

## Comparative Study of High-Linoleic Acid Vegetable Oils for the Production of Conjugated Linoleic Acid

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Conjugated linoleic acid (CLA) is found in small quantities in dairy and beef products. Obtaining optimum dietary CLA levels from these sources requires an increased intake of saturated fat. A 20% CLA soy oil was produced by UV photoisomerization of soy oil linoleic acid (LA), which is naturally low in saturated fat, but no other high-LA vegetable oils have been studied for their potential as CLA-rich oils. The objectives of this research were to (1) compare flax, sunflower, corn, soy, and high-LA safflower oils as sources of CLA-rich vegetable oils using laboratory-scale equipment, (2) compare CLA yields obtained by laboratory-scale and pilot-scale equipment, and (3) compare the oxidative stabilities of laboratory-scale processed oils. High-LA safflower oil produced the most CLA; soy oil produced slightly less, followed by corn, with flax producing very little and sunflower none at all. Minor oil components and turbidity reduced CLA yields, suggesting that oils should be highly refined before CLA production. The pilot-scale system was more effective than the laboratory-scale system due to greater light exposure and larger surface area to volume ratio of the oil samples. The oxidative stabilities of high-LA safflower oil and soy oil were similar before or after irradiation, indicating that these oils are the most suitable for high-CLA production.

**KEYWORDS:** Linoleic acid; vegetable oil; CLA; corn oil; flax oil; sunflower oil; flax seed oil; high-LA safflower; soy oil

### INTRODUCTION

Conjugated linoleic acid (CLA) is a mixture of positional and geometric isomers of linoleic acid (octadecadienoic fatty acid) found naturally in dairy and beef products. The most common CLA isomers are *cis*-9,*trans*-11-octadecadienoic acid and *trans*-10,*cis*-12-octadecadienoic acid (1), but other isomers are also present in food, including *trans*-9,*trans*-11 and *trans*-10,*trans*-12 (2). The National Academy of Sciences concluded that CLA “is the only fatty acid shown unequivocally to inhibit carcinogenesis in experimental animals” (3). Studies indicate that CLA plays antidiabetic, anti-obesity, and antiatherogenic roles (4, 5). CLA appears naturally at levels of 0.3–0.8% (w/w) of the fat in beef and dairy products, making it difficult to consume the recommended 3 g of CLA per day (6, 7) that is necessary to produce the desired physiological effects, without consuming undesirable amounts of saturated fats and cholesterol. For this reason, an alternative source that contains high levels of CLA that is low in saturated fat and cholesterol would be an excellent addition to the human diet.

CLA can be produced by several means, including biosynthesis by fermentation by anaerobic rumen bacteria (8) in bovines. CLA has also been produced by controlled fermentation (9–12) and organic synthesis (13), but these methods are complicated and time-consuming.

Photoisomerization of linoleic acid (LA) in soy oil has been demonstrated to be a simple and effective alternative method of CLA synthesis (14) that can be completed on a laboratory

scale (15) using a UV–visible lamp with 0.15% (w/w) iodine as a catalyst with standard photochemical irradiation apparatus. However, this method is very time-consuming, requiring 144 h to synthesize 20% (w/w) CLA. A pilot-scale method has been developed and optimized by Jain et al. (16) so that CLA-rich soy oil can be produced on a pilot scale yielding 20% (w/w) CLA in 12 h. This requires 0.35% (w/w) iodine catalyst and a customized illuminated laminar flow unit (ILFU) consisting of borosilicate glass plates and three 450 W UV–/visible lamps, to increase the oil’s exposure to ultraviolet light. The advantage of our photoisomerization technique is that CLA is produced in a food source, whereas previous methods, such as that of Yang and Liu (13), produced CLA as an inedible free fatty acid. However, only soy oil has been used to study photoisomerization of oil LA. Other vegetable oils that contain appreciable amounts of LA, such as flax, sunflower, corn, and high-LA safflower oils, are available but have not been evaluated as a source of CLA-rich oil. Therefore, the objectives of this research were to compare flax, sunflower, corn, soy, and high-LA safflower oils as sources of CLA-rich vegetable oils using laboratory-scale processing equipment; to compare laboratory-scale and pilot-scale CLA yields in flax, sunflower, corn, soy, and high-LA safflower oils; and to compare the oxidative stabilities of CLA-rich flax, sunflower, corn, soy, and high-LA safflower oils prepared with laboratory-scale processing equipment.

