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Photocatalytic Production and Processing of Conjugated Linoleic Acid-Rich Soy Oil

VISHAL P. JAIN AND ANDREW PROCTOR*

Department of Food Science, University of Arkansas, 2650 Young Avenue, Fayetteville, Arkansas 72704

Daily intake of conjugated linoleic acid (CLA), an anticarcinogenic, antiatherosclerotic, antimutagenic agent, and antioxidant, from dairy and meat products is substantially less than estimated required values. The objective of this study was to obtain CLA-rich soybean oil by a customized photochemical reaction system with an iodine catalyst and evaluate the effect of processing on iodine and iodo compounds after adsorption. After 144 h of irradiation, a total CLA yield of 24% (w/w) total oil was obtained with 0.15% (w/w) iodine. *Trans*,*trans* isomers (17.5%) formed the majority of the total yield and are also associated with health benefits. The isomers *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA, associated with maximum health benefits, formed approximately 3.5% of the total oil. This amount is quite significant considering that total CLA obtained from dairy sources is only 0.6%. ATR-FTIR, ^{1}H NMR, and GC-MS analyses indicated the absence of peroxide and aldehyde protons, providing evidence that secondary lipid oxidation products were not formed during the photochemical reaction. Adsorption processing vastly reduced the iodine and iodocompounds without CLA loss. Photocatalysis significantly increased the levels of CLA in soybean oil.

KEYWORDS: Conjugated linoleic acid (CLA); soybean oil; iodine; photochemical reaction system; oxidation; adsorption

INTRODUCTION

Conjugated linoleic acid (CLA) is a group of geometrical and positional linoleic acid isomers found naturally in meat and milk products of ruminant animals. The most common CLA isomers are *cis*-9,*trans*-11-octadecadienoic acid and *trans*-10,*cis*-12-octadecadienoic acid (*1*). CLA concentrations in meat and dairy products typically range between 0.3 and 0.8% CLA/g of fat, of which 73−93% of the total CLA is made up by the *cis*-9,*trans*-11 isomer (*2*). In vitro and animal studies indicate that CLA suppresses carcinogenesis (*3*, *4*), reduces atherosclerosis (*5*), enhances the immune system (*6*), reduces body fat (*7*), acts as an antimutagenic agent and antioxidant, and lowers insulin resistance (*8*). Ip et al. extrapolated results from animal studies and estimated that about 3 g of CLA/day is required to obtain beneficial health effects in humans, although such a standard has not been established (*9*). Human intake of CLA from natural food sources, dairy (0.55% total fat) and beef (0.60% total fat), is around 10% of the suggested recommended value (*9*−*11*).

CLA can be chemically synthesized by the dehydration of hydroxyl fatty acids and alkali- or photoisomerization of unsaturated fatty acids or linoleic acid-rich oil. Low yields, extensive purification steps, and inseparability of isomers limit commercial use of most of these chemical methods (*12*). The biochemical pathway through enzymatic interesterification of lipids using algal or bacterial enzymes is another method of synthesis. However, lipase-catalyzed interesterification resulted in a high level of oxidation of the oil and low yields of CLA (*13*). Seki et al. synthesized CLA methyl esters by exposing linoleic acid methyl esters, dissolved in solvents, to a light source in the presence of iodine as a sensitizer (*14*). However, methyl esterification, solvent removal, and separation of CLA restrict the commercial use of this method. Gangidi and Proctor (*15*) photoisomerized soy oil, with iodine as a catalyst, in the absence of solvents and indicated that CLA can be synthesized simply and inexpensively by direct photocatalysis. A simple overhead irradiation system was used, and soy oil in a beaker was irradiated with a 100-W UV lamp from a distance of 45 cm. However, long exposure time and low yields limit the use of this technique. The low yields may have been primarily due to minimal exposure of the oil to light.

None of the previous studies examined the effect of stirring on the overall CLA production. Stirring may be important for uniform exposure of oil to light. Thus, the goal of this study was to increase the CLA yields in soy oil under optimized photoirradiation conditions as a step toward developing a commercially viable process. The specific objectives were to (1) use a customized UV photoirradiation system to obtain CLA-rich soy oil with a minimum of iodine catalyst with no lipid oxidation, (2) determine the effect of stirring on CLA yields using a customized reaction system, and (3) remove iodine and any other iodo compounds while retaining a high level of CLA in the oil.

* Corresponding author. Phone: (479) 575-2980; fax: (479) 575-6936; e-mail: aproctor@uark.edu.

