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Antioxidant Evaluation and Oxidative Stability of Structured Lipids from Extravirgin Olive Oil and Conjugated Linoleic Acid

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Structured lipid (SL) was synthesized from extravirgin olive oil (EVOO) and conjugated linoleic acid (CLA) via a lipase-catalyzed reaction. CLA provides a variety of health benefits, but it is not consumed in free fatty acid form. The synthesized SL olive oil contained 42.5 mol % CLA isomers, and the major isomers were *cis*-9,*trans*-11-CLA (16.9 mol %) and *trans*-10,*cis*-12-CLA (24.2 mol %). The antioxidant activity determined by the radical scavenging capacity with the 2,2-diphenyl-1-picrylhydrazyl radical was lower in SL olive oil than in EVOO. The oxidative stability was also lower in SL olive oil since it had a higher peroxide value, ρ-anisidine value, and 2-thiobarbituric acid reactive substances values during 20 days of storage at 60 °C. This observation could be due to the reduction in the natural phenolic compounds (97%) and tocopherols (56%), and the incorporated CLA with two conjugated double bonds in the SL olive oil. The oxidative stability of SL olive oil was increased by added rosemary extracts at concentrations of 100, 200, and 300 ppm. The present study suggests that the SL olive oil may be a suitable way to incorporate or deliver CLA into human diets. However, the addition of a proper antioxidant would be required for improving its oxidative stability.

KEYWORDS: Conjugated linoleic acid; DPPH•; olive oil; phenolics; oxidative stability; structured lipids

INTRODUCTION

Conjugated linoleic acids (CLAs) are a mixture of positional and geometric isomers of linoleic acid (LA) containing conjugated double bonds. *cis*-9,*trans*-11-CLA is the principal isomer formed by rumen microorganisms, and it is found predominantly in meat and dairy products from ruminant animals,[1,2] whereas commercially produced CLA preparations contain *cis*-9,*trans*-11 and *trans*-10,*cis*-12 as the main isomers. Broad physiological impacts of CLA in animal models have been reported that include reducing risks of cancer and atherosclerosis, enhancing immune response, and reducing fat gain.[1,3−7] These numerous biological impacts result from separate and/or synergetic actions of the two main isomers *cis*-9,*trans*-11- and *trans*-10,*cis*-12-CLA.[6]

The consumption of dietary CLA has decreased in recent years due to the replacement of animal lipids by plant lipids that contain little CLA. To consume more CLA and derive health benefits, the enrichment of CLA in food has been attempted through modification of lipids (fats and oils), in which synthesis of structured lipids (SLs) is the most desirable method. CLA as a free fatty acid would not be a suitable form for use as edible oil. In SLs existing fatty acids in triacylglycerol (TAG) molecules are replaced with functional fatty acids (i.e., CLA) by chemical or enzymatic reactions; thus, nutritive or therapeutic benefits can be expected.[8−10] In the previous work, desirable changes were reported in the chemical and physical properties of the original lipids after modification.[11] However, the removal of natural antioxidants such as tocopherols was also observed in the SLs, leading to reduction in oxidative stability.[11,12]

Extravirgin olive oil (EVOO) is the major cooking oil in the Mediterranean diet. Of all vegetable oils, EVOO is the best source of monounsaturated fatty acids (72−75% oleic acid), which lower the level of homocysteine and total and LDL cholesterol in plasma, which are risk factors for cardiovascular diseases.[13] EVOO also contains natural antioxidants such as tocopherols and phenolic compounds, which play an important role in the oxidative stability of EVOO. However, the refining process alters the oxidative stability of olive oil, leading to a lower total antioxidant activity (TAA) value than that of EVOO.[14]

As a delivery medium for enriching CLA, SL methodology was selected in this study. Commercially produced CLA isomers were incorporated into EVOO through enzyme-catalyzed acidolysis to synthesize SL olive oil. After synthesis of SL, the incorporation of CLA, phenolic content, and factors affecting the oxidative stability were investigated. In addition, the oxidative stability with rosemary extracts added as a natural antioxidant was evaluated by following the peroxide value (POV), ρ-anisidine value (AV), and 2-thiobarbituric acid reactive substances (TBARS) value during 20 days of storage at 60 °C.