### MATERIALS AND METHODS

**Materials.** The following vegetable oils were used: high-LA safflower (Liberty Vegetable Oil Co., Santa Fe Springs, CA), soy (Wesson, ConAgra

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Foods, Inc., Omaha, NE), corn (Mazola, ACH Food Companies, Inc., Memphis, TN), sunflower (The Hain Celestial Group, Inc., Melville, NY), and flax (Spectrum Organic Products, LLC, The Hain Celestial Group, Inc.).

Resublimed iodine (EM Science, Cherry Hill, NJ) was used as a catalyst for isomerization. Sodium methoxide and anhydrous sodium sulfate (EM Science, Darmstadt, Germany) were used for methyl ester preparation for oil fatty acid analysis by gas chromatography. Commercial heptadecanoic acid (17:0) methyl esters (Sigma-Aldrich, St. Louis, MO) were used as standards for GC analysis. Activated carbon (Norit Americas Inc., Marshall, TX) was used to remove iodine from the oil samples after photoirradiation and before oxidative stability analysis.

**Comparison of Flax, Sunflower, Corn, Soy, and High-LA Safflower Oils as Sources of CLA-Rich Vegetable Oils Using Laboratory-Scale Processing Equipment.** *Photoisomerization.* A laboratory-scale customized photochemical reaction unit (Ace Glass Inc., Vineland, NJ) and an ultraviolet light source and methodology described by Jain and Proctor (15) were used. Duplicate 700 g oil samples from each oil source were deaerated by sonication for 10 min and then wrapped with aluminum foil to prevent light exposure while being heated to 70 °C to dissolve 0.15% iodine (w/w). The oil was transferred to the reaction vessel, and the photochemical system was connected to a water supply so that the temperature of the oil could be controlled between 22 and 25 °C while being closely monitored with a Traceable Big-Digit Memory Thermometer sensor (VWR International, Friendswoods, TX). The photochemical reaction system was placed on a magnetic stirrer to facilitate continuous oil stirring during the 168 h irradiation. Ten milliliter samples were collected every 24 h of the irradiation for fatty acid analysis.

*LA and CLA Fatty Acid Analysis by GC-FID.* Oil fatty acid analysis, including the LA and CLA contents, was conducted on each duplicate oil sample both before irradiation and after each day of irradiation. Two replicates from each duplicate oil sample were converted to fatty acid methyl esters (FAMES), which were injected in triplicate by an autosampler CP8400 for GC-FID analysis. This analysis was conducted according to the method of Christie et al. (17).

To convert each replicate to methyl esters, 100 mg of oil was weighed into a 25 mL centrifuge tube in duplicate, and 500  $\mu$ L of 1% heptadecanoic acid methyl ester (w/w) (17:0, internal standard), 2 mL of toluene, and 4 mL of 0.5 M sodium methoxide in methanol were added. The centrifuge tube was heated to 50 °C for 10 min and then cooled for 5 min. To inhibit formation of sodium hydroxide, which could hydrolyze methyl esters to free fatty acids, 200  $\mu$ L of glacial acetic acid was added to the centrifuge tube. Five milliliters of distilled water was then added 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 borosilicate glass vial.

Methyl esters were analyzed by GC using an SP 2560 fused silica capillary column (100 m  $\times$  0.25 mm i.d.  $\times$  0.2  $\mu$ m film thickness; Supelco Inc., Bellefonte, PA) with an FID (model 3800, Varian, Walton Creek, CA). Triplicate 2  $\mu$ L samples, prepared in hexane, were injected by an autosampler CP8400 (Varian), and gas chromatograms were collected by Galaxie Chromatography Workstation 1.9.3.2 (Varian). CLA concentrations were calculated by using the following equation:

$$\text{isomer concn} = \frac{[\text{internal std concn} \times \text{peak area} \times \text{response factor}]}{\text{internal std peak area}}$$

