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

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A COMPARATIVE STUDY OF HIGH-LINOLEIC ACID VEGETABLE OILS FOR THE PRODUCTION OF CONJUGATED LINOLEIC ACID

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Abstract

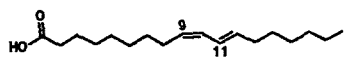
Conjugated linoleic acid (CLA) has anti-carcinogenic, anti-diabetic, and anti-atherogenic properties and is present in very small quantities in dairy and beef products. Obtaining optimum dietary CLA levels from these sources requires an undesirable increased intake of saturated fat. A 20% CLA soy oil has been produced by UV photoisomerization of linoleic acid (LA) in soy oil, which is naturally low in saturated fat. However, 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 UV photoirradiation processing equipment, and 2) compare the oxidative stabilities of laboratory-scale processed oils. Seven hundred g of each oil was irradiated with 0.15% iodine catalyst on a laboratory-scale for 168 hours. Oil fatty acid analysis was done before and after processing as fatty acid methyl esters by gas chromatography-flame ionization detection (GC-FID) analysis. Oxidative stabilities of laboratory-scale processed oils were measured gravimetrically for up to 24 days at 64°C. 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. Low CLA yields were due to carotenoids and lipid oxidation in flax oil and carotenoids and turbidity in sunflower oil. The results show that high LA oils should be highly refined before they are used for CLA production. There was no significant difference between the oxidative stabilities of high LA safflower oil and soy oil before or after irradiation, indicating that these oils are the most suitable for high-CLA production, although high LA safflower oil is more expensive.

Introduction

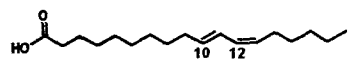
Conjugated linoleic acid (CLA) is a mixture of positional and geometric isomers of linoleic acid (octadecadienoic fatty acid) found naturally in dairy and beef products. The most common CLA isomers are *cis*-9,*trans*-11-octadecadienoic acid and *trans*-10,*cis*-12-octadecadienoic acid (*Lawson et al. 2001*), but other isomers are also present in food, including *trans*-9,trans-11 and *trans*-10,*trans*-12 (*Griinari and Bauman 1999*).

Studies indicate that CLA plays anti-diabetic, anti-obesity, and anti-atherogenic roles (*McGuire and McGuire 1999; Khanal and Olson 2004*). Despite its nutritional benefits, CLA appears naturally at levels of 0.3 – 0.8% of the fat in beef and dairy products, making it difficult to consume the recommended 3 g of CLA per day (*Ip et al. 1994; Ma et al. 1999*) 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 but is low in saturated fat and cholesterol would be an excellent addition to the human diet.

FIGURES



cis-9,trans-11-octadecadienoic acid



trans-10,cis-12-octadecadienoic acid

Figure. 1 – The most common CLA isomers.

Figure. 1 – The most common CLA isomers.

CLA can be produced by several means, including biosynthesis through fermentation by anaerobic rumen bacteria (*Kepler et al. 1966*) in bovines. CLA has also been produced by controlled fermentation (*Martin and Jenkins 2002; Lee et al. 2003; Vahvaselka et al. 2004; Lin et al. 2005*) and organic synthesis (*Yang and Liu 2004*), 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 (*Gangidi and Proctor 2004*) that can be completed on a laboratory scale (*Jain and Proctor 2006*) using a UV/visible lamp with 0.15% iodine as a catalyst with standard photo-chemical irradiation apparatus. However, this method is very time-consuming, requiring 144 hours to synthesize 20% CLA. A pilot-scale method has been developed and optimized by Jain et al. (*2008a*) so that CLA-rich soy oil can be produced on a pilot scale yielding 20% CLA in 12 hours. This requires 0.35% iodine catalyst and a customized illuminated laminar flow unit (ILFU) consisting of borosilicate glass plates and three 450W UV/visible lamps, to increase the oil's exposure to ultraviolet light. This photoisomerization method produces CLA in a food source, whereas previous methods, such as that by Yang and Liu (*2004*), produced

CLA as an inedible free fatty acid. Only soy oil has been used to study photoisomerization of oil LA, but other high LA vegetable oils, such as flax, sunflower, corn, and high LA safflower oils are available. However, they have not been evaluated as a source of CLA-rich oil.

