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Effect of Minor Oil Constituents on Soy Oil Conjugated Linoleic Acid Production

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Conjugated linoleic acid (CLA) is produced by photoisomerization of soy oil linoleic acid. Yields increase with the degree of oil refining, but the effect of specific minor oil components is not known. Therefore, the objectives were to determine the effect of each soy oil minor component on CLA yields and oxidative stability after processing, to determine the effect of soy oil minor constituent interactions on CLA yields and oxidative stability, and to determine how soy oil Magnesol adsorption pretreatment affects CLA yields. Soy oils with varying levels of peroxides, tocopherols, phospholipids, free fatty acids (FFA), and lutein were each UV irradiated, and the CLA content and oxidative stability were determined. A peroxide value of above 0.8 greatly decreased CLA yields, as did phospholipids above 500 ppm. Tocopherols enhanced CLA production at low levels and reduced yields at high concentrations, while lutein and FFA had little effect. High CLA yields corresponded with a lower oil oxidative stability. The interactions between the minor components showed similar trends as seen in the single component study. These findings were supported by observations that Magnesol adsorption removed large quantities of phospholipids and peroxides from soy oil and greatly increased CLA yields but reduced the oxidative stability. Minor components, particularly peroxides and phospholipids, need to be removed from the oil to optimize CLA yields.

KEYWORDS: Conjugated linoleic acid (CLA); refining; minor constituents; interactions; Magnesol

INTRODUCTION

Conjugated linoleic acid (CLA) is a group of geometric and positional isomers of linoleic acid (LA) with conjugated double bonds (*1*). The principle dietary sources of CLA are meat and dairy products of ruminants, which mainly consist of the *cis*-9, *trans*-11- and the *trans*-10,*cis*-12-CLA isomers (*2*). In vitro and animal studies have shown several health benefits of CLA such as its anticarcinogenic, antiatherogenic, and antidiabetic effects, reduction of body fat and increase of lean body mass, enhancement of immune system, and increase in the rate of bone formation (*3*). Human clinical trials have also confirmed some of these health benefits (*3*−*6*). Recently, *trans*,*trans*-CLA isomers, particularly the *trans*-9,*trans*-11-CLA isomer, have shown anticarcinogenic activity due to induction of apoptosis (*7*). Studies by Storey et al. (*8*) indicated that *trans*,*trans*-CLA reduced the ultraviolet radiation (UVR)-induced secretion of interleukin (IL-8), prostaglandin E2 (PGE2), and tumor necrosis factor (TNF-α), which are mediators responsible for inflammation and carcinogenesis in human skin cells.

A pilot scale photoisomerization method has been developed recently by Jain et al. (*9*) to produce CLA from soy oil LA. This method produced ∼20% total CLA after irradiation of refined bleached deodorized (RBD) soy oil for 12 h in the presence of 0.35% iodine catalyst. Approximately 75% of the total CLAs were *trans*,*trans*-isomers, while the remaining were the *cis*,*trans*- and *trans*,*cis*-isomers. A more recent study on the effect of the degree of soy oil processing on CLA yields prior to the photoirradiation step showed that the CLA yields increased with an increasing degree of oil refining (*10*). Crude soy oil gave the lowest CLA yield of 0.2% total CLA, while alkali RBD soy oil gave the highest yield of 16.3% total CLA. CLA processed from crude soy oil had the highest oxidative stability of 13 days of induction time, whereas CLA from RBD soy oil had a low oxidative stability with 8 days of induction time. Increasing CLA yields are probably due to the removal of oil components that inhibit CLA production (*10*). Decreasing oxidative stability with increasing degree of refining is due to the removal of oil components with antioxidant properties (*11*). However, there have been no reports of how each soy oil minor component affects CLA yields and oxidative stability after photoirradiation.

The objectives of this research were to (1) determine the effect of each minor soy oil constituent, peroxides, phospholipids, free fatty acids (FFAs), lutein, and tocopherols on the CLA yield in soy oil, (2) determine the effect of each minor oil constituent on the oxidative stability of CLA-rich soy oil, (3) determine the effect of soy oil minor constituent interactions on CLA yields and oxidative stability, and (4) determine how soy oil Magnesol adsorption pretreatment affects CLA yields.