*Tocopherol Analysis.* Tocopherol content (including  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocopherols) was determined in duplicate for each sample oil before irradiation using method AOCS Ce 8-89 of the American Oil Chemists' Society (18). Standard stock solutions of  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocopherols in methanol (10 mg/10 mL) were prepared, and the absorbance of the solution was measured at 292 nm by a photodiode array detector to calculate the concentration ( $\mu$ g/mL). Appropriate volumes of the stock solution of the tocopherol standards were mixed to obtain a mixed-tocopherol standard working solution and further diluted with hexane to give a solution containing between 1 and 5  $\mu$ g/mL of each tocopherol. The HPLC analytical column (250  $\times$  4 mm) was packed with microparticulate silica (5  $\mu$ m). The HPLC mobile phase consisting of propan-2-ol in hexane (0.5:99.5, v/v) was pumped throughout the column at a flow rate of 1 mL/min for at least 30 min. About 2 g of the homogenized oil sample was

weighed accurately into a 25 mL volumetric flask with hexane. Mixed tocopherol standard working solution (20  $\mu$ L) was injected onto the column, and the area of the tocopherol peaks was recorded using an integrator. Then 20  $\mu$ L of the oil sample was injected and the tocopherols present were identified by reference to the chromatograms obtained from the standards. The areas of the tocopherol peaks were recorded. Two determinations each consisting of duplicate injections were conducted for each oil sample.

*Turbidity Analysis.* Duplicate samples of each oil replicate were analyzed for turbidity prior to irradiation. Each duplicate was diluted 1:10 by volume in hexane and measured by absorption at 600 nm.

*Carotenoid Analysis.* Duplicate samples of each oil replicate were quantified as lutein, the major soy oil carotenoid, even though other carotenoids were present. Each oil duplicate was diluted 1:10 by volume in hexane and measured by absorption at 445 nm, using the method to determine soy lutein content (19).

*Lovibond Color Analysis.* The color of each undiluted oil replicate was measured in duplicate before irradiation in terms of yellow, red, blue, and neutral units by the Lovibond Tintometry RYBN scale (20, 21). This technique relates the color of light transmitted through the oil and its cuvette with the color of light transmitted from the same light source through a set of glass slides of standard size and color (22).

*Phospholipid Analysis.* Duplicate samples from each replicate were analyzed for phospholipid content prior to irradiation. The phospholipid concentration of unirradiated oil duplicates was analyzed by inductively coupled plasma optical emission spectroscopy using a Spectro Flame Modula EOP model FSMEA 85D inductive coupled plasma spectroscopy unit (23).

**Comparison of Flax, Sunflower, Corn, Soy, and High-LA Safflower Oils as Sources of CLA-Rich Vegetable Oils Using Pilot-Scale Processing Equipment.** *Photoisomerization.* The pilot-scale irradiation method (16) was adapted for using small oil samples using the method of Lall et al. (24). The illumination system consisted of three 450 W UV-visible lamps (Ace Glass Inc.) and reflective surfaces to increase UV ray penetration of the oils. Duplicate 5 g oil samples from each oil were degassed by sonication for 10 min and heated to 70 °C to dissolve 0.35% iodine (w/w) while wrapped with aluminum foil to prevent light exposure during heating. The 5 g aliquots of each oil were placed in 7 mL borosilicate glass vials (~1 cm in diameter) and attached to the illuminated laminar flow unit of a photoirradiation unit at 3.14 mW as described by Jain et al. (16) and illuminated for 12 h.

*LA and CLA Fatty Acid Analysis by GC-FID.* Oil fatty acid analysis, including the LA and CLA contents, was conducted on oils before and after irradiation according to the method of Christie et al. (17). Two 100 mg aliquots of each oil duplicate were converted to methyl esters and quantified by gas chromatography with an SP 2560 fused silica column and flame ionization detector, as described previously.

*UV Intensity Analysis.* UV intensity in both processing systems was measured with a digital photometer (Industrial Fiber Optics Inc., Tempe, AZ) in the absence of oil. For the laboratory-scale system, the measurements were taken at the outer edge of the borosilicate glass reaction vessel, with the light source located inside the vessel. For the pilot-scale system, the measurements were taken with the IFLU midway between the photometer and the light source.

