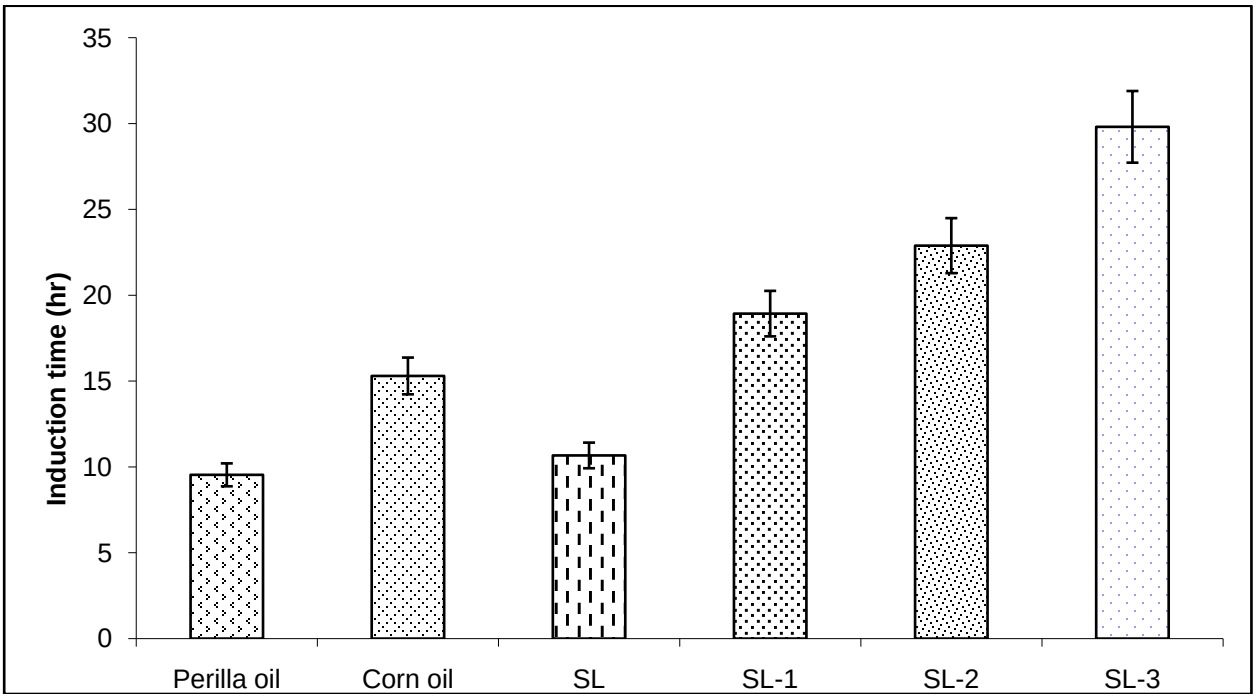

Figure4.Induction period for perilla oil (PO), corn oil (CO), structural lipid (SL) and SL with different antioxidants by the rancimat test. Structural lipid was synthesized with the molar ratio of PO:CO (1:1) at 59°C for 16h.Lipozyme TL IM (10% of the total substrate) was used for the reaction.SL: without antioxidant, SL-1: SL+ rosemary200 ppm, SL-2: SL+ BHT200 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 lipids is desirable as it helps to decrease the ω6/ω3 ratio. Having low ω6/ω3 ratio of oils/lipids is also desirable for preventing heart disease and improving blood LDL level. The synthesized SL were shown to be a good source of ALA, as well as having low ω6/ω3 ratiolipids. SL also shows higher oxidative stability than PO, since it contains a balance of saturated and unsaturated fatty acids. This study also shows the effectiveness ofan antioxidant to prevent the oxidation of SL.

References

1. Sinclair, A. J., Attar-Bashi, A. M., & Li, D. (2002). What is the Role of α-Linolenic Acid for Mammals? ***Lipids,*** 37, 1113-1123.

2. Fortin, P. R., Lew, R. A., Liang, M. H., Wright, E. A., Beckett, L. A., Chalmers,T. C., &Sperling, R. I. (1995). Validation of a meta-analysis: The effects of fish oil in rheumatoid arthritis**. *Journal of Clinical Epidemiology*,** 48:1379-1390.

3. Harris, W. S. (1997). n-3 Fatty acids and serum lipoproteins: human studies. ***The American Journal of Clinical Nutrition***, 65: 1645-1654.

4. Sandars, T. A. B., Oakley, F. R., Miller, G. J., Mitropoulous, K. A., Crook, D., & Oliver, M. F. (1997). Influence of n-6 versus n-3 polyunsaturated fatty acids in diets low in saturated fatty acids on plasma lipoproteins and hemostatic factors. ***Arteriosclerosis, Thrombosis and Vascular Biology,*** 17, 3449-3460.

5. Davidson, M. H., Stein, E. A., Bays, H. E., Maki, K. C., Doyle, R. T., Shalwitz, R. A., Ballantyne, C. M., & Ginsberg, H. N. (2007). Efficacy and tolerability of adding prescription omega-3 fatty acids 4g/d to Simvastatin 40mg/d in hypertriglyceridemic patients: An 8-week, randomized, double-blind, placebo-controlled study. ***Clinical Therapeutics,*** 29: 1354-1367.