MATERIALS AND METHODS

Materials. RBD oil was obtained from Riceland Foods (Stuttgart, AR) and used as the control. Resublimed iodine crystals (EM Science, Cherry Hill, NJ) were used as a catalyst. Crude soy phospholipids and soy

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FFAs were obtained from Riceland Foods, commercial mixed soy tocopherols were from Archer Daniels Midland (Decatur, IL), and lutein was from Chromadex (Irvine, CA). Magnesol, commercial magnesium silicate, was obtained from The Dallas Group of America, Inc. (Whitehouse, NJ).

Determination of the Effect of Minor Soy Oil Constituents on CLA Yields. *Effect of Peroxide Concentrations on CLA Yields.* Two hundred grams of RBD soy oil was incubated for 9 days at 60 °C in a 1000 mL beaker to accelerate the oxidation process with maximum surface area. The beaker was wrapped with aluminum foil to prevent photooxidation. Oil samples of 20 g were taken from 200 g of incubated oil every 24 h, and the PV of each sample was measured in duplicate using the micro-AOCS method (*12*). PVs obtained ranged from 0.8 to 50.6.

Iodine (0.35%) was then dissolved in the 20 g oil samples for photoirradiation by an adaptation of the original photoisomerization method of Jain et al. (*9*), specially designed for smaller quantity samples (*13*). Triplicate 5 g soy oil samples were taken from the 20 g aliquots of each PV oil sample and were placed in 7 mL borosilicate vials. The vials were attached to the glass plate of the photoisomerization unit on the oil side at places of uniform maximum UV intensity. The intensity of UV light from the lamps on the glass plate was measured using a digital photometer (Industrial Fiber Optics, Tempe, AZ). Irradiation was carried out for 12 h at 47 °C (*9*).

The CLA content of each sample was determined using the FAME-gas chromatography (GC) analysis in duplicate (*14*, *15*). The *cis*,*trans*-CLA isomer yields and the total CLA (*cis*,*trans*- and *trans*,*trans*-isomers) yields were reported.

Oil Preparation for Minor Oil Component Analysis. Iodine was added to 500 g of RBD soy oil to achieve a 0.35% solution by heating the oil to 70 °C (*9*). This oil was used as the control for the study of each minor component. The tocopherol, phospholipid, FFA, and lutein concentrations in the control oil were 1100, 0, 200, and 0.8 ppm, respectively. This oil was then used for the addition of all of the further minor component studies.

Effect of Tocopherol Concentrations on CLA Yields. Six 20 g oil samples were taken from the 500 g of the oil and iodine solution, and soy tocopherols were added to five of these to obtain a range of oil samples having tocopherol concentrations of 1100, 1400, 1700, 2000, 2300, and 2600 ppm. These concentrations covered a range representing RBD oil (1100 ppm) to crude oil (2600 ppm). Triplicate 5 g samples taken from 20 g oil aliquots were placed in 7 mL borosilicate vials, and the vials were irradiated as described previously (*13*). The CLA isomers produced in each sample were measured in duplicate using FAME-GC analysis (*14*, *15*).

Effect of Phospholipid Concentrations on CLA Yields. Six 20 g oil samples were taken from the 500 g of the oil and iodine solution, and crude soy phospholipids were added to five of these to obtain a range of oil samples having phospholipid concentrations of 0, 200, 500, 1000, 2000, and 3000 ppm. These concentrations covered a range representing those present in RBD oil (0 ppm) to crude oil (3000 ppm). Triplicate 5 g samples taken from the 20 g oil aliquots were placed in 7 mL borosilicate vials and irradiated as described previously (*13*). The CLA isomers produced in each irradiated oil sample were then measured in duplicate by GC analysis of the FAMEs (*14*, *15*).

Effect of FFA Concentrations on CLA Yields. Six 20 g oil samples were drawn from the 500 g of the oil with iodine, and soy FFAs were added to five of these to obtain a range of oil samples having FFA concentrations of 200, 300, 700, 1200, 2200, and 3200 ppm. These FFA concentrations covered a range of those present in RBD oil (200 ppm) to crude oil (3200 ppm). Triplicate 5 g samples taken from the 20 g oil aliquots were placed in 7 mL borosilicate vials and irradiated as described previously (*13*). GC analysis of FAMEs was carried out in duplicate to measure the CLA yields (*14*, *15*).

