Studies of Reaction Variables for Lipase-Catalyzed Production of Alpha-Linolenic Acid Enriched Structured Lipid and Oxidative Stability with Antioxidants

Kanika Mitra, Jung-Ah Shin, Jeung-Hee Lee, Seong-Ai Kim, Soon-Taek Hong, Chang-Keun Sung, Cheng Lian Xue, and Ki-Teak Lee

Abstract: Alpha-linolenic acid (ALA) enriched structured lipid (SL) was produced by lipase-catalyzed interesterification from perilla oil (PO) and corn oil (CO). The effects of different reaction conditions (substrate molar ratio [PO/CO 1:1 to 1:3], reaction time [0 to 24 h], and reaction temperature [55 to 65 °C]) were studied. Lipozyme RM IM from *Rhizomucor miehei* was used as biocatalyst. We obtained 32.39% of ALA in SL obtained under the optimized conditions (molar ratio—1:1 [PO:CO], temperature—60 °C, reaction time—15 h). In SL, the major triacylglycerol (TAG) species (linolenoyl-linolenoyl-linolenoyl glycerol [LnLnLn], linolenoyl-linolenoyl-linoleoyl glycerol [LnLnL]) mainly from PO and linoleoyl-linoleoyl-oleoyl glycerol (LLO), linoleoyl-oleoyl-oleoyl glycerol (LOO), palmitoyl-linoleoyl-oleoyl glycerol (PLO) from CO decreased while linolenoyl-linolenoyl-oleoyl glycerol (LnLnO) (18.41%), trilinolein (LLL) (9.06%), LLO (16.66%), palmitoyl-linoleoyl-linoleoyl glycerol (PLL) (9.69%) were increased compared to that of physical blend. Total tocopherol content (28.01 mg/100 g), saponification value (SV) (192.2), and iodine value (IV) (161.9) were obtained. Furthermore, oxidative stability of the SL was also investigated by addition of 3 different antioxidants (each 200 ppm of rosemary extract [SL-ROS], BHT [SL-BHT], catechin [SL-CAT]) was added into SL and stored in 60 °C oven for 30 d. 2-Thiobabituric acid-reactive substances (TBARS) value was 0.16 mg/kg in SL-CAT and 0.18 mg/kg in SL-ROS as compared with 0.22 mg/kg in control (SL) after oxidation. The lowest peroxide value (POV, 200.9 meq/kg) and longest induction time (29.88 h) was also observed in SL-CAT.

Keywords: alpha-linolenic acid, corn oil, oxidation, perilla oil

Introduction

Alpha-linolenic acid (ALA) is an unsaturated ω3 fatty acid (C18:3). After diet, it may further convert to other biological metabolites proven to prevent or decrease blood clotting, cancer, cardiovascular disease, inflammatory process, and arthritis (Harris 1997; Sandars and others 1997; Sinclair and others 2002). Among various plant oils, perilla oil (PO) is one of the rich source of ω3 ALA (Shin and others 1994). PO is able to decrease plasma histamine level (Shin and others 2000) and increase higher hypocholesterolemic ability (Watanabe and others 2000). On the other hand, corn oil (CO) contains high amount of ω6 fatty acid (mainly linoleic acid, C18:2) that is generally rich in many vegetable oils. It is known that consumption of imbalanced ω3/ω6 fatty acids may result in negative health effects in the body. Such dietary ratio of ω3 and ω6 fatty acid can be balanced by simple blending or lipase-catalyzed modification (such as ω3-rich PO and ω6-rich soybean oil in Mitra and others 2010), yet few studies were published about modifying CO with PO. Lipid oxidation is one of major quality problems in the processing and storage of fats and oils and emulsion foods (Yang and others 2002). On the other hand, addition of antioxidant in lipids can prevent the oxidative deterioration, and extend the shelf life of fats and oils. It is known that catechin from green tea extract is one of the promise antioxidant among natural phenolic compounds, while rosemary extract shows noticeable antioxidative effect due to the presence of phenolic diterpenes such as carnosic acid and carnosol (Lee and others 2003). In the present study, ALA-enriched structured lipid (SL) was obtained by lipase-catalyzed interesterification from PO and CO. Effects of different reaction conditions (time, temperature, and substrate molar ratio) on interesterification were studied, and compositions of fatty acids and chemical characteristics were examined. Besides, the effectiveness of rosemary extract and catechin as an antioxidant was investigated to prevent or retard the oxidation of SL under accelerated storage condition.