Oxidative stability is a vital oil quality parameter describing an oil's resistance to rancidity. Lipid oxidation is due to the formation and subsequent degradation of lipid hydroperoxides, which lead to rancid food products as well as the formation of hazardous free radicals (*Kanner and Rosenthal 1992*). These free radicals have been linked with cancer, atherosclerosis, hypertension, senile dementia in Alzheimer's, and various forms of amyloidosis (*Harman 1984*). Oils that have a high oxidative stability are superior in quality, safer for human consumption, and more palatable. Currently, there have only been studies on the oxidative stability of CLA-rich soy oil. If other high-linoleic acid vegetable oils can be converted to CLA-rich products via photoisomerization of linoleic acid, characterizing their oxidative stabilities will be key to determining their usefulness as a food product.

Various minor components of vegetable oils will have a significant impact on soy oil CLA yields and oxidative stability, and the amount of these various minor components decreases as degree of processing increases (*Jain, et al. 2008b*). The reason for refining vegetable oils is to remove substances that reduce oil quality (*Jawad 1983*). The steps of refinement are degumming, bleaching, winterizing, steam distillation, neutralizing, and deodorizing (*Hoffman 1989*). Minor components that are removed in these stages include tocopherols, pigments (*especially carotenoids like lutein*), and phospholipids (*Lindley 1998*).

Oil Tocopherols: The tocopherol content of oils is dependent on plant genotype, climate conditions, and processing and storage conditions (*Rabascall and Riera 1987*). Tocopherols are found in vegetable oils in four different forms: α-, β-, γ- and δ-tocopherols. Very low concentrations of tocopherols are sufficient to protect vegetable oils from oxidation. Usually one tocopherol molecule can protect between 10^3 and 10^8 polyunsaturated fatty acid molecules (*Kamal-Eldin et al. 1996*). The method through which tocopherols prevent oxidation involves the transfer of a hydrogen radical from the 6th hydroxyl group of the tocopherol chroman ring to the lipid peroxy radical producing a lipid hydroperoxide and a tocopheroxy radical. The tocopheroxy radical is more stable than the lipid peroxy radical due to resonance stabilization, which causes the rate of oil oxidation to decrease in the propagation stage of autoxidation (*Choe and Min 2006*). The free radical scavenging activity is the highest in -tocopherol, making it the most potent of all the tocopherol antioxidants, followed by γ-, β-, and α-tocopherol (*Ikeda and Fukuzumi 1977; Chu and Lin 1993*) .These natural antioxidants are removed during each step of the oil refining process (*Jung et al. 1989; Kellens 1997; Alpaslan et al. 2001*). It is also known that the various refining steps may affect oxidative stability of vegetable oils (*Jung et al. 1989; Yoon and Kim 1994*). The average loss of total tocopherol content during the refining process was found to be greater than 30% (*Tasan and Demirci 2004*).

Oil Pigments: Pigments present in vegetable oils include carotenoids (both α-, β-, and γ-carotenes and xanthophylls), chlorophyll, diketones, and browning products (*Cowan 1976*). Carotenoid pigments, such as lutein, shown in Figure 3, are known for their ability to protect against oxidation by absorbing light and preventing photooxidation (*Choe and Min 2006*).

Vegetable oil colors are measured commercially in terms of yellow, red, blue, and neutral units by the Lovibond® Tintometry RYBN Scale (AOCS Method Cc 13e-92). This technique compares 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 standard glass slides of various colors (*Estey 1935*). Pigments found in vegetable oils are reduced by the bleaching stage, which uses bleaching clays under low pressure and high temperature (*Cowan 1976; Mounts and Khym 1980; Subramanian et al. 1998*).

Oil Turbidity: Turbidity is a measure of cloudiness in a liquid. Oil components that contribute to turbidity include saturated triglycerides, waxes, free fatty acids, hydrocarbons, and sterols (*Popov et al. 1970; Morrison and Robertson 1975; Caupeil 1977*). These components are present in refined, bleached, and deodorized (RBD) oils in ppm and ppb concentrations, making their removal difficult (*Morrison and Robertson 1975*). Studies on vegetable oil turbidity particularly implicate sunflower oil mainly due not only to its wax content but also its saturated fatty acids, especially stearic and palmitic acid (*Leibovitz and Ruckenstein 1981*). The wax content in sunflower oil decreases with degree of processing (*Leibovitz and Ruckenstein 1984*) and may vary with seed variety, geographical growing area, and seasonal growing conditions (*Moulton 1988*). Turbidity can be determined by measuring absorbance at 600 nm (*Przybylski et al. 1993*).