Effect of Lutein Concentrations on CLA Yields. Six 20 g oil samples were taken from the 500 g of oil with iodine, and the carotenoid pigment lutein was added to five of these to obtain a range of oil samples having lutein concentrations of 0.8, 10.8, 20.8, 30.8, 40.8, and 50.8 ppm. These lutein concentrations covered a range representing RBD oil (0.8 ppm) to crude oil (50.8 ppm). Triplicate 5 g samples taken from 20 g oil aliquots were placed in 7 mL borosilicate vials, and the vials were irradiated as described previously (*13*). The CLA isomers were measured in duplicate by FAME-GC analysis (*14*, *15*).

Table 1. Combinations of Specific Concentrations of Soy Oil Minor Components Derived from Full Factorial Design of the Experiment

	concn (ppm)			
combination no.	tocopherol	phospholipid	FFA	lutein
1	1400	500	300	10.8
2	1400	500	300	40.8
3	1400	500	1200	10.8
4	1400	500	1200	40.8
5	1400	3000	300	10.8
6	1400	3000	300	40.8
7	1400	3000	1200	10.8
8	1400	3000	1200	40.8
9	2000	500	300	10.8
10	2000	500	300	40.8
11	2000	500	1200	10.8
12	2000	500	1200	40.8
13	2000	3000	300	10.8
14	2000	3000	300	40.8
15	2000	3000	1200	10.8
16	2000	3000	1200	40.8
17	2600	500	300	10.8
18	2600	500	300	40.8
19	2600	500	1200	10.8
20	2600	500	1200	40.8
21	2600	3000	300	10.8
22	2600	3000	300	40.8
23	2600	3000	1200	10.8
24	2600	3000	1200	40.8

Determination of the Effect of Each Minor Soy Oil Constituent on the Oxidative Stability of CLA-Rich Soy Oil. The PVs of the CLA-rich soy oils obtained from oils irradiated at different peroxide levels were measured after processing in duplicate using the micro-AOCS method (*12*). For the other four minor components, the formation of primary oxidation products and the relative oxidation induction times were determined by weight change during incubation at 60 °C (*16*). Triplicate 500 mg samples of each irradiated oil were placed in disposable aluminum dishes and incubated at 60 °C to determine weight increase as an indication of peroxide formation and weight loss as peroxide breakdown. The samples were weighed every 24 h, and the results were expressed as a percentage weight change per 500 mg of oil. This method was not used for oils with varying PVs as the induction time had already been reached.

Interactions between Soy Oil Minor Constituents and Their Effects on Soy Oil CLA Yields and Oxidative Stability. For a practical full factorial design of the experiment to study the interactions between the minor constituents and their effects on CLA yields, specific concentrations of each minor component were selected on the basis of the results obtained in the single component study. The tocopherol concentrations selected were 1400, 2000, and 2600 ppm, which covered a range of CLA yields greater than, equal to, and less than the control RBD soy oil, respectively. The phospholipid concentrations used were 500 and 3000 ppm, as they gave similar and lower CLA yields than the control RBD soy oil, respectively. The lutein concentrations of 10.8 and 40.8 ppm were selected for the same reason. The FFA concentrations used for the interaction study were 300 and 1200 ppm because these concentrations gave similar CLA yields as that of the control oil, and higher concentrations of FFA would produce greater oxidation while reducing CLA yields. All of the concentrations selected were subject to full factorial design of experiment, which gave 24 combinations (**Table 1**).

Iodine catalyst (0.35%) was added to 500 g of RBD soy oil. Twenty gram samples were taken, and the minor components were added to achieve the 24 combinations. Triplicate 5 g samples were taken from the 20 g aliquots and placed in 7 mL borosilicate vials for photoirradiation as described previously (*13*). The CLA isomers obtained were then analyzed in duplicate using the FAME-GC method (*14*, *15*). The oxidative stability of the samples was determined using the oxidation weight studies (*16*). The CLA yields obtained and their oxidation induction times were fit in a full

factorial model in the JMP Version 5.0.1 (SAS Inst. Inc., Cary, NC), and the interaction plots were obtained.

Determination of How Soy Oil Magnesol Adsorption Pretreatment Affects CLA Yields. Magnesol is commercial magnesium silicate and is popularly used to treat oils after frying, to slower their degradation by removing suspended solids and oxidation products, mainly peroxides. In this experiment, Magnesol was used to get rid of minor components from the oil and to enhance CLA yields (paper in preparation).