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MATERIALS AND METHODS

Materials. A CLA fatty acid mixture comprising 94% total CLA isomers (70 CLA) produced from safflower oil was a gift from Livemax Co. (Sungnam, Korea). The main CLA isomers were *cis*-9,*trans*-11-CLA (36.5%), *trans*-10,*cis*-12-CLA (54.5%), and other CLA isomers (3.3%). EVOO was provided by C.J. Co. (Seoul, Korea), and immobilized enzyme from *Rhizomucor miehei* (Lipozyme RM IM) was purchased from Novozymes A/S (Copenhagen, Denmark). All solvents were HPLC grade and obtained from Fisher Scientific (Norcross, GA). Heptadecanoic acid (C17:0), standards of phenol, tocopherol, and phytosterols, and Folin−Ciocalteu reagent were from Sigma Chemical Co. (St. Louis, MO). Rosemary extracts (ROS•CON) were kindly provided by Pinus Tki d.d. (Race, Slovenia), and its major components were carnosic acid (24.6%, w/w) and carnosol (3.9%).

Synthesis of Structured Lipids. Enzyme (RM IM lipase, 39.25 g, 5 wt % total substrates) was added to a mixture of EVOO (400 g) and CLA (385.1 g, 1:3 molar ratio) in a 1 L stirred-tank batch-type reactor. The dimensions of the reactor were 11 cm internal diameter and 15 cm height having a double water jacket. A semicircular-shaped (9 cm diameter and 3.2 cm height) stirring blade was used with a speed controller. The blade was placed at a distance of 1 cm from the bottom of the reactor. The reaction mixture was incubated for 24 h at 55 °C in a water bath circulator, and the mixing speed was set at 300 rpm. After incubation, hexane (300 mL) was added to the reaction products, and the enzyme was removed by filtration through a filter paper by applying a vacuum. The reaction product was transferred into a separatory funnel, mixed with 3−4 drops of phenolphthalein solution, and titrated with 0.5 N KOH solution in 20% ethanol (pH end point 7.2). Then saturated NaCl (60 mL) solution was added, the resulting solution was mixed vigorously, and the hexane phase was isolated. After the solution was passed through an anhydrous sodium sulfate column, the hexane was evaporated in a rotary evaporator under a vacuum. This procedure for SL production was repeated three times, and the products were combined.

Fatty Acid Analysis. The fatty acid composition of the oils was determined by converting fatty acids into the corresponding fatty acid methyl esters (FAMEs) as described previously.[11] The gas chromatographic analysis was carried out with a Hewlett-Packard 6890 gas chromatograph (GC) equipped with an autoinjector and a flame-ionization detector (Agilent Technologies, Little Falls, DE) using afused-silica capillary column (SP-Wax, 60 m × 0.25 mm i.d., CARBOWAX 20M poly(ethylene glycol); Supelco, Bellefonte, PA). The temperatures of the injector and detector were 250 and 260 °C, respectively. The oven temperature was programmed from 100 °C for 5 min, increased to 220 °C at a rate of 4 °C/min, and held for 20 min. The carrier gas was nitrogen, and the total gas flow rate at the inlet was 52 mL/min (constant-flow mode) with split mode 50:1. FAMEs were identified by comparison with the relative retention times of standard mixtures (GLC-461; Nu-Chek Prep, Elysian, MN). Heptadecanoic acid was used as an internal standard, and triplicate analyses were performed.

Solid-Phase Extraction (SPE) for Phenolic Compounds. A diol-bonded-phase SPE cartridge (3 mL; Supelco Co., Bellefonte, PA) was used for the extraction of phenolics from oil samples. The cartridge was conditioned with methanol (6 mL) and hexane (6 mL). Each 2.5 g sample of EVOO and SL olive oil was dissolved in hexane (6 mL) and then transferred to the SPE cartridge. The cartridge was washed with hexane (2 × 3 mL) and hexane/ethyl acetate (90:10, v/v; 2 × 4 mL). The phenolic compounds were eluted with methanol (15 mL) into a collection tube, and the solvent was evaporated to dryness under nitrogen.[15]

Colorimetric Evaluation of Total Phenolics. The total phenol content, expressed as gallic acid equivalents (GAEs, mg/kg), was determined colorimetrically using the Folin−Ciocalteu reagent as described by Mosca et al.[16] The phenolic residue obtained from the SPE cartridge was dissolved in 7% acetic acid (400 μL) and the resulting solution vortexed. An aliquot (100 μL) was mixed with Folin−Ciocalteu reagent (50 μL). After 3 min, saturated sodium carbonate solution (50 μL) was added, and then the resulting solution was diluted with water (2.5 mL) and vortexed to mix. The solution was kept in the dark for 90 min, and the absorbance at 765 nm was determined against the blank. The standard curve was prepared with 0, 0.05, 0.1, 0.2, 0.4, and 0.8 mg/mL gallic acid.

Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) Determination of Phenolics. Qualitative and quantitative evaluation of phenolics was performed by RP-HPLC using gallic acid as an internal standard. The phenolic residues of EVOO and SL olive oil were dissolved in methanol (300 μL), and a 20 μL aliquot was injected into the HPLC system. The HPLC system was composed of a Yonglin SP930D dual pump (Yonglin, Anayang, Korea) and a UV830 detector set at 278 nm. A Nova-Pak C18 column (4 μm, 150 × 3.9 mm i.d.; Waters, Milford, MA) was used, and the elution was carried out at a flow rate of 1.0 mL/min. The mobile phases were (solvent A) water/acetic acid (95:5, v/v) and (solvent B) methanol/acetonitrile (50:50, v/v). The solvent gradient was from 95% A−5% B to 70% A−30% B in 25 min, 65% A−35% B in 10 min, 60% A−40% B in 5 min, and 30% A−70% B in 10 min, followed by 100% B in 10 min for maintenance.

Analysis of Tocopherols and Phytosterols. Quantitative analysis of tocopherol in the oils was performed with HPLC (Yonglin, Anayang, Korea) at 295 nm. The column was a Chromsep Catridge, LiChrosorb Diol (5 μm, 3 × 100 mm; Chromapack, Rartian, NJ). The mobile phase was a mixture of hexane fortified with 0.1% acetic acid, and the flow rate was 0.5 mL/min. Standards of α-, γ-, and δ-tocopherol were used for quantification.[17] For analysis of phytosterols, the oils (each 100 mg) were saponified by adding 2 N KOH (2 mL) in ethanol and heating for 15 min at 80 °C. After cooling, distilled water (2 mL) was added, and the unsaponifiable fraction including phytosterols was extracted by mixing with 2 mL of hexane. The hexane phase was collected and passed through an anhydrous sodium sulfate column. A Hewlett-Packard 6890 GC instrument with a flame-ionization detector (Agilent Technologies) and an Ultra-2 column (5% diphenyl/95% dimethylsiloxane, 30 m × 0.25 mm × 0.25 μm) was used. The column was held at 280 °C for 10 min, and the temperatures of the injector and detector were 300 and 300 °C, respectively. 5α-Cholestane was used as an internal standard, and triplicate analyses were performed.

2,2-Diphenyl-1-picrylhydrazyl Radical (DPPH•) Quenching Test. The total free radical scavenging capacities of EVOO and SL olive oil were estimated by DPPH• and compared with those of antioxidants such as tocopherol, 2,6-di-*tert*-butyl-4-methylphenol (BHT), and gallic acid, which were prepared at 1 and 5 mM concentration. SPE phenolic extracts of the oil samples (each 2.5 g) dissolved in methanol (1 mL) were mixed into a DPPH• methanolic solution (100 μM, 2 mL) and the resulting solutions vortexed. The absorbance of each reaction mixture at 517 nm was measured against a methanol blank at 10 and 30 min. The DPPH• scavenging capacities (RSCs) were calculated using the following equation:

$$\text{RSC}(\%) = [1 - (\text{absorbance of the sample}/\text{absorbance of the blank})] \times 100$$

Rancimat Test. The oxidative stability of EVOO and SL olive oil (each 3 g) was evaluated by the Rancimat 743 instrument Metrohm AG (Herisau, Switzerland). The airflow and temperature were set at 20 L/h and 98 °C, respectively, and the results were expressed as induction time (h).

Oxidative Stability with Rosemary Extracts. The EVOO and SL olive oil (each 50 g) were uniformly mixed with rosemary extract (0, 100, 200, or 300 ppm) by an ultrasonicator. The oil samples in a 100 mL beaker (three batches for each oil) were incubated in an oven for 20 days at 60 °C. For the evaluation of oxidative stability, their POVs, AVs, and TBARS values were determined at 0, 5, 9, 17, and 20 days according to the AOCS Official Methods.[17]

Statistical Analysis. The Statistical Analysis System software (SAS, Cary, NC) was used to perform statistical computations. Analysis of variance (ANOVA) with Duncan's multiple range test was performed to determine the significance of the difference at $p < 0.05$ (*18*).

