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Effect of the Degree of Processing on Soy Oil Conjugated Linoleic Acid Yields

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Photoirradiation processing can be used to rapidly synthesize conjugated linoleic acid (CLA) in large quantities in soy oil. The objective of this study was to evaluate the effect of the level of refining of soy oil on CLA yields and oxidative properties after photoirradiation. Crude, alkali-refined, alkali-refined bleached, and alkali-refined bleached and deodorized (RBD) soy oils were photoirradiated in a pilot-plant processing system for 12 h with 0.35% iodine catalyst at 47 °C. RBD soy oil gave the highest total CLA yield of 16.3% of total oil with 4.3% *cis*,*trans*- and *trans*,*cis*-CLA isomers. Oxidative stability as measured by weight gain during incubation at 64 °C showed that iodine decreased the induction time of soy oil samples by 2−4 days. Photoirradiation processing further decreased the induction time by 2 days as a result of loss of total tocopherols. Iodine addition increased the titratable acidity in all the samples of soy oil. However, the level of refining affects this increase of titratable acidity, and RBD soy oil was found to be the most stable. The study indicates that RBD soy oil was the most suitable candidate for photoirradiation processing. Thus, soy oil should be alkali-refined, bleached, deodorized, and then photoprocessed followed by a secondary adsorption step to remove the iodine catalyst to obtain a RBD CLA-rich soy oil.

KEYWORDS: Conjugated linoleic acid (CLA); photoirradiation; refining; iodine; oxidation

INTRODUCTION

Conjugated linoleic acid (CLA) is a group of geometric and positional isomers of linoleic acid naturally found in beef and dairy products at 0.2−0.9% fat level mainly consisting of *cis*-9,*trans*-11 CLA and *trans*-10,*cis*-12 CLA (*1, 2*). In vitro and in vivo results suggest that CLA shows anticarcinogenic properties, reduces atherosclerosis risk, affects growth rate favorably, enhances the immune system, and improves feed efficiency (*3–7*). Clinical trials showed that 1.8 g CLA/day significantly reduced body fat in healthy exercising men and decreased mean body mass index (BMI) (*8, 9*). Risérus and others showed that CLA decreases abdominal fat in obese men with metabolic syndrome (*10*). Recently, the *trans*,*trans*-CLA isomers have shown to have health benefits, in particular, *trans*-9,*trans*-11 CLA isomer inhibits bovine aortic endothelial cell proliferation by apoptotic pathway and proliferation of human leukemic cell lines (*11*). Storey et al. showed that *trans*,*trans*-CLA isomers decreased the ultraviolet radiation (UVR)-induced secretion of interleukin (IL-8), prostaglandin E2 (PGE2), and tumor necrosis factor (TNF-α) in human skin cells responsible for UVR-induced inflammation and carcinogenesis (*12*).

Recently, a pilot-plant scale photoirradiation process was developed to photoisomerize soy oil linoleic acid (LA) to conjugated linoleic acid in large quantities (*13*). Refined, bleached, and deodorized soy oil with 0.35% iodine catalyst was irradiated for 12 h to yield ∼22% CLA. However, there are no reports of the effect of the level of refining on CLA yields and oxidative stability after photoirradiation. Several minor components such as free fatty acids, tocopherols, carotenoids, pigments, trace metals, and peroxides are removed during each degree of refining, which may have a significant effect on CLA yields and oxidative stability (*14–17*).

The goal of this research was to determine the degree of processing of soy oil necessary prior to the photoirradiation step to obtain optimum CLA yields. The specific objectives were to evaluate the effect of the degree of refining on (a) CLA yields and (b) oxidative stability after photoirradiation.

MATERIALS AND METHODS

Materials. Soybean oil processed to varying degrees; crude oil (crude), alkali-refined oil (REF), alkali-refined bleached oil (RB), and alkali-refined bleached and deodorized oil (RBD) samples were obtained from Archer Daniels Midland (Decatur, IL). Resublimed iodine crystals were used as a catalyst (EM Science, Cherry Hill, NJ). Commercial CLA methyl ester (Sigma-Aldrich, St. Louis, MO) containing a mixture of *cis*-9,*trans*-11 CLA, *trans*-10,*cis*-12 CLA, and *trans*,*trans*-CLA isomers and heptadecanoic acid methyl ester (17:0; Sigma-Aldrich) were used as standards.