Twenty grams of RBD soy oil was vortexed for 20 min with 10% Magnesol (w/w) in a 50 mL beaker and filtered, and this was analyzed for minor oil constituents. Phospholipids were measured using Spectro Flame Modula EOP model FSMEA 85D inductively coupled plasma spectroscopy (ICP) unit (AOAC 968.08), FFAs were measured using the AOCS Ca 5a-40 titration method, tocopherols were measured using the AOCS Ce 8-89 high-performance liquid chromatography method, and lutein was analyzed by measuring the absorbance at 445 nm in a UV−vis spectrophotometer (*17*). Iodine (0.35%) was then added to the Magnesol pretreated oil for irradiation. Triplicate 5 g samples of the oil were placed in 7 mL borosilicate vials and irradiated using the same photoisomerization method as described previously (*13*). After irradiation, the CLA yields were analyzed in duplicate using the FAME-GC method (*14*, *15*). The oxidation induction times of the CLA-rich oils were determined by weight change (*16*).

Statistics. Analysis of variance (ANOVA) was conducted on the data using the JMP Version 5.0.1 (SAS Inst. Inc.). A Student's *t* test was used to differentiate mean values, with significance defined at $P < 0.05$.

RESULTS AND DISCUSSION

Determination of the Effect of Minor Soy Oil Constituents on CLA Yields. *Effect of Peroxide Concentrations on CLA Yields.* **Figure 1** shows the effect of peroxide levels in the soy oil before irradiation on the CLA yields. The control RBD soy oil with a PV of 0.8 produced ~16% total CLA. However, the CLA yield decreased significantly even with a small increase in PV, finally reaching ~7% at a PV of 11. The CLA yield remained relatively constant at 7% as PV increased to 11. A similar trend was observed in the case of the *cis*,*trans*-CLA isomers, where they decreased from 4.3% at 0.8 PV to 2% at 11 PV and remained the same on further increase in PV. As PV increased from 0.8 to 11, the residual LA levels increased from 36.6 to 45.3%, and there was no significant difference in the LA levels on further PV increase. This indicates reduced conversion of LA to CLA with an increase in the peroxide levels. LA and linolenic acid (LNA) are most likely to oxidize in soy oil. They produce conjugated diene hydroperoxides, which are excellent UV absorbers, so probably all of the UV light is not available for CLA formation; hence, the decreasing CLA yields with increasing PVs. Therefore, PV is an important factor affecting CLA yields.

Effect of Tocopherol Concentrations on Soy Oil CLA Yield. **Figure 2** shows the CLA yield obtained from soy oil with varying tocopherol concentrations. The control RBD soy oil, containing 1100 ppm tocopherols, produced 14.3% total CLA, which increased slightly but significantly to 16.3% on addition of tocopherols up to 1400 ppm. However, the CLA yield decreased significantly in the presence of tocopherol concentrations greater than 1400 ppm and was 12.1% total CLA at 2600 ppm tocopherols. The control RBD soy oil produced 4.2% *cis*,*trans*-CLA isomers, which increased to 4.85% in the presence of 1400 ppm tocopherols and decreased to 3.3% on further tocopherol addition up to 2600 ppm. Tocopherols are natural antioxidants due to

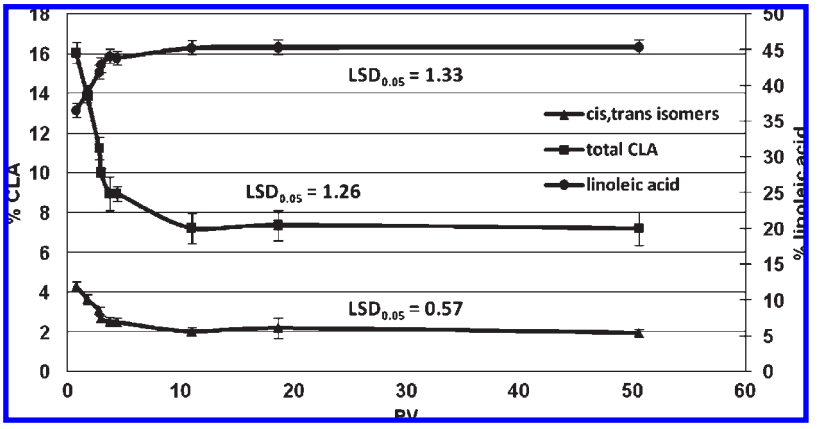

Figure 1. CLA yields and residual LA obtained by photoisomerization of soy oil LA with oils of various peroxide values (PVs).