RESULTS AND DISCUSSION

Fatty Acid Composition and Chemical Characteristics. The most abundant fatty acid present in EVOO was oleic acid (C18:1*n*−9), a monounsaturated fatty acid (MUFA) constituting

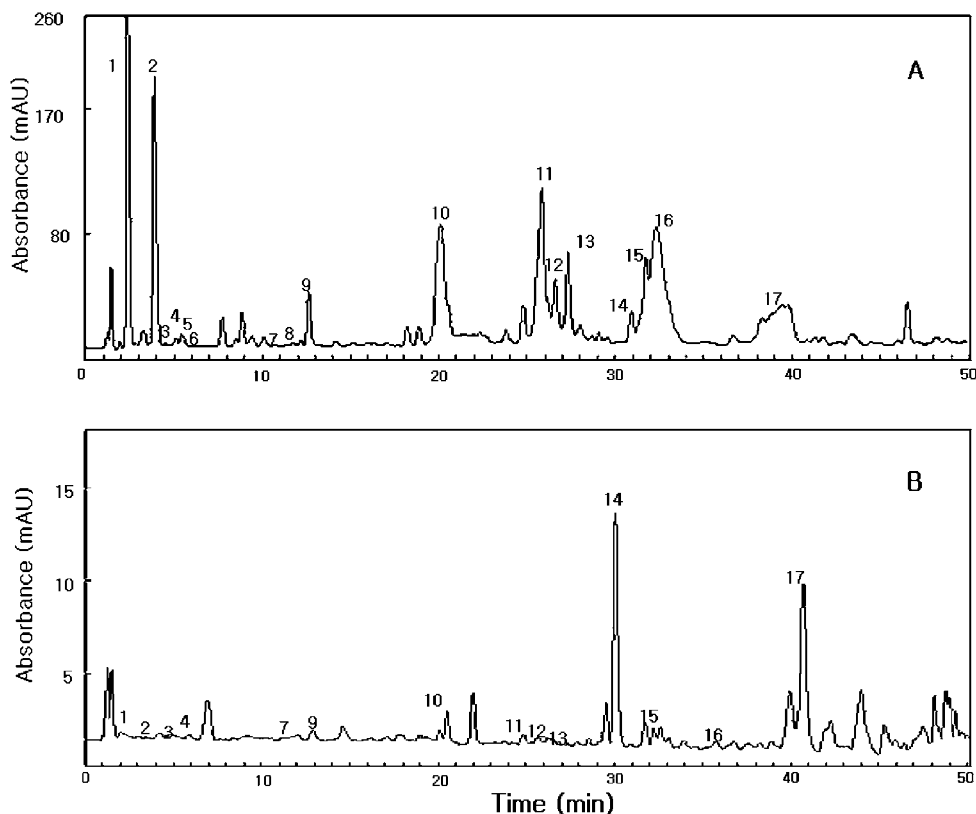

Figure 1. Reversed-phase HPLC separation of the SPE phenolic extracts: (A) EVOO, (B) SL olive oil. Peak numbers: (1) hydroxytyrosol; (2) tyrosol; (3) *p*-hydroxyphenylacetic acid; (4) caffeic acid; (5) vanillic acid; (6) syringic acid; (7) *p*-coumaric acid; (8) ferulic acid; (9) peak I, RT 13.4; (10) peak II, RT 20.4; (11) peak III, RT 26.2; (12) cinnamic acid; (13) peak IV, RT 27.7; (14) peak V, RT 31.5; (15) apigenin; (16) peak VI, RT 32.9; (17) peak VII, RT 40.1. SL olive oil was synthesized from EVOO and CLA via a lipase-catalyzed reaction.

Table 1. Fatty Acid Composition (mol %) of EVOO and SL Olive Oil[a]

fatty acid	EVOO	SL olive oil
C16:0	12.6 ± 0.0	5.9 ± 0.1
C16:1*n*–7	1.0 ± 0.0	0.6 ± 0.1
C18:0	3.1 ± 0.1	1.5 ± 0.1
C18:1*n*–9	75.7 ± 0.1	43.1 ± 0.8
C18:2*n*–6	6.6 ± 0.0	6.0 ± 0.0
C18:3*n*–3	0.8 ± 0.1	0.4 ± 0.0
cis-9,*trans*-11-CLA	ND[b]	16.9 ± 0.3
trans-10,*cis*-12-CLA	ND	24.2 ± 0.8
other CLA isomers	ND	1.4 ± 0.0
total CLA isomers	ND	42.5 ± 0.1

[a] Values are means of three determinations. [b] Not detected.