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CLA Synthesis by Photoirradiation. The soy oil samples (Crude, REF, RB, and RBD) with 0.35% iodine catalyst were photoisomerized in the pilot-plant scale regime for 12 h at 47 °C oil temperature (*13*). Samples were irradiated in duplicates.

CLA Content by FAME Analysis. Methyl esters were prepared from the photoisomerized oil by a base-catalyzed method to reduce the formation of conjugated *trans*,*trans*-isomers during analysis (*17*). Photoisomerized soybean oil (100 mg) was weighed into a 25 mL centrifuge tube, and 500 μL of 1% heptadecanoic acid methyl ester (17:0, internal standard), 2 mL of toluene, and 4 mL of 0.5 M sodium methoxide in methanol were added to the centrifuge tube and then purged with nitrogen gas. The centrifuge tube was heated to 50 °C for 10−12 min and then cooled for 5 min. To inhibit formation of sodium hydroxide, which could hydrolyze methyl esters to free fatty acids, glacial acetic acid (200 μL) was added to the centrifuge tube. Distilled water (5 mL) was added to the centrifuge tube followed by 5 mL of hexane, and the tube was vortexed for 2 min. The hexane layer was extracted and dried over anhydrous sodium sulfate in a 7 mL glass vial. Another 5 mL of hexane was added to the centrifuge tube, the tube was vortexed for another 2 min, and the hexane layer was dried over anhydrous sodium sulfate prior to methyl ester analysis.

Methyl esters were analyzed by gas chromatography (GC) using a SP 2560 fused silica capillary column (100 m × 0.25 mm i.d. × 0.2 μm film thickness; Supelco Inc., Bellefonte, PA) (*18*) with a flame ionization detector (FID) (model CP 3800, Varian, Walton Creek, CA). The fatty acid methyl ester samples were injected in triplicate by an autosampler CP8400 (Varian), and gas chromatograms were obtained by Galaxie Chromatography Workstation 1.9.3.2 (Varian). Different concentrations of FAME standards of C16:0−C18:3 and CLA isomers were injected in the GC-FID. The detected amount of each standard was plotted against the injected amount of that standard. Inverse of the slope of the plot gave the response factor of that standard. Oil samples were then injected in the GC-FID and CLA concentrations were calculated by the following equation:

$$\text{isomer conc} = \frac{\text{internal standard conc (5 mg)} \times \text{peak area} \times \text{relative response factor}}{\text{internal standard peak area}}$$

Absorbance and Turbidity Determination. 0.35% Iodine was mixed with soy oil samples, and a 20% solution of oil in hexane was prepared. The absorbance of each sample was measured in a 1 cm quartz cuvette, using a diode array spectrophotometer (Hewlett-Packard 8452A). The spectrophotometer is attached to a UV-cell-temperature controller (Agilent 89090A), which maintains the required temperature (25 °C) within the cuvette. Absorbance spectra were obtained by the UV−visible ChemStation A.10.01 software package (Agilent technologies, Santa Clara, CA), and absorbance in the UV range (220−380 nm) and visible range were reported. The turbidity of oil samples were obtained by measuring the absorbance at 600 nm.

Oil Oxidation Determination by Weight Change. The primary oxidation product formation and relative oxidation induction time were determined by weight gain during incubation at 64 °C (*19*). Soy oils processed to varying degrees of refining, oils with 0.35% iodine, and oils with 0.35% iodine irradiated in the pilot-plant system were incubated at 64 °C to determine the weight increase as an index of peroxide formation and weight loss as that of peroxide breakdown About a 0.5 g sample was taken in a disposable aluminum dish (5.7 cm i.d. × 1.6 cm d.; VWR International) in triplicate. The samples were weighed every 24 h. Results were expressed as percentage weight change per 500 mg of oil.

Total Tocopherol Content. The total tocopherols content of all soy oil samples and photoirradiated soy oil samples were determined after photoirradiation using the AOCS Ce-8-89 method (*20*).

Peroxide Value. Peroxide value of soy oils processed to varying degrees of refining and oils irradiated in the pilot-plant system were determined to evaluate the effect of irradiation on oxidative stability. An AOCS Cd 8-53 method was used to analyze the samples in triplicates (*20*).