MS 20110667 Submitted 5/27/2011, Accepted 9/8/2011. *Authors Mitra and Kim are with Dept. of Food and Nutrition, Chungnam National Univ., Daejeon 305–764, South Korea. Authors Shin, Hong, Sung, Xue, and K.-T. Lee are with Dept. of Food Science and Technology, Chungnam National Univ., Daejeon 305–764, South Korea. Author J.-H. Lee is with Inst. of Agricultural Science, Chungnam National Univ., Daejeon 305–764, South Korea. Author J.-H. Lee is presently with US Dept. of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, PA 19038, U.S.A. Direct inquiries to author K.-T. Lee (E-mail: ktlee@cnu.ac.kr).*

Materials and Methods

Materials

Refined, bleached, and deodorized CO and nonrefined PO extracted from roasted perilla seeds were purchased from local market (Daejeon, Korea). RM IM (150 IUN/g of catalytic activity with a

doi: 10.1111/j.1750-3841.2011.02464.x

Journal of Food Science A Publication of the Institute of Food Technologists

bulk density of 350 to 450 kg/m^3, a particle size of 0.3 to 0.6 mm, and a water content of 2 to 3 w/w%) from *Rhizomucor miehei* was purchased from Novozymes A/S (Bagsvaerd DK-2880, Denmark). All solvents were of high performance liquid chromatograph (HPLC) grade and obtained from Fisher Scientific (Norcross, Ga., U.S.A.). Catechin was a gift from Il-Shin Wells Company (Chungju, Korea). Rosemary extracts (ROS.CON) were kindly provided by Pinus TKi d.d (Race, Slovenia). 2-Thiobabituric acid-reactive substances (TBARS) reagent was obtained from Alfa Aesar (Heysham, Lancashire, U.K.). Butylated hydroxytoluene (BHT) was purchased from Junsei Chemical Co. Ltd (Tokyo, Japan).

Synthesis of SL

Interesterification reactions between PO and CO were carried out according to 3 different parameters; substrate molar ratio (PO/CO 1:1 to 1:3), reaction time (0 to 24 h), and reaction temperature (55 to 65 °C). RM IM (10% by weight of total substrates) was used as biocatalyst. Total 10 g mixture (according to molar ratio of PO and CO) were used for the reactions and placed in a 50-mL vial with a screw cap, and the reactants were mixed with magnetic stirring bar with maximum speed. After reaction, the reaction products were passed through 0.50-μm disposable syringe filter. For studies on chemical characteristics and oxidation stability, the interesterified SL (200 g) was produced (1:1 molar ratio of PO:CO) at 60 °C for 15 h in a shaking water bath (200 rpm).