Table 2. Comparison of EVOO and SL Olive Oil[a]

	EVOO	SL-olive oil
tocopherols (mg/100 g)		
α-tocopherol	10.4 ± 0.2 a	4.5 ± 0.3 b
γ-tocopherol	ND[b]	ND
δ-tocopherol	ND	ND
phytosterols (mg/100 g)		
campesterol	ND	ND
stigmasterol	ND	ND
β-sitosterol	159.3 ± 17.1 a	102.9 ± 3.7 b
free fatty acids (% oleic)	0.5 ± 0.1 a	0.4 ± 0.1 b
total phenols[c] (mg of GAEs/kg)	85.9 ± 2.5 a	2.4 ± 0.1 b
induction time[d] (h)	42.3 ± 0.5 a	1.9 ± 0.1 b

[a] Values are the means of three samples. Values with different letters in the same row are significantly different among groups ($p < 0.05$). [b] Not detected under this analysis condition. [c] Expressed as gallic acid equivalents using Folin–Ciocalteu reagent. [d] Measured with the Rancimat test apparatus.

75.7 mol % of the total fatty acids as expected (**Table 1**). After the modification of EVOO with a CLA mixture by lipase-catalyzed acidolysis, a reduced content of oleic acid (43.1 mol %) was observed in SL olive oil, which was mostly compensated by the increased CLA content (42.5 mol %). The major CLA isomers incorporated were *cis*-9,*trans*-11-CLA (16.9 mol %) and *trans*-10,*cis*-12-CLA (24.2 mol %). Free fatty acid and peroxide values are often used as a general indicator of oil quality. The free fatty acid value of SL olive oil was less than 0.5%, and this indicated that the deacidification process successfully removed most of the free fatty acids in the reaction product, which are mostly unreacted CLA and fatty acids hydrolyzed from the original EVOO (**Table 2**). In addition, the peroxide value of SL olive oil was also lower than that of EVOO at day 0 (**Table 4**). SL olive oil may be used as a cooking oil on the basis of the oil quality measured.

Tocopherols and Phytosterols. The content of α-tocopherol was reduced by 56% in SL olive oil, and γ- and δ-tocopherol were not detected under our analysis conditions (**Table 2**). A decrease of the tocopherol content in purified SL was previously reported.[12,19] The removal of the natural tocopherols might be due to the processing (i.e., deacidification), and could affect the oxidative stability of SLs. EVOO also contains phytosterols as functional ingredients, which are known to block the absorption of cholesterol from the diet into the bloodstream.[20] β-Sitosterol was the only phytosterol detected. We observed a 53% decrease in β-sitosterol in the SL olive oil compared to the unmodified EVOO substrate (**Table 2**).

Phenolic Compounds. The content of total phenols obtained by the Folin–Ciocalteu colorimetric method is presented in **Table 2**. EVOO was a richer source of phenolics (85.9 mg of GAEs/kg of oil) than SL olive oil (2.4 mg). The high content of phenolic compounds in EVOO imparts a higher stability with regard to autoxidation and a strong fruity flavor composed of bitterness and pungency.[21] **Figure 1** depicts the HPLC separation

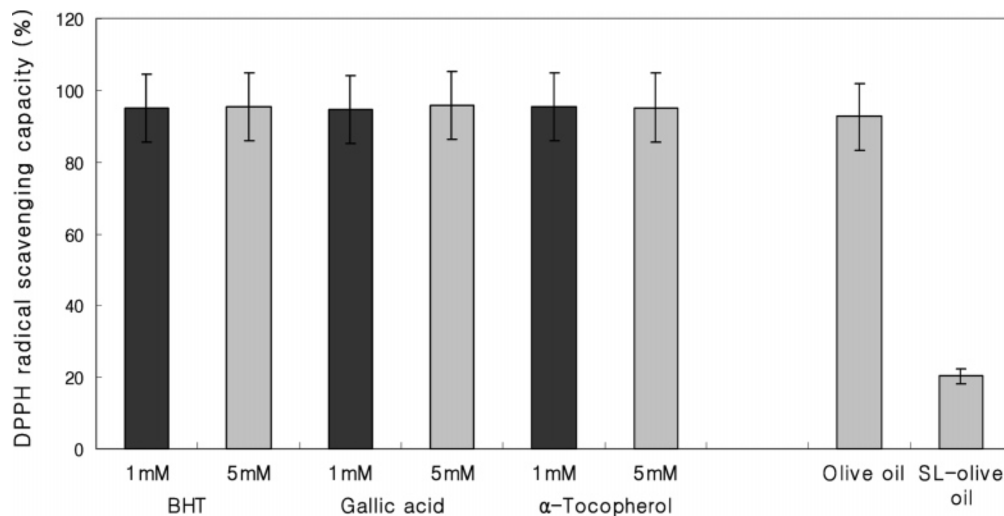

Figure 2. Comparison of DPPH radical scavenging capacity (%) from selected antioxidants (1 and 5 mM) and SPE phenolic extracts of EVOO and SL olive oil (each 2.5 g). SL olive oil was synthesized from EVOO and CLA via a lipase-catalyzed reaction.