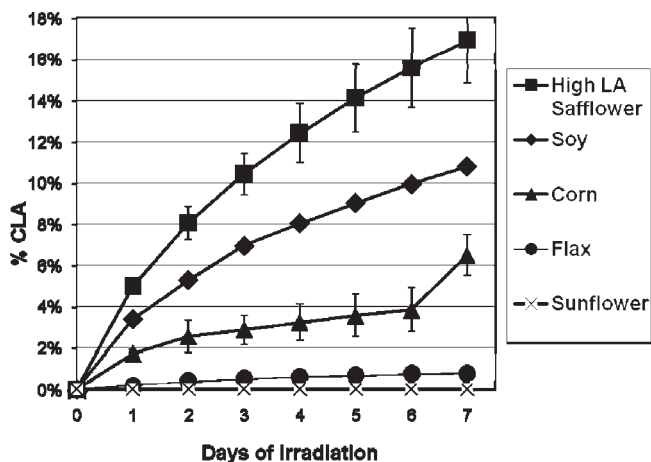
**Comparison of the Oxidative Stabilities of CLA-Rich Flax, Sunflower, Corn, Soy, and High-LA Safflower Oils Prepared with Laboratory-Scale Processing Equipment.** *Sample Preparation.* The sample oils both before and after laboratory-scale irradiation were adsorption processed with 2% SX-51 carbon (Norit Americas Inc.) at 100 °C for 30 min to remove residual iodine. During this time, the beaker filled with oil was wrapped in aluminum foil to prevent light exposure. The carbon-adsorbed oil was then filtered using Whatman GF/A filter paper (VWR International Inc.) to remove the carbon adsorbent.

*Gravimetric Analysis.* The oxidative stability of the irradiated oil and corresponding unirradiated control was determined according to the method of Proctor and Bowen (25). Triplicate 500 mg samples from each duplicate sample were stored at 64 °C and weighed daily for up to 24 days, depending on the oil's rate of oxidation. Data are expressed as percent weight change, indicative of the formation and subsequent degradation of

**Table 1.** Initial and Final Linoleic Acid Values and Final CLA Values after Photoirradiation for 7 Days on the Laboratory-Scale Processing Equipment As Measured by GC-FID<sup>a</sup>

oil	fatty acid concentration (% total oil)		
	initial linoleic acid	final linoleic acid	final CLA
flax	14.7 ± 0.1 a	14.7 ± 0.1 a	0.7 ± 0.1 a
sunflower	34.0 ± 0.4 b	33.9 ± 0.4 b	nd
corn	57.5 ± 1.1 c	49.5 ± 0.6 c	6.5 ± 1.0 b
soy	53.7 ± 0.3 d	42.9 ± 0.4 d	10.8 ± 0.1 c
high-LA safflower	72.6 ± 0.2 e	54.9 ± 2.2 e	16.9 ± 2.0 d

<sup>a</sup> Data with the same letter within the same column are not significantly different, with significance defined at  $P < 0.05$ . nd, none detected.

**Figure 1.** CLA production in high-LA safflower, soy, corn, flax, and sunflower oils by laboratory-scale processing.

lipid hydroperoxides, the primary product of oxidation that leads to rancid products and hazardous free radical formation (26).

**Statistical Analysis.** Analysis of variance (ANOVA) was conducted on all data using JMP version 5.0.1 (SAS Institute Inc., Cary, NC). A Student  $t$  test was used to differentiate mean values, with significance defined at  $P < 0.05$ . Standard deviations were also determined.

## RESULTS AND DISCUSSION

**Comparison of Flax, Sunflower, Corn, Soy, and High-LA Safflower Oils as Sources of CLA-Rich Vegetable Oils Using Laboratory-Scale Processing Equipment.** LA and CLA Fatty Acid Analysis by GC-FID. High-LA safflower oil had the highest initial LA (72.6%), whereas soy and corn oils had intermediate levels (57.5 and 53.7% (w/w), respectively) (Table 1). Sunflower oil had less LA, and flax oil had the least. Oils with the highest initial LA produced the most CLA. However, the soy conversion rates were lower than those reported by Jain and Proctor (15). This may be due to using soy oils that contained different minor component levels, as the greater degree of oil refining, the greater the CLA yields (26). Soy oil produced more CLA than corn oil. Flax oil produced very little CLA with 14% (w/w) initial LA, and sunflower oil produced no CLA at all, despite having 34% initial LA (w/w). The conversions of LA to CLA in each oil were ~23% in high-LA safflower oil, ~20% in soy oil, ~11% in corn oil, ~5% in flax oil, and 0% in sunflower oil. A comparison of soy and corn CLA yields and sunflower and flax CLA yields shows that LA levels alone are not good predictors of CLA production. CLA yields may be reduced by the presence of minor oil components as the more highly the oil is refined, the greater the CLA yield (27). Oil peroxides and phospholipids inhibit CLA yields, possibly by UV absorption (28). Specifically, with the