Fatty acid composition analysis

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

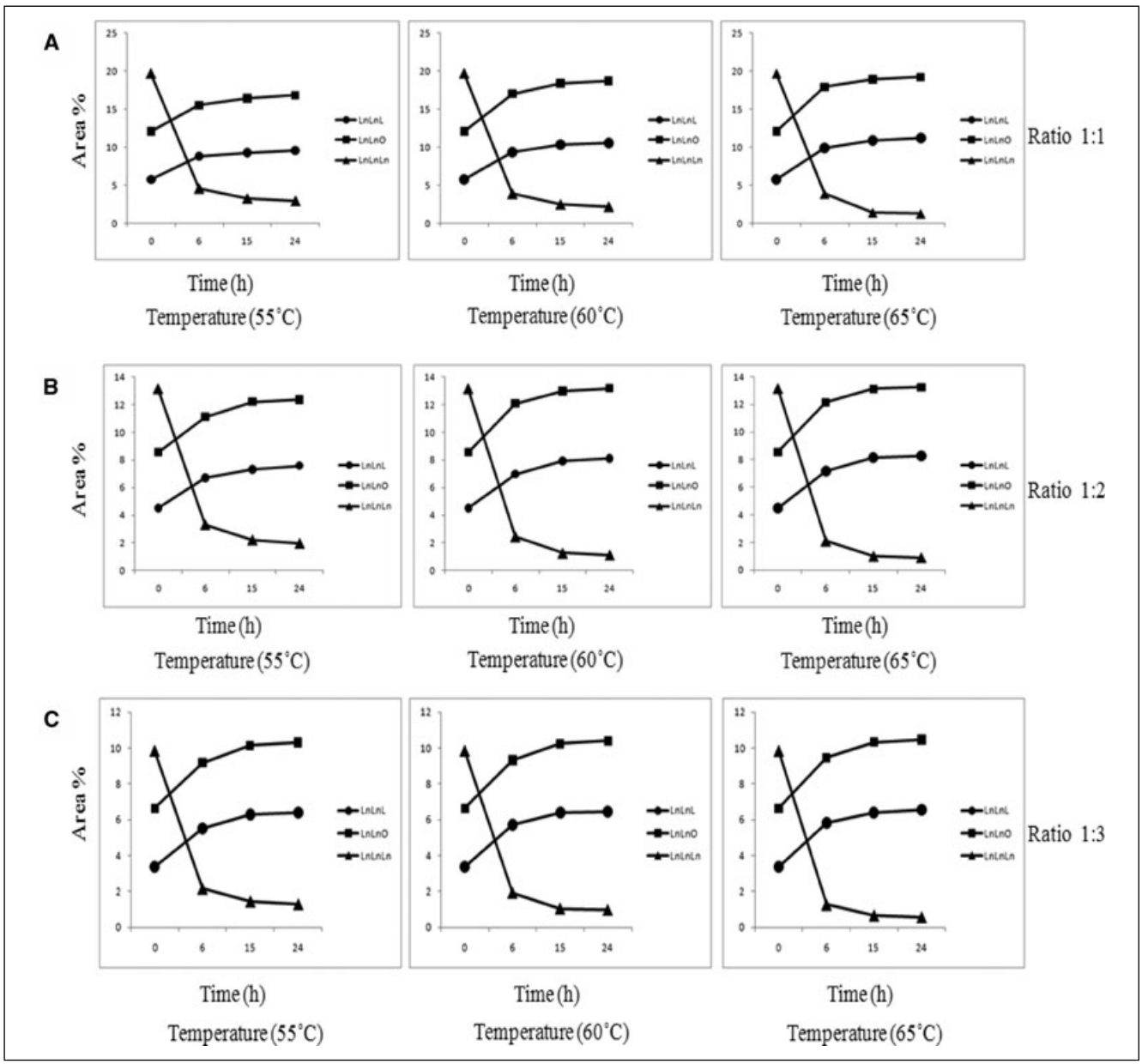

Figure 1–Effect of different reaction time (0, 6, 15, 24 h), substrate mole ratio (PO:CO = 1:1, 1:2, 1:3), and reaction temperature (55, 60, 65 °C) on the changes of triacylglycerol species (LnLnLn, LnLnL, LnLnO).

Table 1–Total and positional distribution of fatty acids (area%) of perilla, corn oil, physical blend, and structured lipid.

	Perilla oil[a]			Corn oil			Physical blend[a]			Structured lipid[a]		
Fatty acid	**Total**	***Sn*-2**	***Sn*-1, 3[c]**	**Total**	***Sn*-2**	***Sn*-1, 3**	**Total**	***Sn*-2**	***Sn*-1, 3**	**Total**	***Sn*-2**	***Sn*-1, 3**
C 16:0	6.27 ± 0.01	0.50 ± 0.10	9.16 ± 0.01	13.01 ± 0.04	1.0 ± 0.20	19.02 ± 0.03	9.68 ± 0.02	0.75 ± 0.01	14.05 ± 0.01	9.38 ± 0.12	0.65 ± 0.02	13.75 ± 0.01
C 18:0	1.96 ± 0.02	0.01 ± 0.02	2.94 ± 0.04	2.21 ± 0.01	0.21 ± 0.01	3.21 ± 0.02	2.07 ± 0.01	0.13 ± 0.01	3.09 ± 0.01	2.09 ± 0.04	0.11 ± 0.01	3.08 ± 0.01
C 18:1	14.54 ± 0.01	16.39 ± 0.03	13.62 ± 0.01	31.93 ± 0.02	32.65 ± 0.01	31.57 ± 0.01	23.23 ± 0.01	24.52 ± 0.02	22.60 ± 0.05	22.20 ± 0.02	24.78 ± 0.01	20.91 ± 0.01
C 18:2	14.08 ± 0.01	17.29 ± 0.01	12.48 ± 0.10	52.23 ± 0.10	65.74 ± 0.01	45.48 ± 0.02	33.15 ± 0.02	41.50 ± 0.02	28.98 ± 0.02	34.05 ± 0.01	40.99 ± 0.12	30.58 ± 0.01
C 18:3	63.15 ± 0.02	65.81 ± 0.01	61.82 ± 0.12	0.62 ± 0.03	0.40 ± 0.01	0.73 ± 0.01	31.87 ± 0.01	33.10 ± 0.01	31.28 ± 0.03	32.39 ± 0.01	33.47 ± 0.12	31.85 ± 0.01
∑SFA	8.23 ± 0.02	0.51 ± 0.01	12.10 ± 0.01	15.22 ± 0.02	1.21 ± 0.01	22.23 ± 0.04	11.75 ± 0.01	0.88 ± 0.01	17.14 ± 0.12	11.47 ± 0.01	0.76 ± 0.02	16.83 ± 0.02
∑USFA	91.77 ± 0.01	99.49 ± 0.06	87.90 ± 0.02	84.78 ± 0.01	98.79 ± 0.01	77.77 ± 0.01	88.25 ± 0.01	99.12 ± 0.01	82.86 ± 0.02	88.53 ± 0.02	99.24 ± 0.02	83.17 ± 0.02
$\omega 6/\omega 3$[b]	0.22 ± 0.01	0.27 ± 0.02	0.20 ± 0.03	84.24 ± 0.01	164.35 ± 0.02	62.30 ± 0.02	1.04 ± 0.01	1.25 ± 0.01	0.93 ± 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]Structured lipid was produced with the molar ratio of 1:1 (PO:CO) at 60 °C for 15 h. Lipozyme RM IM (10% of the total substrate) was used for the reaction. Physical blend was the equal molar ratio mixture of PO: CO (1:1). Data of perilla oil were cited from the reference of Mitra et al. (2010).
[b]$\omega 6$ = C18:2 linoleic acid; $\omega 3$ = C18:3 α-linolenic acid (ALA).
[c]*Sn*−1, 3 (area%) = (3T−*Sn*2)/2, Where T is total fatty acid.

(50/50/1, v/v/v). After drying the plate, the visualized triacylglycerol (TAG) band was separated and analyzed by gas chromatograph (GC) according to the previously explained (Shin and others 2009). Hewlett-Packard 6890 series was used as a gas chromatograph equipped with an auto injector and a flame-ionization detector (Agilent Technologies, Little Falls, Del., U.S.A.). Temperatures of the injector and detector were set at 250 °C and 260 °C, respectively. Fused-silica capillary column (Supelco SP-2560, 100 m × 0.25 mm i.d.; Supelco, Bellefonte, Pa., U.S.A.) was used for analysis. The column was 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. The carrier gas was Nitrogen at 1 mL/min. Fatty acids compositions were identified by comparison with relative retention times of standard mixtures. Duplicate analyses were performed. Fatty acid composition at *sn*-2 position was determined by pancreatic lipase analysis as described previously (Shin and others 2009).