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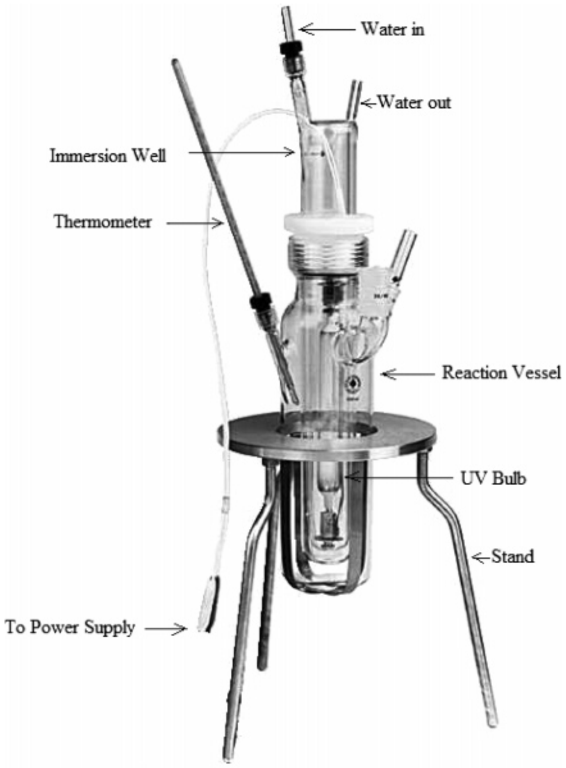

Figure 1. Customized photochemical reaction system, courtesy of Ace Glass, Inc.

MATERIALS AND METHODS

Materials. Refined, bleached, and deodorized soy oil (Wesson; ConAgra, Irvine, CA) was obtained from a local grocery store (Fayetteville, AR) with 52% linoleic acid and 6% linolenic, as measured. Resublimed iodine crystals were used as a catalyst (EM Science, Cherry Hill, NJ). Sodium methoxide and anhydrous sodium sulfate (EM Science, Darmstadt, Germany) were used for methyl ester preparation. 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.

Irradiation System. The photocatalysis reaction system (**Figure 1**) consisted of a borosilicate-glass jacketed reaction vessel of 1000 mL capacity (Ace Glass Inc., Vineland, NJ). The vessel was flat-bottomed to facilitate magnetic stirring. The reaction vessel accommodated a double-walled, borosilicate glass immersion well (Ace Glass Inc.) with inlet and outlet tubes for cooling. The annular space formed between the reaction vessel and the immersion well held the oil for irradiation. The immersion well supported a 100-W UV/vis medium-pressure, quartz, mercury-vapor lamp (Ace Glass Inc.). The lamp operated at 120 V, 60 Hz, and 15 amp power supply. The electric arc reached its peak of attainment in the quartz mercury arc. Visible and ultraviolet radiation was emitted by the ionized mercury vapor, a condition that permits attainment of highest arc efficiency. The lamp had PTFE-covered lead wires, fitted with pin-type connectors, and was connected to a 100-W UV power supply (Ace Glass Inc.) with an input of 120 V and 60 Hz, to supply the extra voltage and current required to initiate the lamp's arc and reduce operational power. The lamp emitted radiation in the UV/vis range of 300 to 700 nm. The assembly was placed in a black-walled cabinet to absorb the outgoing radiations from the assembly to ensure a safe operating environment. The reaction vessel and the immersion well were connected to a water supply to maintain the temperature of the oil between 22 and 25 °C, necessary for optimum efficiency and maximum lamp life. The customized reaction system facilitated maximum exposure of the oil to light.

Effect of Time on CLA Synthesis. Seven hundred grams of commercial soy oil was deaerated with a sonicator for 30 min and taken in a 1000-mL beaker wrapped with aluminum foil to prevent exposure of oil to light. Oil was heated to 70 °C, to facilitate iodine solubilization, while flushing with nitrogen to avoid oxidation. Then, 0.25% iodine was added to the oil, and the contents in the beaker were stirred until the iodine completely dissolved (*15*). Heated oil was transferred to the reaction vessel, and the photochemical system was set up. The system was connected to a cooling water supply, and the temperature of the oil was controlled between 22 and 25 °C and closely monitored with a Traceable Big-Digit Memory Thermometer sensor (VWR International, Friendswoods, TX). The assembly was placed on a magnetic stirrer, and the oil was stirred continuously during irradiation. The soy oil was irradiated for 240 h, and two 5 mL samples were collected at 24-h intervals in 10-mL amber colored glass vials. The vials were purged with nitrogen, capped, and immediately refrigerated at 4 °C. The experiment was repeated in duplicate.

Effect of Iodine Concentration on CLA Synthesis. To study the effect of the iodine concentration on the overall CLA yields, the previous experiment was repeated with 0.10, 0.15, and 0.25% iodine in duplicates. CLA yields with 0.10, 0.15, and 0.25% iodine were compared to optimize the iodine concentration.

Effect of Stirring on CLA Synthesis. To study the effect of stirring on total CLA content and oxidation of the oil, the previous experiment was repeated in duplicate, with optimized iodine concentration, without stirring the contents in the reaction vessel.

Methyl Ester Preparation. Methyl esters were prepared from the photoisomerized oil by a base-catalyzed method to reduce the formation of conjugated *trans*,*trans* isomers during analysis (*16*). One hundred milligrams of photoisomerized soybean oil 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. Five miililiters of distilled water 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.