**Table 2.** CLA Isomer Levels after Photoirradiation for 7 Days on the Laboratory-Scale Processing Equipment As Measured by GC-FID<sup>a</sup>

oil	CLA isomer concentration (% total oil)			
	<i>cis</i> -9, <i>trans</i> -11	<i>trans</i> -9, <i>cis</i> -11 and <i>trans</i> -10, <i>cis</i> -12	<i>trans</i> -10, <i>cis</i> -12	<i>trans</i> -, <i>trans</i> - (8,10; 9,11; and 10,12)
flax	nd	nd	nd	0.7 ± 0.1 a
sunflower	nd	nd	nd	nd
corn	0.5 ± 0.5 a,b	0.5 ± 0.4 a	0.6 ± 0.3 a	4.7 ± 0.2 a
soy	1.1 ± 0.0 b,c	1.3 ± 0.0 b	1.1 ± 0.0 b	7.3 ± 0.1 a
high-LA safflower	1.6 ± 0.1 c	2.0 ± 0.3 b	1.6 ± 0.1 c	11.8 ± 1.9 b

<sup>a</sup> Data with the same letter within the same column are not significantly different, with significance defined at  $P < 0.05$ . nd, none detected.

**Table 3.** Tocopherol Content of Oils before Photoirradiation As Measured by HPLC<sup>a</sup>

oil	tocopherol concentration (ppm)			
	total tocopherols	$\alpha$ -tocopherols	$\beta/\gamma$ -tocopherols	$\delta$ -tocopherols
flax	38.9 ± 4.5 a	14.6 ± 2.8 a	27.8 ± 1.7 c	0.3 ± 0.4 a
sunflower	103.9 ± 5.7 b	92.3 ± 5.4 c	11.6 ± 0.3 a	nd
corn	115.6 ± 6.5 b	39.3 ± 1.9 b	76.3 ± 4.6 d	0.2 ± 0.3 a
soy	112.3 ± 4.6 b	35.7 ± 6.8 b	68.9 ± 4.0 e	9.7 ± 0.8 b
high-LA safflower	124.0 ± 15.9 b	109.5 ± 15.3 d	14.5 ± 0.6 b	0.2 ± 0.3 a

<sup>a</sup> Data with the same letter within the same column are not significantly different, with significance defined at  $P < 0.05$ . nd, none detected.

laboratory-scale equipment, the reductions in LA content for the irradiated oils were ~8, ~11, and ~18% for corn, soy, and high-LA safflower oils, respectively. The LA contents of flax and sunflower oils were unchanged.

The highest rate of CLA production for all oils was during the first day of irradiation (Figure 1) and was followed by a decrease in production rate each following day with the exception of day 6 in corn oil. In oils with the most initial LA, the amount of total CLA produced continues to increase after 7 days of irradiation, indicating the benefit of further irradiation for high-LA safflower and soy oils. The rate of CLA formation in corn oil was less than that of soy oil despite having slightly more initial LA. However, flax and sunflower oils did not produce a significant amount of CLA despite the presence of LA. This further suggests that factors other than initial LA, such as minor oil components, may affect CLA yields.

The *trans*-,*trans*-CLA isomers (Table 2) were produced in the greatest yield for all irradiated oils, comprising 70% (w/w) of the total CLA. The remaining isomers were a mixture of *cis*-,*trans*- (9,11 and 10,12) and *trans*-,*cis*- isomers (9,11 and 10,12), which were present in greater quantities than found in animal products (29). The large yield of *trans*-,*trans*-CLA isomers differs from the results of the biosynthesis and bacterial fermentation methods of CLA formation, which mainly produce the *cis*-9,*trans*-11 isomer. However, the *trans*-,*trans*-CLA isomer still leads to CLA's health benefits (30) and is derived from *cis*-,*trans*-CLA isomers. It is probably favored during photoisomerization because of the greater thermodynamic stability of the *trans*-isomer relative to the *cis*-isomer (29).