Analysis of tocopherols

Analysis of tocopherols was performed using a isocratic normal-phase HPLC system (Lee and others 2006). The HPLC system consisted of a Yonglin SP930D dual pump (Yonglin, Anyang, Korea) accompanied with UV detector set a 295 nm. The column was a Chromsep Cartridge, LiChrosorb Diol (5 μm, 3 × 100 mm, Chromapack, Rartian, N.J., U.S.A.). The mobile phase was a mixture of hexane with 0.1% acetic acid, and the flow rate was 1 mL/min. Standards of α-, γ-, and δ-tocopherol (purity 98%) were used for quantification. The area of each peak was integrated by Autochro-2000 software (Yonglin, Anyang, Korea).

TAG species analysis

The separation of TAG species from PO, CO, and SL were conducted by reverse-phase HPLC (RP-HPLC) as described previously (Adhikari and others 2009). Sample (15 mg) was prepared by dissolving in 10 mL chloroform. The HPLC system consisted of Yonglin SP930D dual pump (Yonglin, Anyang, Korea) with Sedex 75 evaporative light-scattering detector (Sedere, Alfortvill, France) operated at 55 °C with nitrogen pressure of 1.7 bar. Twenty microliter of filtered samples were injected and separated on Nova-Pak C18 column (150 × 3.9 mm, Waters, Milford, Mass., U.S.A.). 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 to 44 min, 20% B; 45 to 50 min, 46% B; 51 to 58 min, 100% B, and then back to the initial flow rate.

Oxidative stability

Three different kinds of antioxidant (rosemary extract, BHT, and catechin) were used in this study. Produced SL was uniformly mixed with rosemary extract (200 ppm), BHT (200 ppm), and catechin (200 ppm) by an ultra-sonicator, and placed in an oven at 60 °C for 30 d. Glycerol monooleate (2 mg/g of oil) was used to help dispersion of catechin into SL. For the evaluation of oxidative stability, peroxide value (POV, for measuring hydroperoxides), ρ-anisidine value (AV, for aldehydes, principally 2-alkanal and 2, 4-dienal), and TBARS values (for substances such as malonaldehydes) were measured at every 5 d for 30 d according to the AOCS official methods (AOCS 1998). The induction time, measuring the volatiles as by-products released from the oxidizing oil of PO, CO, and SL, was obtained by Rancimat analyzer (Rancimat 743, Metrohm, Switzerland). The airflow and temperature were set at

20 L/h and 100 °C, respectively. Results were expressed as induction time (h).

Statistical analysis

Analysis was carried out in duplicate, and the variance of results was performed using the General Linear Model Procedure of SAS Statistical Software (SAS 2000). Duncan's multiple range test was applied to evaluate the significance of differences between means at $P < 0.05$.

Results and Discussion

Effect of different reaction conditions on interesterification reactions

By enzymatic interesterified reaction, rearrangements of fatty acids within and between TAG species result in new altered TAG molecules of the restructured fats and oils (Lee and others 2006). In this study, different molar ratio of PO and CO (1:1, 1:2, and 1:3), reaction temperature (55, 60, and 65 °C), and reaction time (0, 6, 15, and 24 h) were selected to monitor the reactions. Because major changes of certain TAG species would be expected during the reaction, 3 TAG species (linolenoyl-linolenoyl-linoleoyl glycerol [LnLnL], linolenoyl-linolenoyl-linolenoyl glycerol [LnLnLn], and linolenoyl-linolenoyl-oleoyl glycerol [LnLnO]) were selected to monitor the reaction. Before the interesterification between PO and CO, LnLnLn was only found in PO thereafter the amount of that TAG species decreased, accompanying with the increased amounts of LnLnL and LnLnO. The effect of different reaction time, temperature, and substrate molar ratio on the interesterification was presented in Figure 1. It showed that the area% of LnLnL and LnLnO was noticeably increased from 0 to 15 h reaction time, while decreased area% of LnLnLn was observed until equilibration (after 15 h reaction) was reached. Differences in reaction temperature did not show noticeable differences on the profiles of TAG species. Figure 1A shows that area% of LnLnL and LnLnO rapidly increased within 6 h in each reaction.

At 60 °C, area% of LnLnL increased from 5.8 (0 h) to 10.3 (15 h) and 11.6 (24 h), while LnLnO increases from 12.1 (0 h) to 18.4 (15 h) and 18.7 (24 h). It is noticeable that after 15 h reaction, equilibration was observed. At 15 h reaction, the area% of LnLnL was 9.9 (at 55 °C), 10.3 (at 60 °C), and 10.9 (at 65 °C), respectively, while area% of LnLnO was 17.84 (at 55 °C), 18.39 (at 60 °C), and 18.94 (at 65 °C). Therefore, it also showed that temperature did not have much affect on the degree of interesterification. Moreover, LnLnLn that originated from PO was reduced from 19.7 area% at 0 h to 2.5 area% at 15 h when the reaction was performed at 60 °C. Previous studies (Adhikari and others 2009; Shin and others 2009) showed that most of the TAG species (equivalent carbon number [ECN] 36 to 50) changed within 1 to 24 hr during interestification reactions. Similar results were observed on the changes of TAG species of our study during the 24 h interesterification with the other two ratios (Figure 1B and C). After monitoring such results, SL that was produced with the molar ratio of 1:1 (PO:CO) at 60 °C for 15 h reaction was used for further studies.