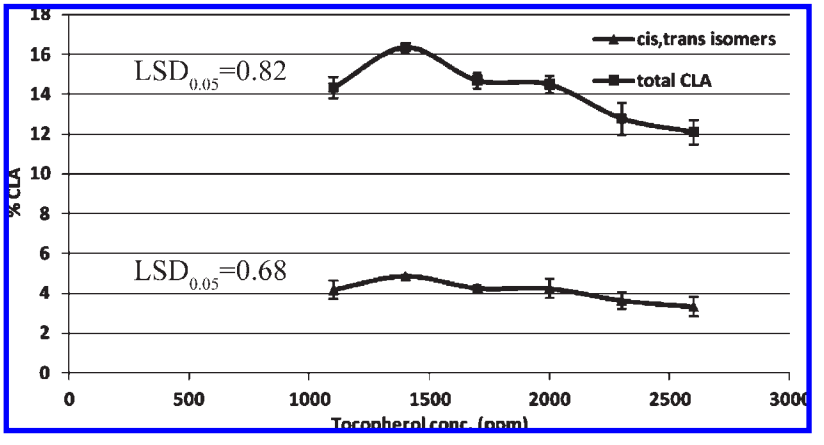

Figure 2. Total and *cis*,*trans*-CLA yields obtained by photoisomerization of soy oil LA with oils of various tocopherol contents.

their free radical scavenging activity. They inhibit the propagation step of the free radical autoxidation mechanism by reacting with various free radicals (*18*). Tocopherols, in concentrations of 1400 ppm, could hence be behaving as antioxidants, reducing the level of peroxides in the oil and thus increasing CLA yield slightly. However, as tocopherol concentrations increase above 1400 ppm, they could be reducing CLA yields by interfering with the free radical formation in the initial step of CLA photoisomerization. This step involves the abstraction of hydrogen from LA by iodine to form a free radical and hydrogen iodide (*19*). Hence, tocopherols could be reversing this first step of CLA synthesis by replacing the removed hydrogen radical.

Effect of Phospholipid Concentrations on Soy Oil CLA Yield. **Figure 3** shows the CLA content in soy oil samples containing various concentrations of phospholipids. The control RBD soy oil with 0 ppm phospholipids produced 13.6% total CLA. There was no significant difference in CLA yield as the phospholipid concentrations increased to 500 ppm. However, the CLA yields declined significantly on further addition of phospholipids above 500 ppm and reached ~4.7% in the presence of 3000 ppm phospholipids. About 4% *cis*,*trans*-CLA was obtained with up to 500 ppm phospholipids, but further addition of phospholipids up to 3000 ppm reduced the *cis*,*trans*-isomer yield to 1.3%. The decrease in CLA yields was not due to phospholipids, causing turbidity as absorbance at 600 nm was less than 0.1 for all samples and not significantly different; therefore, micelles were not formed (data not shown).

Phospholipids are also known to have a synergistic antioxidant effect with tocopherols in oil due to their secondary antioxidant action reducing quinones formed during oxidation of phenolic compounds, formation of Maillard compounds, and hydrogen transfer from the amino group to tocopheroxyl radical regenerating tocopherol (*20*). Hence, with increasing concentrations of phospholipids, more tocopherols could be generated, which could be reducing CLA yields by reacting with the free radicals formed after abstraction of the allylic hydrogen of LA by iodine, thus inhibiting CLA synthesis and reducing CLA yields.

Effect of FFA Concentrations on Soy Oil CLA Yield. **Figure 4** shows the soy oil CLA content obtained with varying FFA concentrations. The control RBD soy oil, containing 200 ppm FFA, produced 16.4% total CLA, which remained relatively unchanged up to 1200 ppm FFA. However, on further addition of FFA to soy oil, the CLA yield showed a significant decrease to 12.4% in the presence of 3200 ppm FFA. A similar trend was observed in the case of the *cis*,*trans*-CLA isomers, where the control RBD soy oil produced 4.8% *cis*,*trans*-CLA, which remained unchanged up to 1200 ppm FFA but decreased significantly to ~3.7% on further FFA addition up to 3200 ppm. Increasing FFA concentrations did not increase turbidity of the oil and was eliminated as a cause of CLA yield reduction. Unsaturated FFA are known to be prooxidants and oxidize more readily than triglycerides (*11*); therefore, trace FFA peroxides could affect CLA yields. Also, the initial PV of the FFA sample when added to the oil, if high, may be reducing CLA yields due to increase in the peroxide levels.