Oil Phospholipids: Phospholipids are a class of lipids in which glycerol is bonded to two fatty acids and a phosphate group which may be attached to an additional chemical group. The classes of phospholipids in soy oil include phosphoglycerides, phosphoinositides, and phytosphingosines (*Cowan 1976*). The maximum antioxidant activity is observed between 3 and 60 ppm. (*Yoon and Min 1987*). The removal of these phospholipids will result in the removal of metals like iron and copper, increasing the oxidative stability of the processed oils, though the mechanism of this action is not well understood (*Cert et al. 2000, Reische et al. 2002*). Oils cannot be deodorized without oil discoloration until after phospholipid removal (*Gutfinger 1978*), which occurs by hydration, followed by centrifugation, during the degumming stage of processing (*Cowan 1976; Lindley 1998*). Degumming reduces the phosphorus content of the oil from 500-900 ppm to 12-170 ppm (*Brown and Snyder 1985*).

The objectives of this research were to compare:

1) Flax, sunflower, corn, soy, and high LA safflower oils as sources of CLA-rich vegetable oils using laboratory-scale processing equipment.
2) The oxidative stabilities of CLA-rich flax, sunflower, corn, soy, and high LA safflower oils prepared with laboratory-scale processing equipment .

Experimental Materials and Methods

Materials

The following vegetable oils were used: High LA safflower (Liberty Vegetable Oil Company, Santa Fe Springs, CA), Soy (Wesson, ConAgra 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., Melville, NY).

Resublimed iodine (EM Science, Cherry Hill, N.J., U.S.A.) 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, U.S.A.) were used as standards for the GC analysis. Activated carbon 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, N.J., USA) and an ultraviolet light source and methodology described by Jain and Proctor (2006) were used. Duplicate 700 g oil samples from each oil source were deaerated by sonication for 10 minutes and then wrapped with aluminum foil to prevent light exposure while being heated to 70°C to dissolve 0.15% iodine. The oil was transferred to the reaction vessel and the photochemical system was connected to a water supply so 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-hour irradiation. 10mL samples were collected every 24 hours 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 content, was conducted on all duplicate sample oils 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. (*2001*).

To convert each oil sample to methyl esters, each duplicate irradiated oil was weighed into a 25-mL centrifuge tube in duplicate, and 500 µL 1% heptadecanoic acid methyl ester (17:0, internal standard), 2 mL toluene, and 4 mL 0.5 M sodium methoxide in methanol were added. The centrifuge tube was heated to 50°C for ten minutes and then cooled for 5 minutes. To inhibit formation of sodium hydroxide, which could hydrolyze methyl esters to free fatty acids, 200 µL glacial acetic acid was added to the centrifuge tube. Then 5 mL distilled water was added followed by 5 mL hexane, and the tube was vortexed for 2 minutes. 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 a SP 2560 fused silica capillary column (100 m × 0.25 mm i.d. × 0.2 µm film thickness; Supelco Inc., Bellefonte, PA) with an FID (model 3800, Varian, Walton Creek, CA). Triplicate 2µL 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 the following equation:

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

Tocopherol Analysis: Tocopherol content (including α-, β-, γ-, and δ-tocopherols) was determined in duplicate for each sample oil before irradiation using the AOCS Ce 8-89 method (American Oil Chemists' Society, 1998). Standard stock solutions of α, β, γ, and δ tocopherols in methanol (10 mg/10 mL) were prepared and the absorbance of the solution was measured at 292 nm to calculate the concentration (µg /mL). Appropriate volumes of the stock solution of the tocopherol standards were mixed to obtain a mixed tocopherol standards working solution and further diluted with hexane to give a solution containing between 1-5 µg/mL of each tocopherol. The HPLC analytical column (250 X 4 mm) was packed with microparticulate silica (5 µm). 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 minutes. 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 µL) was injected onto the column, and the area of the tocopherol peaks was recorded using an integrator. Then 20µL of the oil sample were 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 from each sample oil were analyzed for turbidity prior to irradiation. Each

sample oil duplicate was diluted 1:10 by volume in hexane and measured by absorption at 600nm.