CLA Methyl Ester Analysis by GC. Methyl esters were analyzed by GC using a SP 2560 fused silica capillary column (100 m $\times$ 0.25 mm i.d. $\times$ 0.2 μm film thickness; Supelco Inc., Bellefonte, PA) (*17*) with a FID (model 3800, Varian, Walton Creek, CA). The samples, prepared in hexane, were injected in triplicate by an autosampler (AS 380, HTA s.r.1; Bressica, Italy), and gas chromatograms were printed by a data module printer (Waters, Milford, MA). Commercial CLA methyl ester, methyl linoleate, and mixed methyl fatty esters (Sigma) were used as standards. CLA concentrations were calculated by the following equation:

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

ATR-FTIR Analysis of Lipid Oxidation Products. ATR-FTIR absorbance spectra were obtained using a Nicolet Nexus 670 spectrophotometer (Nicolet Analytical Instruments, Madison, WI) in the range of 700−4000 cm^{-1} (*15*). Spectra were obtained for soy oil samples with 0.00, 0.10, 0.15, and 0.25% iodine and photoisomerized for 144 h for unirradiated soy oil. Samples were placed on an ATR 45° ZnSe-trough plate (Spectra-Tech Inc., Shelton, CT), and the plate was then placed in a slide-mounted horizontal ATR with prealigned fixed mirrors (Spectra-Tech Inc.). The spectra were compared in the range of 3400−3600 cm^{-1} for hydroperoxide formation in the photoisomerized samples.

^{1}H NMR Analysis of Lipid Oxidation Products. Fresh soy oil, soy oil with 100 ppm hexanal, and 144-h photoisomerized oil with 0.15% iodine were analyzed for proton peaks using a ^{1}H NMR spectrometer (Avance 300; Bruker, Rheinstetten, Germany) operating at 300 MHz (*15*). To reduce the oil viscosity for higher resolution between peaks, samples were dissolved in 20% $CDCl_3$ (w/w). The

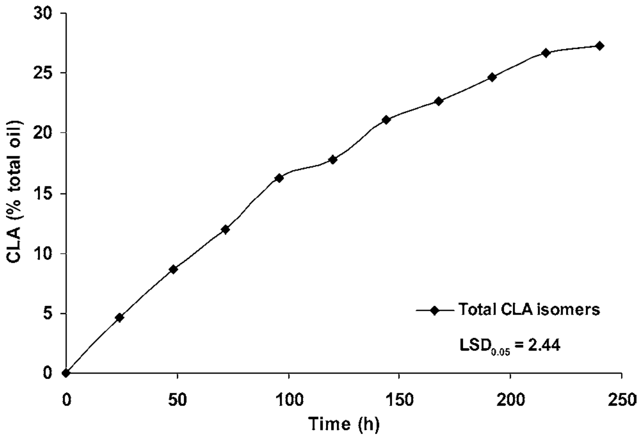

Figure 2. Increase of total CLA isomers (% total oil, w/w) with time of irradiation in soy oil with 0.25% iodine as a photosensitizer.

spectra were examined for aldehyde and hydroperoxide proton peaks in the range of 8−12 ppm.

GC-MS Analysis of Lipid Oxidation Products. The oil samples were analyzed for volatiles by GC-MS as described by Frankel et al. (*18*). Samples were incubated for 30 min at 45 °C in a glass vial, and the volatiles were adsorbed by solid-phase microextraction (SPME) using a carboxen/poly(dimethylsiloxane) (CAR/PDMS) fiber (Supelco Inc., Bellfonte, PA). The volatiles were analyzed by desorbing the volatiles in the injector port (270 °C for 2 min) of a Varian Star 3400 CX gas chromatograph (Varian Analytical Instruments, Walnut Creek, CA) with an autosampler (Varian 8200 Autosampler) and a CP-Sil8 CB-MS fused-silica capillary column (30 m × 0.25 mm i.d. and 0.25 m film thickness; Varian Analytical Instruments). The GC oven temperature was increased from 50 to 200 °C at the rate of 5 °C/min. The initial and final temperatures were held for 5 and 1 min, respectively. The volatiles were identified with a Saturn 2000 mass selective detector (Varian).

Iodine Adsorption from CLA Soy Oil. To adsorb iodine from the irradiated oil, 25 g of irradiated soy oil sample was mixed with 4% (w/w) commercial activated clay Pure-Flo Natural B80/M85-20 (Oil Dri Corporation, Chicago, IL) for 30 min. The contents were then filtered through Whatman filter paper no. 2 to remove the adsorbent. The adsorbed oil was further analyzed for iodine removal by colorimetry and for total CLA isomer content by GC-FID.

Color Determination of Adsorbed CLA Soy Oil by Lovibond Tintometer. Commercial soy oil, 144-h irradiated soy oil with 0.15% iodine, and clay-processed irradiated soy oil were analyzed for color by Lovibond tintometer PFX990 (Salisbury, UK). The data were reported using the Lovibond-RYBN scale, and the results were compared for color reduction as an indication of iodine removal.

Statistical Analysis. Analysis of variance (ANOVA) was conducted on the data using the JMP version 5.0.1 (SAS Inst., Cary, NC) with different iodine concentrations and time as factors for analysis. Least significant differences were calculated to compare mean values, with significance defined at $p < 0.05$.

RESULTS AND DISCUSSION

Effect of Time on CLA Synthesis. Figure 2 shows the effect of irradiation time on total CLA isomers as a percentage of total oil on a weight-by-weight basis. The CLA content increased almost linearly with irradiation time until 200 h. However, the CLA yield increased negligibly after 240 h. Irradiating soy oil for 240 h with 0.25% iodine yielded 27.3% CLA isomers. Because soy oil contains 50% linoleic acid (LA), this represents a 54% conversion rate of LA to CLA. A higher CLA yield can be attributed to enhanced oil exposure to light by use of the customized photochemical reaction system as compared to previous studies by Gangidi and Proctor (*15*).