Effect of interestification reaction on fatty acid compositions

Fatty acid profiles (mol%) determined by GC are presented in Table 1. As expected, the most abundant fatty acids in the

Table 2–Triacylglycerol (TAG) composition (area%) of perilla oil, corn oil, physical blend, and structured lipid.

ECN[a]	Proposed TAG species[b]	Perilla oil[c]	Corn oil	Physical blend[c]	Structured lipid[c]
36	LnLnLn	39.45 ± 0.01	ND	19.65 ± 0.01	1.49 ± 0.01
38	LnLnL	13.55 ± 0.02	ND	6.03 ± 0.01	9.89 ± 0.02
40	LnLnO	24.19 ± 0.01	ND	11.99 ± 0.02	18.41 ± 0.02
40	LnLnP	5.57 ± 0.01	ND	2.91 ± 0.01	3.45 ± 0.02
42	LLL	ND[d]	16.12 ± 0.01	8.55 ± 0.02	9.06 ± 0.01
42	OLnL	6.09 ± 0.02	ND	3.01 ± 0.01	4.47 ± 0.01
44	LLO	ND	29.59 ± 0.02	13.98 ± 0.01	16.66 ± 0.02
44	PLL	2.30 ± 0.01	14.38 ± 0.02	8.32 ± 0.03	9.69 ± 0.02
46	LOO	ND	13.48 ± 0.01	6.91 ± 0.01	7.33 ± 0.01
46	PLO	ND	15.50 ± 0.01	8.11 ± 0.02	8.72 ± 0.01
46	PLP	ND	2.26 ± 0.01	1.12 ± 0.01	1.26 ± 0.01
48	OOO	ND	3.30 ± 0.02	1.65 ± 0.01	1.60 ± 0.02
48	POO	ND	5.36 ± 0.01	2.83 ± 0.02	2.58 ± 0.01
	Others[e]	8.85 ± 0.01	ND	4.94 ± 0.01	5.39 ± 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 = Palmitic acid.
[c]Structured lipid was produced with the molar ratio of 1:1 (PO:CO) at 60 °C for 15 h. Lipozyme RM IM (10% of the total substrate) was used for the reaction. Physical blend was the equal molar ratio mixture of PO: CO (1:1). Data of perilla oil were cited from Mitra and others (2010).
[d]ND = not detected.

Table 3–Tocopherol contents and chemical characteristics of perilla oil, corn oil, physical blend, and structured lipid.

Tocopherol (mg/100 g)	Perilla oil[a]	Corn oil	Physical blend[a]	Structured lipid[a]
α-Tocopherol	3.81 ± 0.04[d]	17.71 ± 0.01[a]	9.23 ± 0.01[b]	6.25 ± 0.02[c]
γ-Tocopherol	23.42 ± 0.02[c]	33.91 ± 0.08[a]	26.98 ± 0.02[b]	21.57 ± 0.01[c]
δ-Tocopherol	0.40 ± 0.02[b]	1.28 ± 0.02[a]	0.62 ± 0.01[b]	0.19 ± 0.01[c]
Total	27.63 ± 0.02[c]	52.90 ± 0.01[a]	36.83 ± 0.02[b]	28.01 ± 0.03[c]
Chemical characteristics	Perilla oil	Corn oil	Physical blend	Structured lipid
Saponification value	197.1 ± 0.2[a]	186.1 ± 0.2[c]	191.2 ± 0.1[b]	192.2 ± 0.3[b]
Iodine value	201.8 ± 0.3[a]	121.7 ± 0.2[c]	159.7 ± 0.1[b]	161.9 ± 0.1[b]
Free fatty acid (% oleic)[b]	0.3 ± 0.02[a]	0.26 ± 0.01[c]	0.28 ± 0.01[b]	0.29 ± 0.01*a*[b]

[a]Structured lipid was produced with the molar ratio of 1:1 (PO:CO) at 60 °C for 15 h. Lipozyme RM IM (10% of the total substrate) was used for the reaction. Physical blend was the equal molar ratio mixture of PO: CO (1:1). Data (tocopherols) of perilla oil were cited from the reference of Mitra et al (2010).
[b]Free fatty acid (% oleic) = Acid value/1.99.
All data are mean values ± standard deviations of duplicate measurements. The same letters within row are not significantly different ($P < 0.05$).