Carotenoid Analysis: Duplicate samples from each sample oil were quantified as lutein, even though other carotenoids were present. Each oil duplicate was diluted 1:10 by volume in hexane and measured by absorption at 445nm, using the method to determine soy lutein content (*Proctor and Snyder 1987*).

Lovibond Color Analysis: The color of each undiluted oil was measured in duplicate before irradiation in terms of yellow, red, blue, and neutral units by the Lovibond® Tintometry RYBN Scale (AOCS Method Cc 13e-92, AOCS Method Cc 13j-97). 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 (*Estey 1935*).

Statistical Analysis: Analysis of variance (ANOVA) was conducted on all data using JMP Version 5.0.1 (SAS Inst. Inc., Cary, N.C., U.S.A.). A Student t test was used to differentiate mean values, with significance defined at $P < 0.05$. Standard deviations were also determined.

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., Marshall, TX, USA) at 100°C for 30 minutes 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 control was determined by the method of Proctor and Bowen (*1996*). 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 lipid hydroperoxides, the primary product of oxidation that leads to rancid products and hazardous free radical formation (*Kanner and Rosenthal 1992*).

Statistical Analysis: Analysis of variance (ANOVA) was conducted on all data using JMP Version 5.0.1 (SAS Inst. Inc., Cary, N.C., U.S.A.). 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 oils as sources of CLA-rich vegetable oils

LA and CLA Fatty Acid Analysis by GC-FID: High LA safflower oil had the highest initial LA, while soy and corn oils had intermediate levels (Table 1). Sunflower oil had less LA while 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 (*2006*). This may be due to using a different soy oil source that contained different minor component levels, which are known to reduce CLA yields (*Jain, et al. 2008b*). Corn and soy oils had similar initial LA contents, but corn oil produced less CLA.

Flax oil produced very little CLA with 14% initial LA and sunflower oil produced no CLA at all, despite having 34% initial LA. The conversion of LA to CLA in each oil was: ~23% in high LA safflower oil, ~20% in soy oil, ~11% in corn oil, ~5% in flax oil, and 0% in sunflower oil. Differences may be due to increasing minor component levels in each oil, which have been shown to reduce CLA yields (*Jain et al. 2008b*). A comparison of soy and corn CLA yields and sunflower and flax CLA yields show that LA levels alone are not good predictors of CLA production. The highest rate of CLA production for each oil was during the first day of irradiation (Figure 2) and was followed by a decrease in the rate of CLA production each subsequent day.

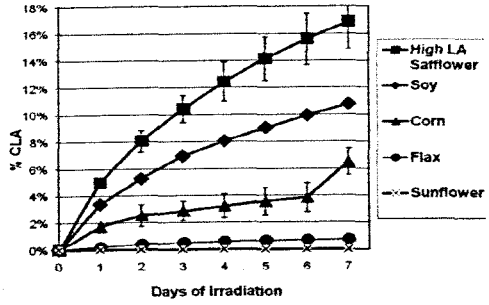
Table 1. Initial and final linoleic acid values and final CLA values after photoirradiation for seven days on the laboratory-scale processing equipment as measured by GC-FID.

Table 1. Initial and final linoleic acid values and final CLA values after photoirradiation for seven days on the laboratory-scale processing equipment as measured by GC-FID.			
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	0.0% ± 0.0 a
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 d
High LA Safflower	72.6% ± 0.2 e	54.9% ± 2.2 e	16.9% ± 2.0 d

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

In oils with the most initial LA, the amount of total CLA produced continues to increase after seven 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. In addition, flax and sunflower oils did not produce

Figure 2. CLA production in high LA safflower, soy, corn, flax, and sunflower oils by laboratory-scale processing.



a significant amount of CLA despite the presence of LA. This further suggests that factors other than initial LA may affect CLA yields .