Effect of Lutein Concentrations on Soy Oil CLA Yield. **Figure 5** shows the CLA yield obtained from soy oil containing varying concentrations of lutein pigment. A total CLA of 14%

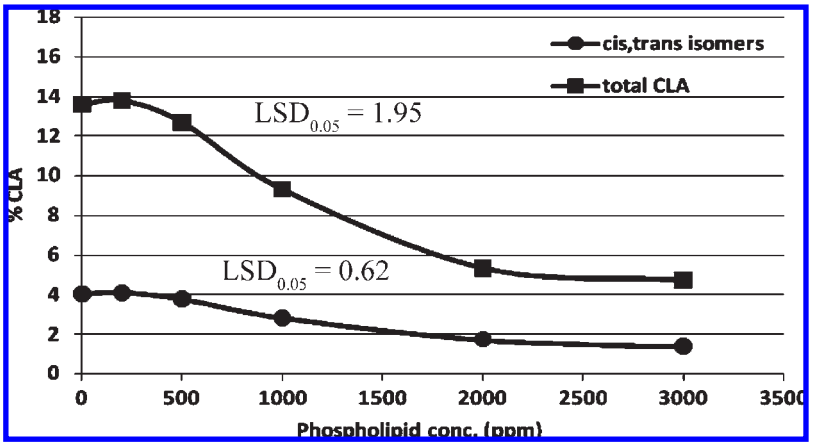

Figure 3. Total and *cis*,*trans*-CLA yields obtained by photoisomerization of soy oil LA with oils of various phospholipid contents.

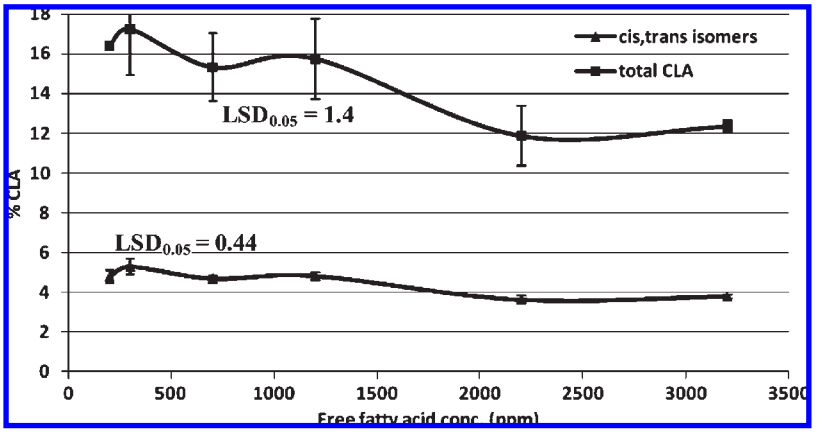

Figure 4. Total and *cis*,*trans*-CLA yields obtained by photoisomerization of soy oil LA with oils of various FFA contents.

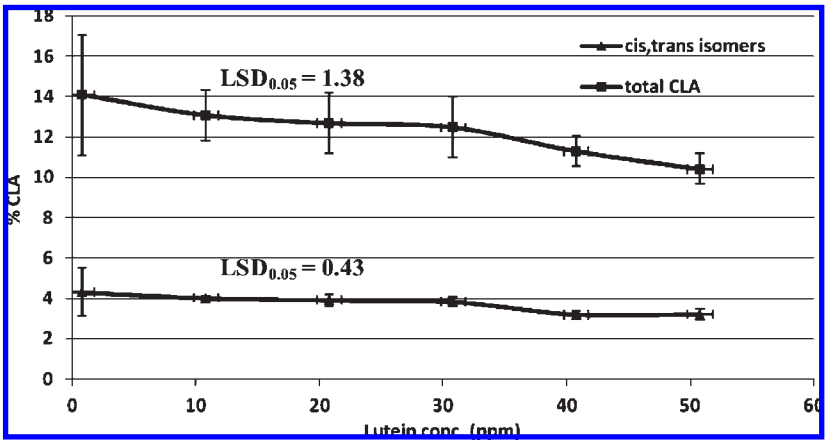

Figure 5. Total and *cis,trans*-CLA yields obtained by photoisomerization of soy oil LA with oils of various lutein contents.