PO and CO were ALA (alpha-C18:3, 63.15%) and linoleic acid (C18:2, 52.23%), respectively. PO contained 91.77% of total unsaturated fatty acids (∑USFA) whereas CO contained 84.78% of ∑USFA. After interesterification, 88.53% USFA was found on the TAG backbone of the SL (Table 1). Oleic (22.20%), linoleic acid (34.05%), and ALA (32.39%) were the major USFA in the SL, having approximately 1:1 ($\omega6/\omega3$ fatty acid) ratio, in which CO originally had very high ratio of 84:1 ($\omega6/\omega3$ fatty acid). It is known that recommended healthy dietary ratio of $\omega3$ and $\omega6$ fatty acid ranged from 1:1 to 1:4 (Simopoulos 2002, 2006). The health beneficial effects of oils are greatly influenced not only by total fatty acid compositions but also by the distributions of fatty acids on stereo specific numbering (*sn*) positions of TAG molecules. Furthermore, fatty acid 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. Positional fatty acid composition is also presented in Table 1. High contents of unsaturated fatty acids (99.24%) were observed at *sn*-2 position in the SL, which contained 24.78% of oleic acid, 40.99% of linoleic acid, and 33.47% of ALA. Our previous study (Mitra and others 2010) partially supports this result where after interesterification reaction, ALA ($\omega3$) was incorporated in SL successfully resulting in the decreased ratio of $\omega6/\omega3$.

Effect of interestification reaction on TAG profiles

Changes of TAG species due to rearrangement of fatty acid during the reaction are one of the most important phenomenon from lipase-catalyzed interesterification (Lee and others 2006; Alim and others 2008). To observe such changes, reverse-phase HPLC was applied. In Table 2, the most abundant TAG species in PO were LnLnLn (39.45%), LnLnL (13.55%), and LnLnO (24.19%), whereas major TAG species in the CO were trilinolein (LLL) (16.12%), linoleoyl-linoleoyl-oleoyl glycerol (LLO) (29.59%), and palmitoyl-linoleoyl-oleoyl glycerol (PLO) (15.50%). Shin and others 2009 reported that after interestification reaction, in SL, ALA liberated from TAG molecule (LnLnLn) was incorporated into

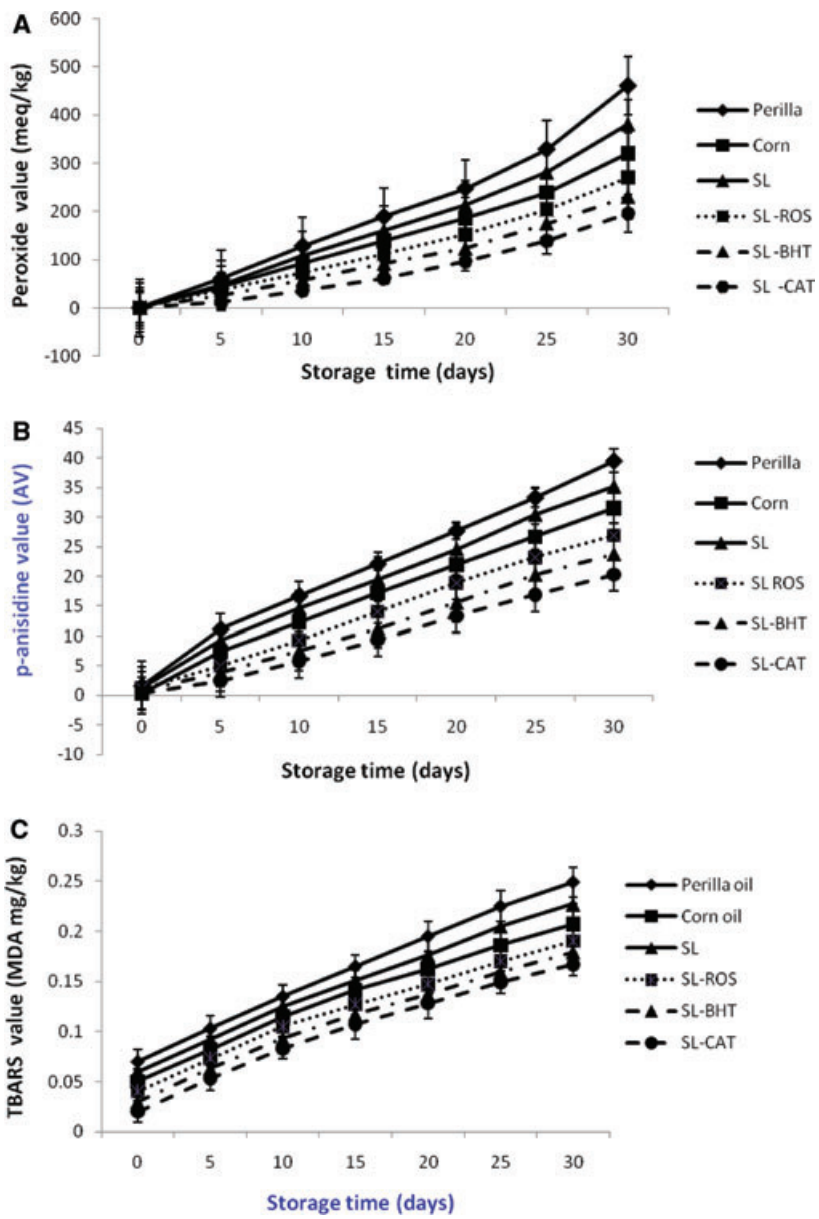

Figure 2–(A) Effect of antioxidant addition on peroxide value (POV); (B) Effect of antioxidant addition on p-anisidine value (AV); (C) Effect of antioxidant addition on 2-thiobabituric acid-reactive substances (TBARS) value. Perilla oil, corn oil, and structured lipid (SL) without or with antioxidant were oxidized at 60 °C for 30 d. SL, without antioxidant; SL-ROS, SL + 200 ppm addition of rosemary extract; SL-BHT, SL + 200 ppm addition of BHT; SL-CAT, SL + 200 ppm addition of catechin.