The *trans-,trans-* isomers CLA isomers (Table 2) were produced in the greatest yield for all irradiated oils, comprising 70% 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 (*Jain and Proctor 2007a*). The large yield of *trans-,trans-* 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 (*Gavino et al. 2003; Kuan-Lin et al. 2005*) and is derived from cis-,trans- CLA isomers. It is probably favored during photoisomerization because of the greater stability of the *trans-* isomer relative to the cis- isomer (*Jain and Proctor 2007b*).

Table 2. CLA isomer levels after photoirradiation for seven days on the laboratory-scale processing equipment as measured by GC-FID.

Table 2. CLA isomer levels after photoirradiation for seven days on the laboratory-scale processing equipment as measured by GC-FID.				
Oil	cis-9,trans-11	trans-9,cis-11 and cis-10,trans-12	trans-10,cis-12	trans-,trans- (8,10, 9,11, and 10,12)
Flax	0.0% ± 0.0 a	0.0% ± 0.0 a	0.0% ± 0.0 a	0.7% ± 0.1 b
Sunflower	0.0% ± 0.0 a	0.0% ± 0.0 a	0.0% ± 0.0 a	0.0% ± 0.0 a
Corn	0.5% ± 0.5 a,b	0.5% ± 0.4 a	0.6% ± 0.3 b	4.7% ± 0.2 b
Soy	1.1% ± 0.0 b,c	1.3% ± 0.0 b	1.1% ± 0.0 c	7.3% ± 0.1 b
High LA Safflower	1.6% ± 0.1 c	2.0% ± 0.3 b	1.6% ± 0.1 d	11.8% ± 1.9 c

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

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 (data not shown). Flax oil had a significantly lower tocopherol concentration of less than 40 ppm. For high LA safflower and sunflower oils, α-tocopherols were the most prominent, while β/γ-tocopherols were the most common in soy, flax, and sunflower oils. δ-tocopherols only reached significant levels in soy oil. The δ-tocopherols and γ-tocopherols are the most potent radical scavenging antioxidants of all tocopherols (*Ikeda and Fukuzumi 1977; Chu and Lin 1993*), and may therefore hinder the free radical mechanism through which LA is isomerized to CLA. Since δ-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 (*Jain and Proctor 2006, 2008a*). It is difficult to know which oils have the highest γ-tocopherol content, because γ-tocopherols and β-tocopherols co-elute during HPLC analysis. It can be reported that the oils with the highest β/γ-tocopherol content were soy oil and corn oil, with ~70 ppm, whereas the other oils studied were all below 30ppm of β/γ-tocopherols. This may explain why both soy and corn oils yielded less CLA than expected from their initial LA contents.

Turbidity Analysis: Turbidity was only measurable in sunflower oil with an absorption of 0.60 ± 0.00 at 600nm (data not shown). Sunflower oil is the primary vegetable oil noted for its turbidity after oil refining (*Leibovitz and Ruckenstein 1981, Leibovitz and Ruckenstein 1984*). 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. "Winterizing" the oil by storing at 4°C will cause precipitation of the waxes and sterols and is commonly done with soy oil.

Carotenoid Analysis: The only vegetable oils to show a significant lutein concentration were flax oil, with 9.8 ppm lutein, and sunflower oil, with 1.2 ppm 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 (*Choe and Min 2006*), possibly contributing to flax oil's low conversion rate (~5%) of LA to CLA.

Phospholipid Phosphorous Analysis: Phosphorous 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 (*Yoon and Min 1987*). The data indicate a very high degree of processing in terms of degumming, which reduces the oil phospholipid phosphorous content from 500-900 ppm to 12-170 ppm (*Brown and Snyder 1985*). Phospholipids sometimes cause turbidity, but this was not an issue in this study.

Comparison of the oxidative stabilities of CLA-rich

Gravimetric Analysis: Table 3 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.

Unirradiated high LA safflower and soy oils each had intermediate induction times without significant difference,

Table 3. 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.

Table 3. 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.			
Oil	Unirradiated	Irradiated, without iodine adsorption	Irradiated, with iodine adsorption on carbon
Flax	1.5 ± 0.7 a,b,c	1.0 ± 0.0 a,b	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 e,f	1.0 ± 1.4 a,b,c
Soy	7.0 ± 1.4 e,f	5.5 ± 0.7 d,e	3.0 ± 0.0 b,c
High LA Safflower	6.0 ± 0.0 e	3.5 ± 0.7 c,d	2.0 ± 1.4 a,b,c

*Data with the same letter are not significantly different, with significance defined at $P < 0.05$.