**Tocopherol Analysis.** Table 3 shows that high-LA safflower, soy, corn, and sunflower oils were found to have a total tocopherol content in excess of 100 ppm, with no significant difference. Flax oil had a significantly lower tocopherol concentration of <40 ppm. For high-LA safflower and sunflower oils,  $\alpha$ -tocopherols were the most prominent, whereas  $\beta/\gamma$ -tocopherols were the most common in soy, flax, and corn oils.  $\delta$ -Tocopherols reached significant levels only in soy oil. The  $\delta$ -tocopherols and

**Table 4.** Carotenoid Content Calculated as Lutein by Absorption at 445 nm and Lovibond RYBN Oil Color before Photoirradiation<sup>a</sup>

oil	carotenoid content (ppm)	red	yellow
flax	9.8 ± 0.5 a	1.5 ± 0.1 c	70.0 ± 0.0 d
sunflower	1.2 ± 0.1 b	0.2 ± 0.1 a	0.6 ± 0.1 a
corn	nd	0.3 ± 0.0 b	1.0 ± 0.0 c
soy	nd	0.2 ± 0.0 a	0.7 ± 0.0 b
high-LA safflower	nd	0.1 ± 0.0 a	0.5 ± 0.0 a

<sup>a</sup> Data with the same letter within the same column are not significantly different, with significance defined at  $P < 0.05$ . nd, none detected.

$\gamma$ -tocopherols are the most potent radical-scavenging antioxidants of all tocopherols (33, 34) and may therefore hinder free radical formation at the methylene carbon  $\alpha$  to both double bonds in LA, which is the first step in LA isomerization to CLA. Because  $\delta$ -tocopherols were present in soy oil at high levels, this may explain why CLA yield in soy oil was lower in this study than in previous studies (16, 17). It is difficult to determine which oils had the highest  $\gamma$ -tocopherol content, because  $\gamma$ -tocopherols and  $\beta$ -tocopherols coelute during HPLC analysis. Oils with the highest  $\beta/\gamma$ -tocopherol content were soy oil and corn oil, with  $\sim 70$  ppm, whereas the other oils studied were all below 30 ppm of  $\beta/\gamma$ -tocopherols (31, 32). This may explain why both soy and corn oils yielded less CLA than expected despite their relatively high LA content.

**Turbidity Analysis.** Turbidity was measurable in only sunflower oil with an absorption of  $0.60 \pm 0.00$  at 600 nm. Sunflower oil is the primary vegetable oil noted for its turbidity after oil refining (33, 34). Turbidity is probably due to residual waxes and sterols, which may interfere with UV light absorption by LA. Although turbidity is low, it may explain why no CLA was produced from sunflower oil.

**Carotenoid Analysis.** Table 4 shows the carotenoid concentration for each oil, calculated as lutein. The only vegetable oils to show a significant lutein concentration were flax oil, with 9.8 ppm of lutein, and sunflower oil, with 1.2 ppm of lutein, both of which produced little to no CLA. High-LA safflower, soy, and corn oils had no measurable lutein content and produced the most CLA. The significant amount of carotenoids in flax oil may have hindered CLA formation due to UV absorbance (35), possibly contributing to flax oil's low conversion rate ( $\sim 5\%$ ) of LA to CLA.

**Color Analysis.** Table 4 describes the oil color as determined by the Lovibond RYBN scale and reflects the carotenoid data. Flax oil was determined to be the most highly colored, with readings of 70.0 for yellow and 1.5 for red, due to lutein content. All other oils analyzed had color readings of  $\leq 1.0$  for all colors of the Lovibond RYBN scale. The large values for color, especially yellow carotenoids, in flax oil may hinder UV light penetration, which would also effectively inhibit the free radical mechanism through which CLA is produced from LA. Either of these possibilities may explain the low conversion rate ( $\sim 5\%$ ) of LA to CLA found in flax oil.