Effect of Iodine Concentration on CLA Content. Figure 3 shows the effect of different iodine concentrations on the total CLA yield as a percentage of total oil on a weight-by-weight basis. No CLA isomers were produced by irradiating soy oil with 0% iodine. This result is consistent with the observation of previous researchers who demonstrated that iodine was necessary for the photosensitizing the isomerization reaction (*15*). Irradiation of soy oil for 144 h with 0.15% iodine produced 23.8% total CLA isomers, which is significantly higher than total CLA isomers produced by irradiating soy oil with 0.10 and 0.25% iodine. Although soy oil with 0.10% iodine started with a higher conversion rate, the isomerization rate subsequently fell below the conversion rates of oil with 0.15 and 0.25% iodine.

Figure 4 shows the amount of *cis*-9,*trans*-11 CLA formed by irradiating soy oil with different iodine concentrations. About 1.8% of the isomer was formed by irradiating soy oil with 0.15% iodine for 144 h. Although there was no significant difference in the *cis*-9,*trans*-11 CLA content of soy oil with 0.15 and 0.10% iodine until 80 h, further irradiation caused a significant difference.

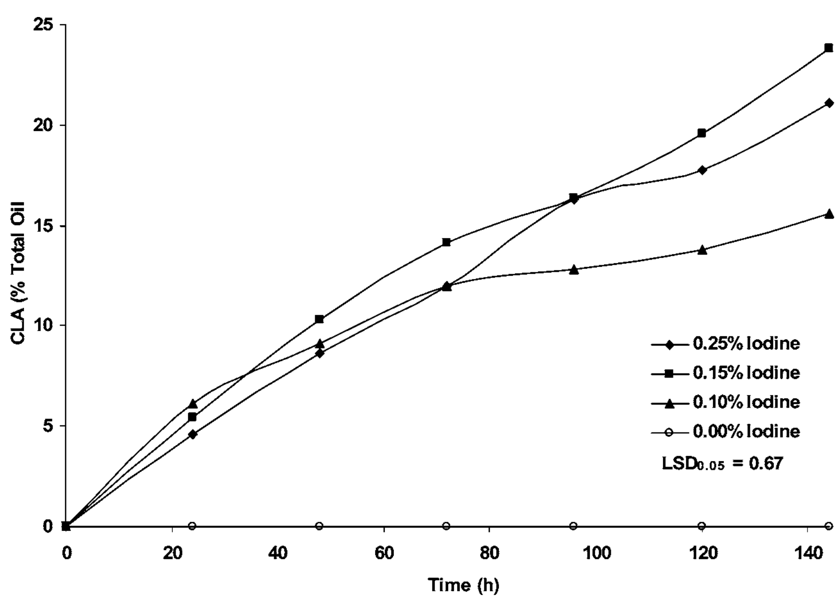

Figure 3. Effect of iodine concentration on total CLA isomers (% total oil, w/w) content in irradiated soy oil.

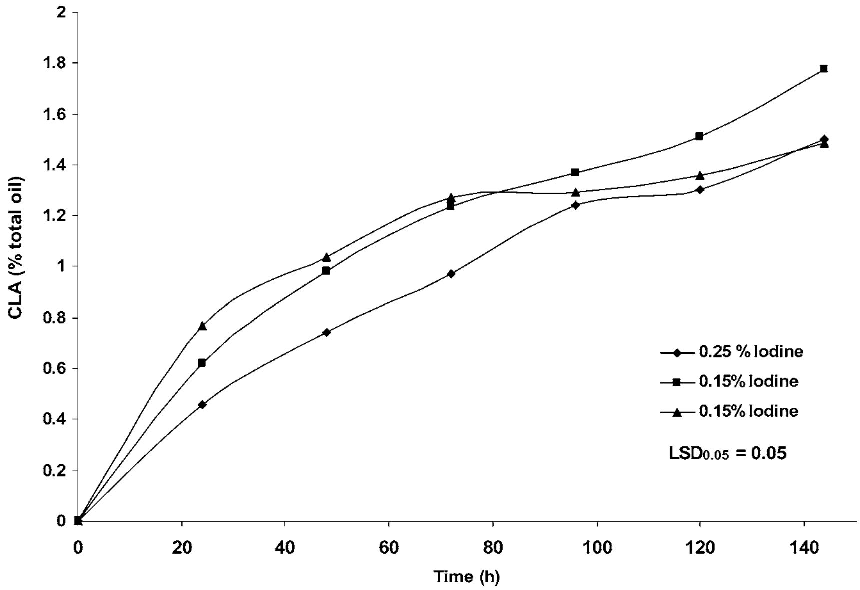

Figure 4. Effect of iodine concentration on *cis*-9,*trans*-11 CLA (% total oil, w/w) content in irradiated soy oil.