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 two times more prone to oxidation than linoleic acid and resulting conjugated dienes absorb in the UV region. Tokle et al. 2009 (in review) 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, 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, since lutein prevents photooxidation, and soy oil's high level of δ-tocopherols of 9.7 ppm , which are very potent antioxidants (*Estey 1935*).

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 like tocopherols and lutein from the oils, increasing the likelihood of oxidation. Though 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.

Conclusion

Soy oil and high linoleic acid safflower oils were the only viable candidates for the production of CLA-rich vegetable oils. Since 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 the oils was as *trans-,trans-* isomers, due to their greater stability. A high linoleic acid 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 the can be used for CLA production.

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, probably due to the presence of iodine free radicals. Iodine removal by carbon adsorption did not stabilize the oils, possibly because of simultaneous adsorption of tocopherols. 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 impact CLA yields. Flax oil's initial linolenic acid ($C_{18:3}$) content was 60%, which also negatively impacts its oxidative stability and makes it unlikely that high CLA yields will result from the low initial amount of initial LA.

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Mentor Comments:

Andrew Proctor details the process Whitney Gammill went through to develop and complete her own unique research project and the importance of the results of her study.

I met Whitney in the Fall of 2006 when she was a student in the 'Food Science Orientation' class. I gave a lecture in this course regarding my research on conjugated linoleic acid (CLA) rich soy oil production by isomerizing soy oil linoleic acid. She then approached me regarding the possibility of working with me on her Honors thesis and began working with me on her project. I was immediately impressed by her conscientious attitude and outstanding academic record. She is a very serious, determined student who has clear academic and career goals. Whitney showed herself to be a gifted laboratory worker and highly motivated to conduct research. She subsequently evaluated the potential of various linoleic acid containing oils to produce CLA, which have significant health benefits.

Whitney quickly learned our techniques to photo-isomerize oil linoleic acid to CLA. She also rapidly developed the skills necessary to conduct oil fatty acid analysis by conversion of fatty acids to methyl esters, which she quantifies by gas chromatography. She confidently used our novel photo-isomerization vegetable oil processing techniques and related lipid analytical methods to contribute new findings to the growing field of CLA studies. Whitney has made more progress than I anticipated in the time frame available, while working in the laboratory with minimum supervision.

Whitney has the academic performance, laboratory skills and personal qualities to enable her to succeed as a researcher. She has the ability to comprehend important concepts rapidly and ask crucial questions necessary to pursue a line of inquiry. Her thorough understanding of chemistry and the related sciences and her desire to understand the theoretical basis of the research project is essential to produce quality work and evidence of her attitude and potential to make a serious impact with her future work. She also has a remarkable ability to focus on a task and follow it through to completion without succumbing to discouragement along the way. Her willingness to do laboratory work primarily during nights and weekends, without being requested to do so, is one example of her determination to achieve research success. I believe her tenacity and maturity will serve her well in the future and deserves the recognition of this award. Although the research is part of an ongoing research program on CLA production, Whitney has very much made this her own project and developed the knowledge, understanding and expertise to effectively communicate the significance of the research and her findings.

This paper is an abbreviated version of her Honors thesis. Whitney's research is significant and timely since dietary increase in CLA is related to reducing obesity and obesity related diseases. The importance of her work was recognized in 2008 by the American Oil Chemists Society (AOCS), when she presented her studies at the AOCS Annual meeting. The AOCS Health and Nutrition Division awarded her first prize in the poster competiton where she was competing against graduate student researchers from throughout the world. She was also awarded the prestigious Barry M Goldwater Scholarship in 2008.

The impact of this investigation was enhanced this year by a recent study conducted by Dr. Devareddy at UofA that showed that 1% dietary CLA-rich oil, from our laboratory, decreased the serum cholesterol of obese rats by 38%. This highlights the nutritional and health importance of CLA-rich oil production and will be an important stimulus to commercialization.

The CLA-rich soy oil production process was patented in 2008 and a new company, 'Ultra V Technologies' formed to bring the process to commercialization. Whitney's findings will be important in selecting oils for CLA production and determining to what degree they should be refined.