**Phospholipid Phosphorus Analysis.** Phosphorus was not detected in high-LA safflower, soy, corn, or sunflower oils at any level but was detected in flax oil at 5.79 ppm and is within the range in which phospholipids provide antioxidant activity (36). The data indicate a very high degree of processing in terms of degumming, which reduces the oil phospholipid phosphorus content from 500–900 to 12–170 ppm (37). Therefore, lack of light penetration due to turbidity caused by phospholipids is highly improbable.

**Comparison of Flax, Sunflower, Corn, Soy, and High-LA Safflower Oils as Sources of CLA-Rich Vegetable Oils Using Pilot-Scale Processing Equipment.** LA and CLA Fatty Acid Analysis by

**Table 5.** Linoleic Acid Concentration before and after Processing and CLA Concentration in Flax, Sunflower, Corn, Soy, and High-LA Safflower Oils with Pilot-Scale Equipment<sup>a</sup>

oil	linoleic acid concentration (% total oil)		CLA concentration (% total oil)
	before	after	
flax	13.7 ± 0.0 c	12.9 ± 0.1 b	1.0 ± 0.1 a
sunflower	25.0 ± 0.0 c	18.8 ± 1.2 b	4.7 ± 1.1 a
corn	57.3 ± 0.1 c	48.3 ± 0.2 b	7.6 ± 0.2 a
soy	54.8 ± 0.2 c	41.2 ± 0.8 b	13.3 ± 0.7 a
high-LA safflower	73.0 ± 0.2 c	47.9 ± 0.0 b	22.8 ± 0.0 a

<sup>a</sup> Data with the same letter within the same row are not significantly different, with significance defined at  $P < 0.05$ .

**Table 6.** Light Intensity for the Laboratory-Scale and Pilot-Scale Processing Systems<sup>a</sup>

light source	light intensity (mW)
laboratory-scale light source (one 100 W UV-vis light bulb)	0.5 ± 0.2 a
pilot-scale light source (three 450 W UV-vis light bulbs)	2.5 ± 0.7 b

<sup>a</sup> Data with the same letter within the same column are not significantly different, with significance defined at  $P < 0.05$ .

**GC-FID.** Table 5 shows the before and after irradiation concentrations of linoleic acid in flax, sunflower, corn, soy, and high-LA safflower oil with the corresponding CLA yields after irradiation. With the pilot-scale equipment, flax, sunflower, corn, soy, and high-LA safflower oils showed reductions in LA content of  $\sim 1$ ,  $\sim 19$ ,  $\sim 9$ ,  $\sim 14$ , and  $\sim 25\%$ , respectively.

High-LA safflower oil had consistently greater CLA yields than the other oils, followed by soy oil. Although sunflower oil processed with the laboratory-scale equipment did not yield measurable amounts of CLA, when processed with the pilot-scale equipment, a significantly greater amount of CLA was measured (4.7%), despite having significantly less initial LA.

The light intensity of the pilot-scale system was about 5 times greater than the light intensity of the laboratory-scale system, as determined by a digital photometer (Table 6), which is the most likely reason for the difference in LA conversion to CLA between the two systems. However, the larger oil surface area to volume ratio in the pilot plant system may also result in more efficient light penetration as 7 g of oil in a glass vial was used for the pilot-scale irradiation compared to 700 g of oil in the laboratory-scale reaction vessel (16).

**Comparison of the Oxidative Stabilities of CLA-Rich Soy, High-LA Safflower, Corn, Sunflower, and Flax Oils Prepared with Laboratory-Scale Processing Equipment.** Gravimetric Analysis.

Table 7 summarizes the induction times for each oil's oxidation before irradiation, after irradiation, and after activated carbon treatment to remove residual iodine. Unirradiated corn and sunflower oils each had the longest induction time of all the oils without significant difference, indicating high oxidative stability, probably due to high tocopherol content (Table 3).