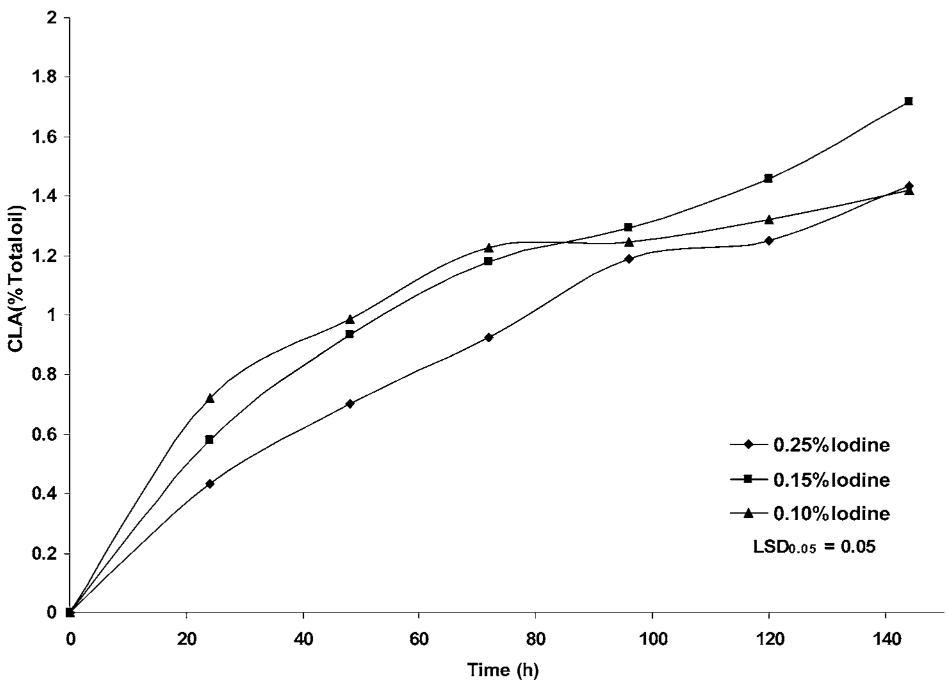

Figure 5. Effect of iodine concentration on *trans*-10,*cis*-12 CLA (% total oil, w/w) content in irradiated soy oil.

Figure 5 shows the amount of *trans*-10,*cis*-12 CLA formed with various iodine concentrations. This isomer had a similar trend to *cis*-9,*trans*-11 CLA and was formed in almost similar amounts. The *trans*,*trans* group of isomers mainly consists of *trans*-8,*trans*-10 CLA, *trans*-9,*trans*-11 CLA, and *trans*-10,*trans*-12 CLA (*19*).

Figure 6 shows the amount of *trans*,*trans* CLA isomers formed by irradiating soy oil with different iodine concentrations. The amount of these isomers formed in soy oil increases with time and shows a similar trend to other isomers. With 0.15% iodine and after 144 h of irradiation, 17.5% of these isomers is formed. Although 74% of total CLA isomers is accounted for by the *trans*,*trans* CLA isomers, these isomers have been shown to have beneficial effects, in particular, reduction in fat deposition (*20*). Soy oil fatty acid profiles as by partial GC-FID chromatograms before and after photoirradiation are shown in **Figure 7**. The data show that CLA isomers are produced at the expense of linoleic acid but also with some loss of linolenic acid with *trans*,*trans* being the major CLA isomers.

A comparison of CLA content in a diary product, beef, and soy oil from photoirradiation studies is shown in **Table 1**. Cow's whole milk contains CLA at 0.65% total fat level. However, about 92% of the total CLA content is composed of the *cis*-9,*trans*-11 CLA isomer (*10*). The *trans*-10,*cis*-12 CLA isomer is present at less than 0.01% total fat level. The CLA content of beef is also about 0.6% of the total fat, but beef has far less *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA isomers than is found in dairy (*11*). Gangidi and Proctor synthesized CLA by photoirradiation and obtained 0.61 and 0.59% total fat of *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA, respectively. The total CLA content was not reported (*15*). The customized photochemical reaction system significantly increased the total CLA yield to 23.8% and the yield of the two most important isomers to 3.5%.