other TAG molecules (ECN 42 to 46) comparing to that of physical blend. Composition of each TAG species in physical blend and SL was also compared in our study. In SL, the major TAG species (LnLnLn, LnLnL) mainly from PO and LLO, linoleoyl-oleoyl-oleoyl glycerol (LOO), PLO from CO decreased while LnLnO (18.41%), LLL (9.06%), LLO (16.66%), palmitoyl-linoleoyl-linoleoyl glycerol (PLL) (9.69%) were increased compared to that of physical blend. Those results showed that substantial changes in TAG species were occurred in SL. The results of reverse-phase HPLC showed that SL contained very low amount of LnLnLn (1.49%) species in its TAG molecules, while SL also contained 32.39% of ALA in its total fatty acid composition. These two results indicated that the ALA originally from PO (presented mainly as component of LnLnLn) was widely distributed into other TAG molecules of CO, changing composition of TAG species in SL.

Tocopherol analysis and chemical properties

Tocopherols are known as antioxidants that are naturally found mainly in plants at various amounts, in which several isomers (mainly α, γ, and δ) are found. Tocopherols content in the PO, CO, and SL are presented in Table 3. PO (27.63 mg/100 g) and SL (28.01 mg/100 g) contained significantly less amount of total tocopherols compared to CO (52.90 mg/100 g), in which CO especially contained significantly higher amount of γ-tocopherol (33.91 mg/100 g) than PO and SL ($P < 0.05$). In SL, α-tocopherol (6.25 mg/100g) and γ-tocopherol (21.57 mg/100g) with negligible amount of δ-tocopherol were detected. When total amount of tocopherols was compared between physical blend and SL, reduced amount of tocopherols was observed in SL due to loss of tocopherols during the reaction and purification processes (Lee and others 2006; Alim and others 2008). Table 3 also compared the saponification value (SV) and iodine value (IV) of physical blend and SL. It is known that SV is an estimate of the average molecular weight of the constituent fatty acids in lipid, and IV is a measurement of the double bonds in acyl group of lipid (that is, unsaturated fatty acid). In the result SV and IV of SL were 192.2 and 161.9, which were not significantly different from the values of physical blend, suggesting that SL is composed of different TAG species without significantly different chemical characteristics such as SV and IV (Table 2 and 3). All samples showed less than 0.3% free fatty acid content.

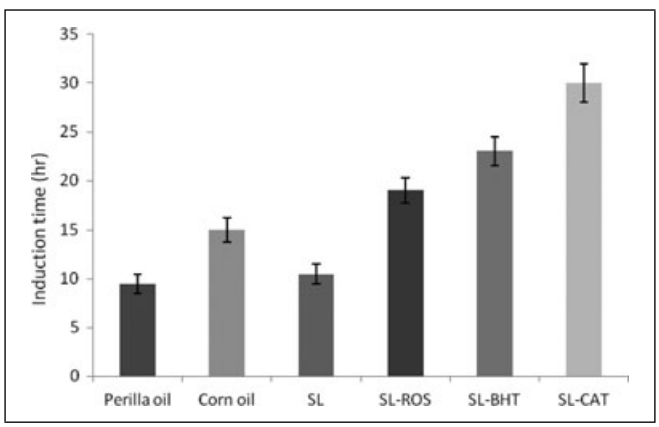

Figure 3–Induction time (h) of perilla oil, corn oil, structured lipid (SL), and SL with addition of different antioxidants. SL, without antioxidant; SL-ROS, SL + 200 ppm addition of rosemary extract; SL-BHT, SL + 200 ppm addition of BHT; SL-CAT, SL + 200 ppm addition of catechin.

Oxidative stability

Loss of tocopherols (acts as natural antioxidants) was observed after interesterification in Table 3, leading to further study on oxidative stability of SL. Each 200 ppm of rosemary extract (SL-ROS), BHT (SL-BHT), catechin (SL-CAT) was added into SL and stored in the 60 °C oven for 30 d with the control (SL without any antioxidant), PO and CO. The results of POV, AV, and TBARS value showed that 200 ppm addition of any rosemary extract, BHT, and catechin in SL could retard oxidation of SL (Figure 2A to C). Compared to PO, SL was more stable to oxidation, having lower POVs for 30 d (Figure 2A). After 15 d, POVs of PO and SL increased more rapidly than those of CO, SL-ROS, SL-BHT, and SL-CAT in which POV of SL-CAT increased up to 201 meq/kg, whereas control (SL without any antioxidant) increased to 381 meq/kg after 30-d oxidation.