Moisture. The moisture content of soy oils processed to varying degrees of refining, oils with 0.35% iodine, and oils irradiated in the pilot-plant system was determined using the AOCS Ca 2c-25 method (*20*).

Table 1. Fatty Acid Analysis of Crude, Alkali-Refined, Alkali-Refined Bleached, and Alkali-Refined Bleached and Deodorized Soy Oil Samples after UV Irradiation in the Pilot-Plant System for 12 h with 0.35% Iodine Catalyst[a]

soy oil sample	*cis-trans-* and *trans-cis*-CLA[b] (% total oil)	total CLA (% total oil)
crude	0.2 d	0.2 d
alkali-refined	1.7 c	6.4 c
alkali-refined bleached	2.8 b	10.3 b
alkali-refined bleached and deodorized	4.3 a	16.3 a

[a] Data expressed as means; $n = 4$. Values within a column with different letters differ significantly, $p < 0.05$. [b] Comprises *cis*-9,*trans*-11 CLA, *trans*-10,*cis*-12 CLA, *trans*-9,*cis*-11 CLA, and *cis*-10,*trans*-12 CLA isomers.

Titratable Acidity. The titratable acidity was measured by accurately weighing about 56.4 g of oil sample in an Erlenmeyer flask. Hot neutralized alcohol (95% ethyl alcohol) and 2 mL of phenolphthalein indicator solution (1% in 95% alcohol) were added to the flask. It was titrated with standard sodium hydroxide solution, shaking vigorously until the appearance of first pink color of the same intensity as that of the neutralized alcohol before the addition of the sample, with the color persisting for 30 s.

$$\text{titratable acidity (\%)} = \frac{\text{mL of alkali} \times N \times 40.0}{\text{wt of the sample}}$$

Statistics. Analysis of variance (ANOVA) was conducted on the data using the JMP Version 5.0.1 (SAS Inst. Inc., Cary, NC). A Student *t* test was used to differentiate mean values, with significance defined at $P < 0.05$.

RESULTS AND DISCUSSION

CLA Content by FAME Analysis. Table 1 shows the CLA content in soy oil samples, processed to various degrees of refining, after irradiation. Only 0.2% total CLA was synthesized in crude soy oil sample composed of *cis*-9,*trans*-11 CLA, *trans*-10,*cis*-12 CLA, *trans*-9,*cis*-12 CLA, and *cis*-10,*trans*-12 CLA isomers (*cis*,*trans*-/*trans*,*cis*-CLA). However, there was a significant increase in CLA yields as the degree of refining was increased. The Alkali-refined (REF) soy oil sample contained 6.4% total CLA with 1.7% *cis*,*trans*-/*trans*,*cis*-CLA. Approximately 75% of the total CLA was *trans*,*trans*-CLA composed of *trans*-9,*trans*-11 CLA, *trans*-10,*trans*-12 CLA, and *trans*-11,*trans*-13 CLA. The highest CLA content was obtained in refined bleached deodorized (RBD) soy oil with 16.3% CLA isomers. Thus, as the degree of refining was increased, the total CLA content of soy samples increased.

Since the more highly processed oil produced higher levels of CLA, UV absorbance and turbidity of the oil samples were obtained to see if these oil characteristics (UV absorbance and turbidity) were possible yield determining factors. **Figure 1a** shows the absorbance profile of iodine in hexane and soy oil samples processed to varying degrees of refining in the UV range. Iodine, a catalyst for the photoirradiation reaction, absorbs only in the lower UV range from 210 to 250 nm. The absorbance profiles of soy oil samples suggest that RBD soy oil absorbs the most in the 220−290 nm range, as compared to crude soy oil. The extent of UV radiation absorbed by soy oil samples increases as the degree of refining increases. The extent of UV radiation absorbed by the soy oil samples can be correlated to the amount of CLA isomers synthesized in each sample. **Figure 1b** shows the absorbance profile of the soy oil samples in the visible range from 375 to 500 nm, indicating the presence of carotenoids in the samples. As expected, the amount of carotenoids decreased with increasing degree of processing.