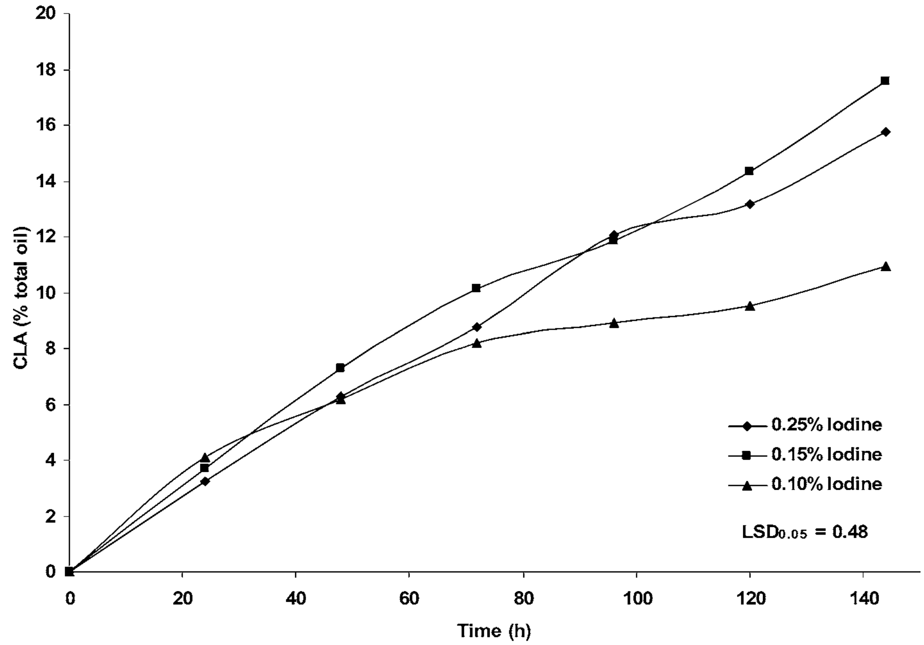

Figure 6. Effect of iodine concentration on *trans,trans*-CLA isomers (% total oil, w/w) content in irradiated soy oil.

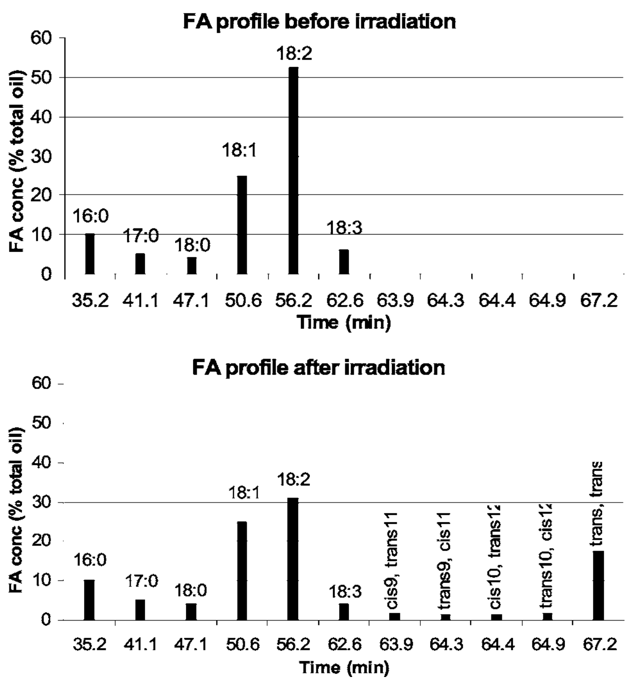

Figure 7. Soy oil fatty acid profiles as shown by partial GC-FID chromatograms before and after photoirradiation.

The increase in the total CLA content by the customized photoirradiation system may be attributed to the decrease in distance between light source and oil.

Oxidation Analysis. ATR-FTIR Analysis. Figure 8 shows the FTIR spectra of photoisomerized soy oil using an attenuated total reflectance accessory with a ZnSe-trough plate. The spectra in the range of 3400−3600 cm^{-1} showed no peroxide peak and indicated no significant oxidation in oil. The spectra of soy oil with 0.10, 0.15, and 0.25% iodine irradiated for 144 h were similar to the spectra of fresh soy oil and indicated the absence of peroxides in the irradiated oil samples.

^{1}H NMR Analysis. ^{1}H NMR spectra of refined, bleached, and deodorized soy oil showed no aldehyde proton peak in the range of 9.0 and 10.5 ppm. Soy oil spiked with 100 ppm hexanal showed an aldehyde proton peak at 9.4 ppm. Spectra of the

Table 1. Comparison of Percentage of CLA from a Dairy Product (Whole Milk), Beef, and Photoirradiation Studies

	CLA isomer concentration [% total oil (w/w)]			
			photoirradiation	
fatty acid	dairy (*10*)[a]	beef (*11*)	ref *15*[b]	customized system[c]
cis-9,*trans*-11 CLA	0.63	0.10	0.61	1.78
trans-10,*cis*-12 CLA	<0.01	0.02	0.59	1.72
total CLA	0.65	0.60	NR[d]	23.79

[a] Number in parentheses indicates refs from which data were obtained. [b] Overhead irradiation system (*15*). [c] Present study using a customized photoirradiation system. [d] Total CLA yield not reported.

144-h irradiated soy oil sample with 0.15% iodine showed no proton peak in the 9.0−10.5 ppm range and indicated the absence of aldehydes and peroxides (data not shown). These data confirmed the data from ATR-FTIR analysis.

GC-MS Analysis. Soy oil with 1 ppm hexanal showed a peak at a retention time of 2.9 min. Fresh soy oil and the 144-h irradiated oil sample with 0.15% iodine showed no measurable hexanal peak at 2.9 min. This indicated that the irradiated oil sample had less than 1 ppm hexanal and confirms the data obtained from ATR-FTIR and ^{1}H NMR (data not shown).

Effect of Stirring on CLA Content. Table 2 shows a comparison of the CLA isomers formed in irradiated soy oil with 0.15% iodine concentration with and without stirring the contents in the reaction vessel. The results indicated that stirring uniformly exposed oil to light, and hence, a better conversion rate was achieved. No stirring may be one of the reasons for low yields of CLA isomers as experienced in previous studies by Gangidi and Proctor (*15*). ^{1}H NMR and GC MS analysis of the two samples showed no significant differences in oxidation data (data not shown).