Table 3. Phenolic Composition of EVOO and SL Olive Oil[a,b]

no.	phenolic compd	EVOO	SL olive oil
1	hydroxytyrosol	8.28 ± 0.25 a	0.01 ± 0.00 b
2	tyrosol	6.28 ± 0.27 a	0.02 ± 0.02 b
3	*p*-hydroxyphenylacetic acid	0.03 ± 0.02 a	0.01 ± 0.00 b
4	caffeic acid	0.33 ± 0.06 a	0.04 ± 0.01 b
5	vanillic acid	0.01 ± 0.01 a	ND[c]
6	syringic acid	0.01 ± 0.00 a	ND
7	*p*-coumaric acid	0.08 ± 0.07 a	<0.01 b
8	ferulic acid	0.09 ± 0.03 a	ND
9	peak I (RT[d] 13.4)	1.56 ± 0.07 a	0.13 ± 0.01 b
10	peak II (RT 20.4)	7.83 ± 0.45 a	0.23 ± 0.05 b
11	peak III (RT 26.2)	8.78 ± 0.96 a	0.06 ± 0.00 b
12	cinnamic acid	2.67 ± 0.07 a	0.08 ± 0.02 b
13	peak IV (RT 27.7)	3.64 ± 0.03 a	0.08 ± 0.01 b
14	peak V (RT 31.5)	1.42 ± 0.37 a	1.46 ± 0.15 b
15	apigenin	4.70 ± 0.87 a	0.04 ± 0.01 b
16	peak VI (RT 32.9)	12.16 ± 1.28 a	0.02 ± 0.01 b
17	peak VII (RT 40.1)	8.09 ± 0.21 a	1.87 ± 0.12 b

[a] Phenolics were expressed as milligrams of gallic acid equivalents per gram of oil. [b] Values with different letters in the same row are significantly different among groups ($p < 0.05$). [c] Not detected. [d] Retention time.

of the SPE phenolic extracts from the oils. Individual peaks were identified by comparing the retention times with the corresponding standards. However, unidentified peaks were also present (**Table 3**). Most representative phenolic compounds identified in EVOO were hydroxyl tyrosol, tyrosol, caffeic acid, cinnamic acid, and apigenin, which represent 34% of the total phenolics.[22] Unidentified phenolic compounds for six peaks represent 63% of the total phenolic compounds. The contents of the individual phenolics in SL olive oil were much lower than those in EVOO, possibly because the deacidification process subsequently removed a considerable amount of phenolic compounds. This result was similar to a previous report concerning the phenolic content between EVOO and refined olive oil in which commercial refined olive oil was found to contain a much lower amount of phenols (8 mg of GAEs/kg of oil) than EVOO (860 mg).[23]

Oxidative Stability. DPPH• was used to evaluate the RSC of SPE extracts of EVOO and SL olive oil. Their RSCs were compared with those of selected antioxidants (gallic acid, α-tocopherol, and BHT). The antioxidants exhibited higher DPPH• scavenging capacities (∼95%) than the oils. SPE extracts of EVOO showed a stronger RSC than those of SL olive oil (**Figure 2**). In addition, the induction time, determined with the Rancimat test, indicated that EVOO was more stable to oxidation than SL olive oil (**Table 2**).

Table 4. POVs of EVOO and SL Olive Oil with Rosemary Extracts during Storage at 60 °C[a]