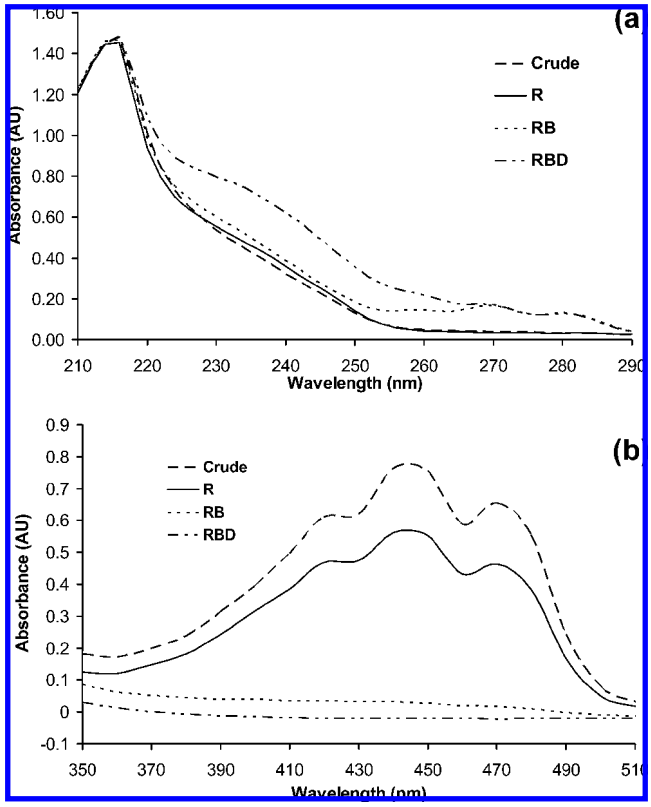

Figure 1. (**a**) Absorbance spectra of soy oils processed to varying degree of refining in the UV range. (**b**) Absorbance spectra of soy oils processed to varying degree of refining in the visible range.

Free iodine molecules form iodine radicals in the presence of UV light. Formation of iodine radicals is essential for the catalytic isomerization of linoleic acid double bonds to form conjugated linoleic acid. However, iodine absorbs in the UV range only from 210 to 250 nm. The UV absorbance of certain minor components in the upper UV range may aid in the excitation of iodine to form iodine radicals and thus positively affect the overall photoirradiation reaction resulting in increased CLA yields (**Figure 1a** and **Table 1**). Turbidity measurements of the oil samples showed that all the oil samples were clear and turbidity was not a factor of consideration for varying yields (data not shown).

Slightly lower CLA yields were observed in RBD soy oil in this study relative to those reported by Jain and Proctor (*13*). Lower CLA yields may be due to use of a different oil source subjected to a different degree of processing. This study shows that the degree of processing affects CLA yield, probably due to differences in oil composition, and is worthy of further study.

Oil Oxidation Determination by Weight Change. Table 2 shows the induction times (ITs) and percent weight gains (PWGs) of soy oil samples as a measure of oxidation stability and amount of breakdown products produced, respectively. Crude soy oil was found to be the most oxidatively stable with an IT of 13 days and PWG of 0.9%. REF, RB, and RBD soy oils had identical ITs of 8 days and similar PWGs. With addition of 0.35% iodine, ITs of all the oils decreased significantly with no significant change in PWGs. However, irradiation of oil samples further decreased the ITs to 4 days for all the samples with no significant change in PWGs.

Tsuzuki et al. found that CLA-containing triacylglycerols were as oxidatively stable as linoleic acid-containing triacylglycerols (*21*). Minemoto et al. showed that activation energies for the CLA isomers were greater than the nonconjugated polyunsaturated fatty acids. However, enthalpy−entropy compensation leads to similar oxidation rates for CLA and PUFA (*22*). Thus, reduction of ITs of irradiated soy oils may not be a result of CLA formation but may be attributed to loss of inherent antioxidants during photoirradiation or the pro-oxidant effect of iodine. Since iodine radicals are involved in catalytic formation of CLA, iodine radicals may extract an electron from lipid molecules initiating lipid radical formation, which can then propagate to form hydroperoxides. Thus, the mode of oxidation of soy oil with iodine remains the same but initiated sooner by iodine.

Table 3 shows the total tocopherol content of soy oils before and after irradiation. Unirradiated crude, REF, and RB soy oils had similar total tocopherol content. RBD soy oil had about 285 ppm of total tocopherols as a result of tocopherol loss during deodorization. As a result of irradiation, we observed a 15−30% loss of total tocopherols in each oil sample, and it can be attributed to the decreased induction times for oxidation of oil samples. This loss of tocopherols may need to be compensated for in an irradiated oil sample to preserve the oil oxidation quality.