Iodine Removal. Clay processing significantly removed iodine from the irradiated oil sample with 0.15% iodine. **Table 3** shows Lovibond tintometer color values with the Lovibond RYBN scale. Iodine adsorption on clay significantly reduced color. The clay-processed irradiated soy oil sample was analyzed for its total CLA and CLA isomer content. The results were compared with CLA content of the irradiated oil sample not

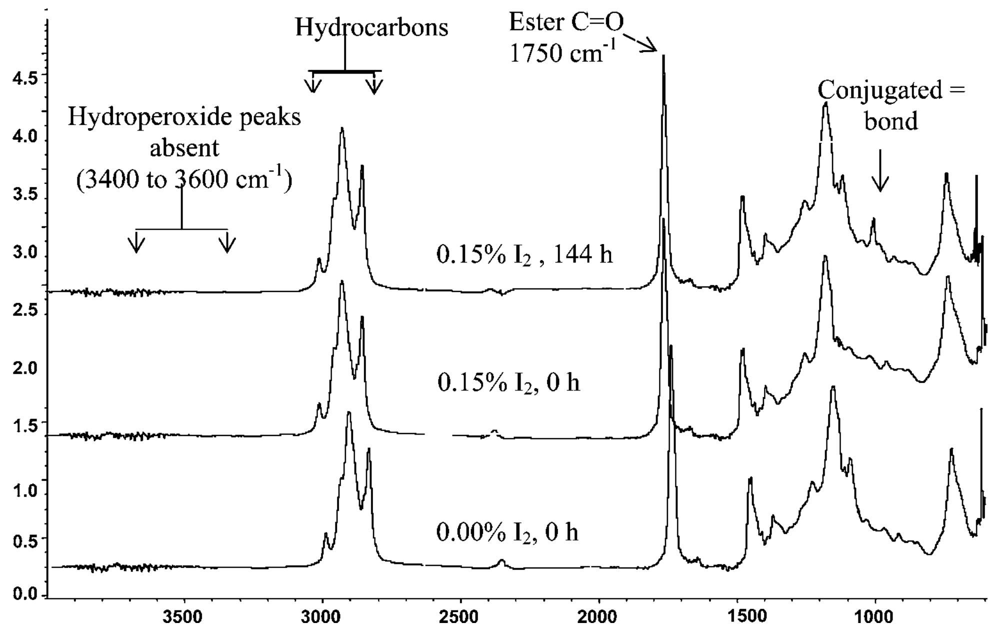

Figure 8. ATR-FTIR spectra of photoirradiated soy oil samples in a range of 4000–700 cm^{-1}.

Table 2. Comparison of CLA Isomers in 144-h Irradiated Soy Oil Containing 0.15 % Iodine with and without Stirring the Contents of the Reaction Vessel[a]

CLA isomer	CLA isomer concentration [% total oil (w/w)] stirring	no stirring
linoleic acid	30.86a	37.21b
cis-9,*trans*-11 CLA	1.78a	1.53b
trans-10,*cis*-12 CLA	1.72a	1.47b
trans,trans CLA[a]	17.56a	12.17b
total CLA	23.79a	17.14b

[a] Constituted by *trans*-8,*trans*-10 CLA, *trans*-9,*trans*-11 CLA, and *trans*-10,*trans*-12 CLA. Values are means, $n = 3$. Means with different letters within a row differ significantly, $p < 0.05$.

Table 3. Lovibond Tintometer Color Values of Clay-Processed Soy Oil as an Indication of Iodine Removal

oil sample	color readings[a] R	Y	B	N
RBD oil[b]	0.0	0.2	0.0	0.0
144-h irradiated oil + 0.15% iodine	1.6	3.4	0.0	0.1
activated clay-processed oil	0.1	0.6	0.0	0.0

[a] Color measured with Lovibond tintometer and reported using the Lovibond RYBN (red, yellow, blue, neutral) scale. [b] Refined, bleached, and deodorized soy oil.

processed with clay. There was no significant difference in the total CLA and CLA isomer content between the two samples (data not shown). This indicated that clay adsorption does not remove any CLA isomer.

This study shows that a high yield of CLA can be obtained by irradiating soy oil with a customized reaction system. The distance between irradiation source and oil, uniform light exposure accomplished by stirring the oil during irradiation, and optimum iodine concentration are the three most important factors affecting CLA yields. *Trans,trans* isomers contributed 75% of the total CLA content because these isomers are very stable. Although a higher yield of CLA was obtained, the long irradiation time still remains a concern. The longer irradiation time may be a result of the thick oil layer to be penetrated by light in the reaction vessel. Varying the oil layer thickness and the light source intensity may help achieve a better yield with a low irradiation time.

ABBREVIATIONS USED

CLA, conjugated linoleic acid; ATR, attenuated total reflectance; FTIR, Fourier transform infrared; NMR, nuclear magnetic resonance; GC, gas chromatography; FID, flame ionization detector; GC-MS, gas chromatography mass spectroscopy; UV, ultra violet; PTFE, poly tetrafluoroethylene; ppm, parts per million; SPME, solid-phase microextraction; CAR, carboxen; PDMS, poly(dimethylsiloxane); I_2, iodine

LITERATURE CITED

(1) Lawson, R. E.; Moss, A. R.; Givens, D. I. The role of dairy products in supplying conjugated linoleic acid to man's diet: a review. *Nutr. Res. Rev.* **2001**, *14*, 153−172.