It is known that AV measures the amount of aldehydes (principally 2-alkenals and 2, 4-dienal) while TBARS value mainly determines the amount of substance such as malonaldehydes, representing secondary oxidation products. During 30 d, the highest AV and TBARS value was observed in PO. With addition of antioxidant, TBARS value on 30 d was 0.16 mg/kg in SL-CAT and 0.18 mg/kg in SL-ROS as compared with 0.22 mg/kg in control (SL). Among compounds used in this study, catechin showed the most effective antioxidative activity on SL. Thus, SL with 200 ppm of any antioxidant used in this study showed lower POV, AV, and TBARS value than the control SL. On the other hand, significantly high amount of total tocopherols (52.90 mg/100 g) in CO may retard the oxidation process compared to PO with high amount of ALA. The result of rancimat test is presented in Figure 3. Prolonged induction time suggests increased oxidative stability (Alim and others 2008). PO containing high content of ALA showed the lowest oxidative stability, while control (SL without antioxidant, induction time = 12.23 h) was more stable to oxidation than PO (induction time = 9.24 h). The longest induction time (29.88 h) was observed in SL-CAT, indicating that catechin was very effective to prevent or retard the oxidation of SL obtained in this study.

Conclusion

ALA-enriched SL was produced by lipase-catalyzed interesterification from PO and CO. It showed that area% of LnLnL and LnLnO was rapidly increased from 0 to 15 h reaction time, while decreased area% of LnLnLn (originally from PO) was observed until equilibration was reached. It was observed that 32.39% of ALA in SL obtained by the optimized conditions (molar ratio—1:1 (PO:CO), temperature—60 °C, reaction time—15 h). TBARS value was significantly lower in SL with catechin (SL-CAT) and SL with rosemary extract (SL-ROS) than in control (SL) after 30-d oxidation. The lowest POV (200.9 meq/kg) and the longest induction time (29.88 h) was also observed in SL-CAT, indicating that catechin was very effective to prevent or retard the oxidation of SL obtained in this study. Our study indicates that the newly synthesized SL could be a better source of ω3 (ALA) fatty acid having low ω6 to ω63 ratio with longest shelf life than those of the others commercial vegetable oils.

Acknowledgments

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (nr 2011-0006110).

References

Adhikari P, Zhu XM, Gautam A, Shin JA, Hu JN, Lee JH, Akoh CC, Lee KT. 2009. Scaled-up production of zero-*trans* margarine fat using pine nut oil and palm stearin. Food Chem 119:1332–8.

Alim MA, Lee JH, Shin JA, Lee YJ, Choi MS, Akoh CC, Lee KT. 2008. Lipase-catalyzed production of solid fat stock from fractionated rice bran oil, palm stearin, and conjugated linoleic acid by response surface methodology. Food Chem 106:712–9.

AOCS. 1998. Official methods and recommended practices of the American Oil Chemists' Society. 5th ed. Champaign, Ill.: AOCS Press.

Harris WS. 1997. N-3 fatty acids and serum lipoproteins: human studies. Am J Clin Nutr 65:1645–4.

Lee JH, Kim MR, Kim RK, Kim IH, Lee KT. 2003. Characterization of lipase-catalyzed structured lipids from selected vegetables oils with conjugated linoleic acid: their oxidative stability with rosemary extracts. J Food Sci 68:1653–8.

Lee JH, Lee KT, Akoh CC, Shin KC, Kim MR. 2006. Antioxidant evaluation and oxidation stability of structured lipids from extra virgin olive oil and conjugated linoleic acid. J Agric Food Chem 54:5416–21.

Mitra K, Lee JH, Lee KT, Kim SI. 2010. Production tactic and physiochemical properties of low w6/w3 ration structured lipid synthesized from perilla and soybean oil. Int J Food Sci Tech 45:1321–9.

Sandars TAB, Oakley FR, Miller GJ, Mitropoulous KA, Crook D, Oliver MF. 1997. Influence of n-6 versus n-3 polyunsaturated fatty acids in diets low in saturated fatty acids on plasma lipoproteins and hemostatic factors. Arterioscl Throm Vas 17:3449–60.

SAS Institute. 2000. Statistical analysis software, release 8.2. Cary, NC.

Shin HS, Kim SW. 1994. Lipid composition of perilla seed. J Am Oil Chem 71:619–22.

Shin JA, Akoh CC, Lee KT. 2009. Enzymatic interesterification of anhydrous butterfat with flaxseed oil and palm stearin to produce low-*trans* spreadable fat. Food Chem 120:1–9.

Shin TY, Kim SY, Kim SH, Kim YK, Park HJ, Chae BS, Jung HJ, Kim HM. 2000. Inhibitory effect of mast cell-mediated immediate-type allergic reactions in rats by *Perilla Frutescens*. Immunopharmacol Immunotoxicol 22:489–500.

Simopoulos, AP. 2002. The Importance of the ratio of Omega-6/Omega-3 essential fatty acids. Biomed Pharmacother 56:365–79.

Simopoulos, AP. 2006. Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic-variation: nutritional implications for chronic diseases. Biomed Pharmacother 60:502–7.

Sinclair AJ, Attar-Bashi AM, Li D. 2002. What is the role of α-Linolenic acid for mammals? Lipids 37:1113–23.

Watanabe MI, 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 Res Int 33:893–900.

Yang MH, Lin HJ, Choong YM. 2002. A rapid gas chromatographic method for direct determination of BHA, BHT and TBHQ in edible oils and fats. Food Res Int 35:627–33.