Peroxide Value. Table 4 shows the peroxide values (PVs) of soy oils before and after irradiation. PVs of unirradiated crude and RBD samples were found to be the lowest at about 0.2 mequiv/1000 g sample. The low PVs of crude and RBD samples can be attributed to native carotenoids and tocopherols present in crude oil and removal of formed hydroperoxides and byproducts from RBD oil during refining. Initial PVs of REF and RB soy oils were found to be significantly higher than crude and RBD oil samples, although they were unchanged after photoirradiation. PVs of crude and RBD soy oil increased and may be attributed to the loss of total tocopherols during irradiation. However, the PV of irradiated RBD soy oil was lower than other irradiated oil samples because metal catalysts and other impurities responsible for oxidation initiation are removed during refining steps and thus decreasing the possibility of triacylglycerol oxidation.

Moisture. High moisture content of oil samples can lead to hydrolysis of triacylglycerol molecules, producing free fatty acids that are less oxidatively stable than triacylglycerols, in the presence of a suitable catalyst. **Table 5** shows the moisture content of soy oil samples, soy oil with 0.35% iodine, and soy oil samples with 0.35% iodine irradiated in the pilot-plant system for 12 h. Crude soy oil had the highest moisture with 0.103% of oil (w/w) whereas no significant change in the moisture content of soy oil was observed as a result of alkali-refining because no water removal step is involved. However, with every additional refining step, moisture content decreases. RBD soy oil had the least moisture with 0.006% of oil weight. Addition of iodine and irradiation processing did not have any consistent effect on moisture content, although RBD soy oil was found to change the least. Stable moisture content would maintain a stable titratable acidity and free fatty acid profile of the oil.

Titratable Acidity. Titratable acidity (TA) was used as a measure of total acidity as a result of free fatty acids and any other acid generated during iodine addition and irradiation processing. Increase in acidity of the oil samples can lead to acid hydrolysis of the oil generating free fatty acids and thus changing the oxidation profile of the oil. **Table 6** shows the TAs in soy oil samples, soy oil samples with 0.35% iodine, and soy oil samples with 0.35% iodine irradiated in the pilot-plant system for 12 h. Every refining step results in the removal of free fatty acids from soy oil, which is reflected by the TAs

Table 2. Induction Time (IT) and Percent Weight Gain (PWG) at an Incubation Temperature of 64 °C by Crude, Alkali-Refined, Alkali-Refined Bleached, and Alkali-Refined Bleached and Deodorized Soy Oil Samples, Soy Oil Samples with 0.35% Iodine Catalyst, and Soy Oil Samples Irradiated in the Pilot-Plant System for 12 h with 0.35% Iodine Catalyst[a]

	oil		oil with 0.35% iodine		irradiated oil with 0.35% iodine	
soy oil sample	IT (days)	PWG (%)	IT (days)	PWG (%)	IT (days)	PWG (%)
crude	13 a	0.9 w	8 b	1.1 w	4 d	0.9w
alkali-refined	8 b	4.2 yz	4 d	5.2 z	4 d	4.0 yz
alkali-refined bleached	8 b	3.7 xy	6 c	4.3 yz	4 d	4.0 yz
alkali-refined bleached and deodorized	8 b	5.4 z	6 c	5.1 z	4 d	5.2 z

[a] Data expressed as means; $n = 6$. Values within columns for a particular analysis with different letters differ significantly, $p < 0.05$.

Table 3. Total Tocopherol Content of Crude, Alkali-Refined, Alkali-Refined Bleached, and Alkali-Refined Bleached and Deodorized Soy Oil Samples and Soy Oil Samples Irradiated in the Pilot-Plant System for 12 h with 0.35% Iodine Catalyst[a]

	total tocopherol content (ppm)	
oil	control oil	irradiated oil with 0.35% iodine
crude	328.3 a	275.5 bc
alkali-refined	330.3 a	241.5 cd
alkali-refined bleached	346.2 a	277.6 bc
alkali-refined bleached and deodorized	285.5 b	201.0 d

[a] Total tocopherol content measured as sum of α, β, γ, and δ tocopherols. Data expressed as means; $n = 4$. Values within a column with different letters differ significantly, $p < 0.05$.