(2) Chin, S. F.; Liu, W.; Storkson, J. M.; Ha, Y. L.; Pariza, M. W. Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. *J. Food Comp. Anal.* **1992**, *5*, 185−197.

(3) Ip, C.; Chin, S. F.; Scimeca, J. A.; Pariza, M. W. Mammary cancer prevention by conjugated dienoic derivative of linoleic acid. *Cancer Res.* **1991**, *51*, 6118−6124.

(4) Shultz, T. D.; Chew, B. P.; Seaman, W. R.; Luedecke, L. O. Inhibitory effect of conjugated dienoic derivatives of linoleic acid and beta-carotene on the in vitro growth of human cancer cells. *Cancer Lett.* **1992**, *63*, 125−133.

(5) Lee, K. N.; Kritchevsky, D.; Pariza, M. W. Conjugated linoleic acid and atherosclerosis in rabbits. *Atherosclerosis* **1994**, *108*, 19−25.

(6) Cook, M. E.; Miller, C. C.; Park, Y.; Pariza, M. W. Immune modulation by altered nutrient metabolism: nutritional control of immune-induced growth depression. *Poultry Sci.* **1993**, *72*, 1301−1305.

(7) Park, Y.; Storkson, J. M.; Albright, K. J.; Liu, W.; Pariza, M. W. Evidence that the *trans*-10,*cis*-12 isomer of conjugated linoleic acid induces body composition changes in mice. *Lipids* **1999**, *34*, 235−241.

(8) Yeong, L. H.; Nancy, K. G.; Michael, W. P. Newly recognized anticarcinogenic fatty acids: Identification and quantification in natural and processed cheeses. *J. Agric. Food Chem.* **1989**, *37*, 75−81.

(9) Ip, C.; Singh, M.; Thompson, H. J.; Scimeca, J. A. Conjugated linoleic acid suppresses mammary carcinogenesis and proliferative activity of the mammary gland in the rat. *Cancer Res.* **1994**, *54*, 1212−1215.

(10) Veth, M. J.; Griinari, M.; Pfeiffer, A. M.; Bauman, D. E. Effect of CLA on milk fat synthesis in dairy cows: comparison of inhibition by methyl esters and free acids and relationships among studies. *Lipids* **2004**, *39*, 365−372.

(11) Beaulieu, A. D.; Drackley, J. K.; Merchen, N. R. Concentrations of conjugated linoleic acid (*cis*-9,*trans*-11-octadecadienoic acid) are not increased in tissue lipids of cattle fed a high-concentrate diet supplemented with soybean oil. *J. Anim. Sci.* **2002**, *80*, 847−861.

(12) Adlof, R. O. Preparation of unlabeled and isotope-labeled conjugated linoleic and related fatty acid isomers. In *Advances in Conjugated Linoleic Acid Research*; Yurawecz, M. P., Mossoba, M. M., Kramer, J. K. G., Pariza, M. W., Nelson, G. J., Eds.; AOCS Press: Champaign, IL, 1999; Vol. 1, pp 21−38.

(13) Lee, J. H.; Kim, M. R.; Kim, H. R.; Kim, L. H.; Lee, K. T. Characterization of lipase-catalyzed structured lipids from selected vegetable oils with conjugated linoleic acid: Their oxidative stability with rosemary extracts. *J. Food Sci*. **2003**, *68*, 1653−1658.

(14) Seki, K.; Kaneko, R.; Kobayashi, K. Photoconjugation of methyl linoleate in the presence of iodine as a sensitizer. *Yukagaku* **1998**, *38*, 949−954.

(15) Gangidi, R. R.; Proctor, A. Photochemical production of conjugated linoleic acid from soybean oil. *Lipids* **2004**, *39*, 577−582.

(16) Christie, W. W.; Sebedio, J. L.; Juaneda, P. A practical guide to the analysis of conjugated linoleic acid. *Inform* **2001**, *2*, 147−152.

(17) Ma, D. W. L.; Wierzbicki, A. A.; Field, C. J.; Clandinin, M. T. Conjugated linoleic acid in Canadian dairy and beef products. *J. Agric. Food Chem.* **1999**, *47*, 1956−1960.

(18) Frankel, E. N.; Neff, W. E.; Selke, E. Analysis of autoxidized fats by gas chromatography-mass spectrometry: VIII. Volatile thermal decomposition of hydroperoxy cyclic peroxides. *Lipids* **1983**, *18*, 264−70.

(19) Sebedio, J. L.; Juaneda, P.; Gregoire, S.; Chardigny, J. M.; Martin, J. C.; Ginies, C. Geometry of conjugated double bonds of CLA isomers in a commercial mixture and in their hepatic 20:4 metabolites. *Lipids* **1999**, *34*, 1319−1326.

(20) Gavino, V. C.; Gavino, G. G.; Pelletier, R. M. *Trans*-9,*trans*-11 conjugated linoleic acid decreases triglyceride accumulation in primary cult adipocytes from human and hamster adipose tissue. Abstract: Workshop on the role of conjugated linoleic acid in human health; March 13−15 2003, Winnepeg, Canada.

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