was produced from the control RBD soy oil containing 0.8 ppm lutein, and there was no significant difference in the CLA yield up to 30.8 ppm lutein. However, with increasing lutein, the CLA yield showed a significant decrease to 10.4% in the presence of 50.8 ppm lutein. The *cis,trans*-CLA isomers obtained also remained constant at about 4% up to 30.8 ppm lutein concentrations but showed a significant decrease to 3.2% on further addition of lutein up to 50.8 ppm. Lutein is a UV absorber that absorbs light and is isomerized itself. Hence, increasing concentrations of lutein reduce the amount of UV light available for conversion of LA to CLA, thus reducing the CLA yields.

In the case of each minor constituent, there was a critical threshold concentration above which the CLA yield decreased significantly. These critical concentrations were 0.8 peroxide value (PV), 1400 ppm tocopherols, 500 ppm phospholipids, 1200 ppm FFA, and 30.8 ppm lutein. Only oils with added tocopherols showed a significant increase in the CLA yield relative to that obtained from the control RBD soy oil. A very small PV had a large impact on reducing CLA yields. Also, oils with added phospholipids showed a large decline in the CLA yield of about 61% from its critical threshold concentration (500 ppm) to the largest concentration (3000 ppm). Tocopherols, FFA, and lutein decreased CLA yields by 41, 25, and 16%, respectively, from their critical threshold concentration to their largest concentration.

Determination of the Effect of Each Minor Soy Oil Constituent on the Oxidative Stability of CLA-Rich Soy Oil. *PV*. **Table 2** shows the changes in the PV of CLA-rich soy oil produced on irradiating soy oil samples with different PVs. The PVs before irradiation are significantly different, but there is no significant difference in the PVs of the CLA-rich soy oils after processing. The final PVs of the CLA-rich soy oils range between 1.9 and 4.2 and probably represent the breakdown of oil peroxides during processing.

Tocopherols. **Figure 6** shows the oxidation induction times of the CLA-rich soy oil with varying tocopherol concentrations determined by gravimetric analysis. It indicates that the control RBD soy oil, containing 1100 ppm tocopherols, gave an induction time of 1 day, which increased to 1.7 days on addition of tocopherols up to 1400 ppm. This induction time showed no significant increase up to 2300 ppm tocopherols, but on further addition of tocopherols up to 2600 ppm, the induction time showed a significant rise to 2.7 days, showing its antioxidant properties.

Phospholipids. **Figure 7** shows the trend in the oxidation induction time of the CLA-rich soy oil produced in the presence of varying phospholipid concentrations determined by gravimetric analysis. Control RBD soy oil containing 0 ppm phospholipids showed an induction time of 2 days, which increased to about 4.3 days on addition of phospholipids up to 1000 ppm and remained constant at 4.3 days on further phospholipid addition. This increased induction time, that is, the increased oxidative stability of the CLA-rich soy oil, could be due to antioxidant activity of the phospholipids (*21*, *22*) and their synergy with tocopherols (*20*) or due to residual tocopherols in phospholipid sample (*18*).

Table 2. Changes in PVs of Soy Oil with Varying Peroxide Levels after Irradiation

oil sample no.	PV before irradiation[a]	PV after irradiation[a]
1	0.8 a	2.5 cd
2	1.8 b	2.9 cd
3	2.8 c	2.3 d
4	3.0 c	2.6 cd
5	3.8 d	1.9 d
6	4.4 e	2.3 d
7	11.0 f	3.3 cd
8	18.7 g	3.4 cd
9	50.6 h	4.2 bc
	$LSD_{0.05}$ = 0.23	$LSD_{0.05}$ = 1.86

[a] Values with the same letter are not statistically different.

FFAs. **Figure 8** shows the oxidation induction time of CLA-rich soy oil in the presence of varying levels of FFA determined by gravimetric analysis. The control RBD soy oil, containing 200 ppm FFA, gave an induction time of 1 day, which remained the same on addition of FFA up to 700 ppm. However, the presence of a higher FFA concentration of 1200 ppm increased the oxidation induction time to 2 days, which remained constant on further FFA addition up to 3200 ppm. Unsaturated FFAs are known to act as prooxidants in soy oil (*11*); however, they slightly increased the induction time of CLA-rich soy oil. This increase could be due to residual tocopherols in the oil, but the exact reason behind this is unclear.