Unirradiated high-LA safflower and soy oils each had intermediate induction times without significant difference, also probably due to high tocopherol content. Unirradiated flax oil had a significantly shorter induction time than the other four oils, indicating the lowest oxidative stability. This is probably due to its low tocopherol content and its high (60%) linolenic acid ( $C_{18:3}$ ) content, as determined by GC-FID. Linolenic acid is 2 times more prone to oxidation than linoleic acid, and the resulting conjugated

**Table 7.** Oxidation Induction Times (Days) of Flax, Sunflower, Corn, Soy, and High-LA Safflower Oils Determined Gravimetrically after Being Processed with Laboratory-Scale Equipment and Incubated at 64 °C<sup>a</sup>

oil	oxidation induction time (days)		
	unirradiated	irradiated, without iodine adsorption	irradiated, with iodine adsorption on carbon
flax	1.5 ± 0.7 abc	1.0 ± 0.0 ab	0.0 ± 0.0 a
sunflower	12.0 ± 1.4 g	8.0 ± 0.0 f	0.5 ± 0.7 a
corn	12.0 ± 1.4 g	7.0 ± 2.8 ef	1.0 ± 1.4 abc
soy	7.0 ± 1.4 ef	5.5 ± 0.7 de	3.0 ± 0.0 bc
high-LA safflower	6.0 ± 0.0 e	3.5 ± 0.7 cd	2.0 ± 1.4 abc

<sup>a</sup> Data with the same letter are not significantly different, with significance defined at  $P < 0.05$ .

dienes absorb in the UV region. Tokle et al. (27) also showed that a small degree of lipid oxidation dramatically reduces soy oil CLA yields by studying the effect of various peroxide concentrations on CLA production. Therefore, the oxidative status of the oils may be responsible for their lower yields.

Irradiated sunflower, corn, and high-LA safflower oils without adsorption treatment had a decrease in induction times compared to the unirradiated oils. High-LA safflower and corn oils had the largest significant decreases in induction time (42%), followed by sunflower oil (33%). Irradiated flax and soy oils did not show a significant difference in induction time from unirradiated flax and soy oils. This may be due to flax oil's high lutein concentration of 9.8 ppm (Table 4), because lutein prevents photooxidation, and soy oil's high level of  $\delta$ -tocopherols of 9.7 ppm (Table 3), which are very potent antioxidants (22).

After irradiation and activated carbon treatment, sunflower, corn, and soy oils showed significant decreases in induction time, indicating that while the carbon adsorption process was meant to remove iodine, it also may remove antioxidants such as tocopherols and lutein from the oils, increasing the likelihood of oxidation. Although flax oil did not show a significant decrease in induction time following this process, it was probably less oxidatively stable relative to the other sample oils before irradiation. High-LA safflower oil did not have a significant decrease in induction time following the activated carbon treatment, which may indicate that there was still significant residual tocopherol antioxidant capacity after adsorption.

Soy oil and high-LA safflower oils were, thus, the only viable candidates for the production of CLA-rich vegetable oils. Because high-LA safflower oil is more expensive than soy oil, soy oil is more commercially viable for CLA production. The majority of CLA produced in all of the oils was as *trans*-, *trans*-isomers, due to their greater stability. A high-LA vegetable oil is important in producing a CLA-rich product, but initial LA content is not the only important factor. Factors such as carotenoid content, conjugated diene oxidation products, and turbidity reduce CLA yields. Sunflower oil did not produce any CLA after laboratory-scale processing despite containing appreciable levels of LA, probably due to oil turbidity reducing light penetration. Flax oil produced <1% CLA after laboratory-scale processing, due to its relatively low LA level, high carotenoid content, and low oxidative stability resulting from its high linolenic acid content. Oils should be high in linoleic acid, highly refined, and minimally oxidized before they can be used for CLA production.

The adapted pilot-scale processing system was the most efficient means of producing CLA-rich oil because of the greater light exposure and the larger surface area to volume ratio of the oil in this system, relative to the laboratory-scale system. This

ensured better light penetration, which appears to be the limiting factor during irradiation. The highest CLA yields were obtained with pilot-scale processing of high-LA safflower oil.

Oxidative stability of the oils before irradiation was protected with significant tocopherol levels, with the exception of flax oil, which was less stable. There was a loss of oxidative stability after processing. The low CLA yields and oxidative stability of flax oil in this study may indicate that the presence of lipid oxidation products prior to irradiation negatively affects CLA yields. Flax oil's initial linolenic acid (C<sub>18:3</sub>) content was 60%, which also negatively affects its oxidative stability and makes it unlikely that high CLA yields will result from the low initial amount of LA.

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