	POV (mequiv/kg of oil)				
	0 days	5 days	10 days	15 days	20 days
			EVOO		
control	10.5 ± 0.7 a	14.5 ± 0.7 a	18.0 ± 0.1 d	23.0 ± 1.4 e	30.5 ± 0.7 d
100 ppm	11.0 ± 0.01 a	13.0 ± 0.1 ab	16.3 ± 0.3 e	20.8 ± 0.3 ef	26.0 ± 0.1 e
200 ppm	11.5 ± 0.7 a	12.0 ± 0.1 b	15.5 ± 0.7 e	18.3 ± 0.3 fg	24.5 ± 0.7 e
300 ppm	10.8 ± 0.3 a	11.5 ± 0.7 bc	15.8 ± 0.3 e	16.5 ± 0.7 g	22.5 ± 0.7 e
			SL Olive Oil		
control	2.8 ± 0.3 b	10.5 ± 0.7 c	41.5 ± 0.7 a	144.0 ± 1.4 a	262.0 ± 2.8 a
100 ppm	3.3 ± 0.3 b	11.0 ± 0.4 c	35.0 ± 1.4 b	117.5 ± 2.1 b	223.2 ± 1.4 b
200 ppm	2.8 ± 0.4 b	6.5 ± 0.7 d	27.5 ± 0.7 c	100.0 ± 2.8 c	221.0 ± 1.4 b
300 ppm	2.3 ± 0.3 b	3.5 ± 0.7 e	15.0 ± 0.1 e	52.0 ± 0.1 d	209.5 ± 2.1 c

[a] Values are the means of three samples. Values with different letters in the same column are significantly different among groups ($p < 0.05$).

Table 5. AVs of EVOO and SL Ilive Oil with Rosemary Extracts during Storage at 60 °C[a]

	AV (mg/kg of oil)				
	0 days	5 days	10 days	15 days	20 days
			EVOO		
control	1.2 ± 0.0 a	1.8 ± 0.2 b	1.5 ± 0.0 cd	3.5 ± 0.2 e	3.6 ± 0.1 d
100 ppm	0.9 ± 0.0 b	2.0 ± 0.7 b	1.6 ± 0.1 cd	3.5 ± 0.1 e	2.1 ± 0.3 e
200 ppm	1.0 ± 0.2 ab	2.2 ± 0.2 b	1.9 ± 0.1 c	3.5 ± 0.0 e	1.4 ± 0.5 f
300 ppm	0.9 ± 0.1 b	2.9 ± 0.0 a	1.8 ± 0.1 cd	3.2 ± 0.1 e	1.2 ± 0.0 f
			SL Olive Oil		
control	0.4 ± 0.6 c	1.6 ± 0.2 bc	4.6 ± 0.1 a	19.4 ± 0.1 a	31.4 ± 0.5 a
100 ppm	0.3 ± 0.0 c	1.0 ± 0.2 c	4.5 ± 0.0 a	17.6 ± 0.0 b	26.6 ± 0.0 b
200 ppm	0.3 ± 0.0 c	1.0 ± 0.1 c	3.3 ± 0.5 b	14.8 ± 0.1 c	26.5 ± 0.2 b
300 ppm	0.3 ± 0.1 c	0.9 ± 0.0 c	1.2 ± 0.3 d	8.9 ± 0.9 d	16.3 ± 0.4 c

[a] Values are the means of three samples. Values with different letters in the same column are significantly different among groups ($p < 0.05$).

The oxidative stabilities of EVOO and SL olive oil were also assessed by obtaining the POV for the initial oxidation stage and the AV and TBARS value for the secondary oxidation during 20 days of storage at 60 °C (**Tables 4−6**). Among the control oils, EVOO was less stable to oxidation than SL olive oil in the early stages (up to 5 days) since EVOO showed a higher POV, AV, and TBARS value. However, afterward, SL olive oil became more susceptible to oxidation, showing a rapid increase in the POV, AV, and TBARS value possibly because

Table 6. TBARS Values of EVOO and SL Olive Oil with Rosemary Extracts during Storage at 60 °C[a]

	TBARS (mg/kg of oil)				
	0 days	5 days	10 days	15 days	20 days
			EVOO		
control	0.0076 ± 0.001 b	0.0058 ± 0.001 a	0.0064 ± 0.001 bc	0.0053 ± 0.001 d	0.0075 ± 0.001 bc
100 ppm	0.0102 ± 0.001 a	0.0054 ± 0.001 a	0.0065 ± 0.001 b	0.0049 ± 0.001 de	0.0076 ± 0.001 b
200 ppm	0.0074 ± 0.001 b	0.0050 ± 0.001 a	0.0060 ± 0.001 bc	0.0048 ± 0.001 de	0.0058 ± 0.001 c
300 ppm	0.0097 ± 0.001 a	0.0052 ± 0.001 a	0.0059 ± 0.001 bc	0.0039 ± 0.001 e	0.0060 ± 0.001 bc
			SL Olive Oil		
control	0.0042 ± 0.001 c	0.0057 ± 0.002 a	0.0084 ± 0.001 a	0.0137 ± 0.001 ab	0.0151 ± 0.001 a
100 ppm	0.0043 ± 0.001 c	0.0063 ± 0.001 a	0.0082 ± 0.001 a	0.0128 ± 0.001 b	0.0145 ± 0.001 a
200 ppm	0.0045 ± 0.001 c	0.0059 ± 0.001 a	0.0062 ± 0.001 bc	0.0143 ± 0.001 a	0.0152 ± 0.001 a
300 ppm	0.0048 ± 0.001 c	0.0053 ± 0.001 a	0.0054 ± 0.001 c	0.0111 ± 0.001 c	0.0137 ± 0.001 a