Table 4. Peroxide Values of Crude, Alkali-Refined, Alkali-Refined Bleached, and Alkali-Refined Bleached and Deodorized Soy Oil Samples and Soy Oil Samples Irradiated in the Pilot-Plant System for 12 h with 0.35% Iodine Catalyst[a]

	peroxide value (mequiv/1000 g sample)	
soy oil sample	oil	irradiated oil with 0.35% iodine
crude	0.2 b	0.9 a
alkali-refined	1.2 a	0.9 a
alkali-refined bleached	0.8 a	0.9 a
alkali-refined bleached and deodorized	0.2 b	0.4 a

[a] Data expressed as means; $n = 4$. Values within a row with different letters differ significantly, $p < 0.05$.

Table 5. Moisture Content of Crude, Alkali-Refined, Alkali-Refined Bleached, and Alkali-Refined Bleached and Deodorized Soy Oil Samples, Soy Oil Samples with 0.35% Iodine Catalyst, and Soy Oil Samples Irradiated in the Pilot-Plant System for 12 h with 0.35% Iodine Catalyst[a]

	moisture content (% w/w)		
soy oil sample	oil	oil with 0.35% iodine	irradiated oil with 0.35% iodine
crude	0.10 a	0.09 ab	0.06 bc
alkali-refined	0.08 ab	0.03 cd	0.08 ab
alkali-refined bleached	0.03 cd	0.03 cde	0.01 de
alkali-refined bleached and deodorized	0.01 e	0.00 de	0.00 de

[a] Data expressed as means; $n = 4$. Values with different letters differ significantly, $p < 0.05$.

of soy oil samples processed to varying degrees of refining. Crude oil had the highest TA with 0.65 mg NaOH/g oil, and RBD oil had the least with 0.06 mg NaOH/g oil. Iodine addition significantly increased the TA in every oil sample and may be a result of several complex reactions between iodine, moisture, and other minor components. TA varied inconsistently with irradiation processing of soy oil samples with 0.35% iodine.

Table 6. Titratable Acidities of Crude, Alkali-Refined, Alkali-Refined Bleached, and Alkali-Refined Bleached and Deodorized Soy Oil Samples, Soy Oil Samples with 0.35% Iodine Catalyst, and Soy Oil Samples Irradiated in the Pilot-Plant System for 12 h with 0.35% Iodine Catalyst[a]

	titratable acidity (mg NaOH/g oil)		
soy oil sample	oil	oil with 0.35% iodine	irradiated oil with 0.35% iodine
crude	0.7 c	1.6 a	1.1 b
alkali-refined	0.2 f	0.3 e	0.3 e
alkali-refined bleached	0.1 g	0.2 f	0.5 d
alkali-refined bleached and deodorized	0.1 g	0.2 f	0.2 f

[a] Data expressed as means; $n = 4$. Values with different letters differ significantly, $p < 0.05$.

However, TAs of RBD soy oil, RBD oil with 0.35% iodine, and irradiated RBD oil was found to be the most stable. Lower moisture content and removal of impurities and metal catalysts, during refining steps, causing triacylglycerol hydrolysis may be responsible for such stable titratable acidity of an irradiated CLA-rich RBD soy oil.

The study suggests that the level of refining affects the CLA yields obtained and the oxidative stability of the oil during photoirradiation. Alkali-refined bleached and deodorized soy oil was found to be the most suitable oil for photoirradiation processing as it gave the highest CLA yield with minimal change in peroxide value. Iodine plays a significant role in the oil oxidative stability and probably needs to be taken into account for stability calculations. Total tocopherol content of the oil decreases with photoirradiation processing and needs to be compensated for after processing.

ABBREVIATIONS USED

CLA, conjugated linoleic acid; BMI, body mass index; UVR, ultraviolet radiation; crude, crude oil; REF, alkali-refined oil; RB, alkali-refined bleached oil; RBD, alkali-refined bleached deodorized oil; GC, gas chromatography; FID, flame ionization detector; AOCS, American oil chemists' society; ANOVA, analysis of variance; IT, induction time; PWG, percent weight gain; PV, peroxide value.

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