6. Shin, T.Y., Kim, S.Y., Kim, S.H., Kim, Y.K., Park, H.J., Chae, B.S., Jung, H.J. &Kim, H.M. (2000). Inhibitory effect of mast cell-mediated immediate-type allergic reactions in rats by *Perillafrutescens*.***Immunopharmacology and Immunotoxicology***, 22, 489-500.

7. Watanabe, M.I., Umekawa, H., Takahashi, T. &Furuichi, Y. (2000). Comparative effects of safflower oil and perilla oil on serum and hepatic lipid levels, fatty acid compositions of serum and hepatic phospholipids, and hepatic mRNA expressions of 3-hydroxy-3-methylglutaryl CoA reductase, LDL receptor, and cholesterol 7alpha-hydroxylase in young and adult rats. ***Food Research International***, 33, 893-900.

8. Simopoulos, A. P. (2002). The Importance of the Ratio of Omega-6/Omega-3 Essential Fatty Acids, ***Biomed. Pharmacother***, 56, 365-379.

9. Simopoulos, A. P. (2006).Evolutionary aspects of diet, the omega-6/omega-3 ratio and geneticvariation: nutritional implications for chronic diseases. ***Biomedicine & Pharmacotherapy,*** 60, 502-507.

10. Kim, Y., Ji, S. K., &Choi, H. (2000). Modulation of liver microsomalmonooxygenasesystem by dietary n-6/n-3 ratios in rat hepatocarcinogenesis. ***Nutrition and Cancer,*** 37, 65-72.

11. Osorio,N. M., Ferreira,D. S., Gusmao,J. H., &Fonseca, M. M. R. (2001). Response surface modeling of the production of ω-3 polyunsaturated fatty acids-enriched fats by a commercial immobilized lipase. ***Journal of Molecular Catalysis B: Enzymatic***, 11, 677-686.

12. Shin, H. S. & Kim, S.W. (1994). Lipid Composition of Perilla Seed, ***Journal of American Oil Chemists' Society***,71, 619–622.

13. Li, Z.Y, &Ward, O. P. (1993). Enzymecatalysedproduction of vegetable oils containing omega-3polyunsaturate fatty acid. ***Biotechnology Letters***, 15, 185-188.

14. Yi, H. J., Fang, C. H. &Chia, H. F. (1998). The incorporation of n-3 polyunsaturatedfatty acids

15. into Acylglycerolsof Borage Oil via Lipase-Catalyzed Reactions. ***Journal of American Oil Chemists' Society***, 75, 961-965.

16. Xu, X., Mu, H., Hoy, C. E., Nissen, J. A. (1999). Production of specifically structured lipids by enzymatic interesterification in a pilot enzyme bed reactor: process optimization by response surface methodology**.** ***Fett/Lipid,*** 101, 207-214.

17. Mitra, K., Lee, J. H., Lee, K. T, Kim, S.A. (2010). Production tatic and physiochemical properties of low ω6/ ω3 ratio structured lipid synthesized from perilla and soybean oil. ***InternationalJournal of Food Science & Technology***, 45, 1321-1329.

18. Yang, M. H., Lin, H. J., Choong, Y. M. (2002). A rapid gas chromatographic method for direct determination of BHA, BHT and TBHQ in edible oils and fats. ***Food Research International***, 35, 627-633.

19. Lee, J. H., Lee, K. T., Akon, C. C. Shin, K. C., & Kim, M. R. (2006). Antioxidant Evaluation and Oxidation Stability of Structured Lipids from Extravirgin Olive Oil and Conjugated Linoleic Acid. ***Journal of Agriculture and Food Chemistry***, 54, 5416-5421.

20. Ozturk, S.,&Cakmakci, S. (2006). The effect of antioxidants on butter in relation to storage temperature and duration. ***European Journal of Lipid Science and Technology***, 108, 951-959.

21. Norata, G. D., Marchesi, P., Passamonti, S., Pirillo, A., Violi, F., &Catapano,A.L. (2007). Anti-inflammatory and anti-atherogeniceffects of cathechin, caffeic acid and trans-resveratrol inapolipoprotein E deficient mice. ***Atherosclerosis***, 191, 265-271.

22. McMillan, B., Riggs, D. R., Jackson, B. J., Cunningham, C. &McFadden, D. W. (2007). Dietary influence on pancreatic cancer growth by catechinand inositol hexaphosphate. ***TheJournal of Surgical Research***, 141, 115-119.

23. Lee, J. H., Kim, M. R., Kim, R. K., Kim, I. H., &Lee K.T. (2003). Characterization of Lipase-Catalyzed Stuctured Lipids from Selected Vegetables Oils with Conjugated Linoleic Acid: their oxidative stability with rosemary extracts**.** ***Journal of Food Science***, 68, 1653-1658.

24. Mitra, K., Kim, S.A., Lee, J. H., Choi, S.W., Lee, K. T. (2010). Production and characterization of α – linolenic acid enriched structured lipids from lipase-catalyzed interesterification. ***Food Science and Biotechnology,*** 19, 57-62.

25. SAS Institute (2000). Statistical Analysis Software, release 8.2. Cary, NC, USA.

26. Lau, P. Y., Hammond, E. G., & Ross, P. F. (1982). Effects of randomization of theoxidation of corn oil. ***Journal of American Oil Chemists' Society***, 59, 407-411.

27. Tan,C.P., &Che, Y.B (2000). Different scanning calorimetric analysis of edible oils: Comparison of thermal properties and chemical composition.***Journal of American Oil Chemists'Society***, 77, 143-155