[a] Values are the means of three samples. Values with different letters in the same column are significantly different among groups ($p < 0.05$).

lipid oxidation progressed with the formation of oxidative products such as hydroperoxides and aldehydes. The POV and AV of the EVOO control increased to 18.0 and 1.5, respectively, at 10 days of storage, but in the SL olive oil control these values increased to higher values (41.5 and 4.6, respectively) and then remained significantly higher than those of EVOO for the remaining days of storage ($p < 0.05$). The TBARS values increased gradually in both oils over the entire storage, but were significantly higher in SL olive oil than in EVOO after 5 days of storage ($p < 0.05$). Therefore, the pattern of increasing TBARS values was similar to that of AVs (**Table 6**).

This result suggests that the different oxidative stability values between oils might be due to the different amounts of phenolic compounds and tocopherols (**Table 2**). EVOO contains various natural antioxidants such as tocopherols, carotenoids, and phenolic compounds which are capable of retarding or preventing lipid oxidation.[24] Phenolic compounds have been reported to be more effective than tocopherols in enhancing the stability toward oxidation.[25] Therefore, the removal of tocopherols or phenolics could result in the oxidative deterioration of structured lipids. Besides, CLA has two conjugated double bonds that can oxidize faster than those of linoleic acid or oleic acid.[26] Thus, the increased content of CLA (42.5 mol %) contributed in part in making SL olive oil more susceptible to oxidation than olive oil, which contained 75.7 mol % C18:1 (oleic acid).

A further investigation was focused on the evaluation of the oxidative stability effects of adding rosemary extracts (100, 200, and 300 ppm), a natural antioxidant, to SL olive oil. The extract contained approximately 24.6% (w/w) carnosic acid and 3.9% carnosol on improving its oxidative stability. Hydroperoxide and aldehyde formation was effectively inhibited by rosemary extracts in SL olive oil as well as in EVOO, showing significantly lower POVs and AVs than the control (without rosemary extracts) (**Tables 4** and **5**). Their antioxidant activities appeared more effective in SL olive oil than in EVOO. The highest concentration extract (300 ppm) showed the lowest POV and AV at 5, 10, 15, and 20 days of storage at 60 °C, indicating that the antioxidant effectiveness increased with the concentration of rosemary extract (ROS·CON).

In conclusion, CLA is a free fatty acid that would not be a suitable form for use as dietary oil. To increase the concentration of CLA in edible oil, SL olive oil was synthesized from EVOO to incorporate CLA via enzyme-catalyzed acidolysis. The oxidative stability of SL olive oil was lower compared to that of EVOO due to the removal of antioxidative ingredients (i.e., phenolics and tocopherols) and the incorporation of CLA. However, the oxidative status of SL olive oil could be improved with the addition of proper antioxidants such as rosemary extract, which contains carnosic acid and carnosol as the major antioxidant components, acting as radical scavengers.[27] In this study rosemary extracts at different concentrations (100−300 ppm) could effectively retard oxidation in SL olive oil. From the viewpoint of functional lipids, SL olive oil contained 42.5 mol % CLA isomers, which may provide a variety of health benefits. A wide selection of olive oils are available in the marketplace, and all olive oils are graded in accordance with the degree of acidity they contain. Extravirgin olive oil having less than 1% acidity contains natural antioxidant compounds, but is the most expensive. This study showed that the production of SL olive oil resulted in the removal of phenolics and tocopherols during the reaction or purification (i.e., alkaline deacidification); therefore, use of cheaper grades of olive oil (or pure olive oil) may be more appropriate. The alkaline refining method was used for removing the free fatty acids from the reaction product in this study. Oils with a high content of free fatty acids require a relatively high amount of alkali, leading to high loss of neutral oils. Thus, a comparison between alkaline refining and physical refining (i.e., distillation) would be needed in a future study.

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