Lutein. **Figure 9** shows the effect of varying lutein concentrations on the oxidation induction time of the CLA-rich soy oil determined by gravimetric analysis. An increase in lutein concentrations from 0.8 to 50.8 ppm showed no significant change in the oxidation induction time of the CLA-rich soy oil, which remained constant at around 0.7−1 days. Thus, lutein does not affect oil oxidation after processing.

Tocopherols and phospholipids showed the greatest increase in induction time of the four components. They were followed by FFA, which had a slight but significant increase. Added lutein caused no change in the induction time. Low CLA yield oils had

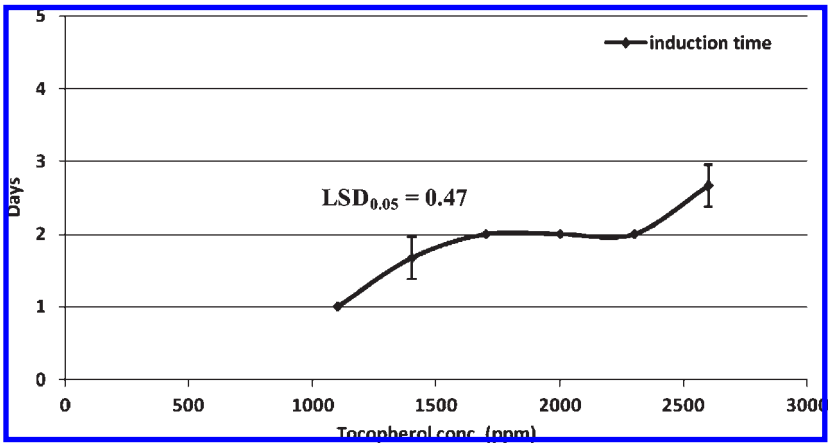

Figure 6. Oxidation induction time of CLA-rich soy oil obtained by photoisomerization of LA with oils of various tocopherol contents.

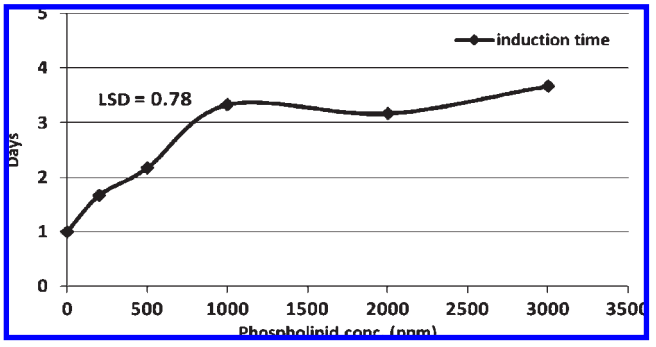

Figure 7. Oxidation induction time of CLA-rich soy oil obtained by photoisomerization of LA with oils of various phospholipid contents.

high induction times. CLA is probably oxidatively unstable relative to LA (*23*, *24*). Therefore, the increase in induction time, and thus oxidative stability, could be either due to antioxidant properties of the minor constituents themselves or due to lower CLA yields.

Interactions between Soy Oil Minor Constituents and Their Effects on Soy Oil CLA Yields and Oxidative Stability. *CLA Yields*. The full factorial model of the CLA yields obtained from oils containing the different combinations of minor components had an R^2 value of 0.95. This model gave a maximum desirability of (15.51 ± 2.06) % total CLA in the presence of 1400 ppm tocopherols, 10.8 ppm lutein, 500 ppm phospholipids, and 1200 ppm FFA.

Figure 10 shows the interactions plot obtained from this model. Graphs 1a and 1b show the trend in total CLA yield with varying concentrations of tocopherols with lutein. CLA yields increased from 11 to 14% in the presence of 2600 ppm tocopherols as the lutein concentration increased from 10.8 to 40.8 ppm. However, in the presence of 1400 and 2000 ppm tocopherols, the CLA yields decreased from 14 to 9% as the lutein concentration increased. This large decrease in CLA yield could be due to UV light absorption of lutein, as described previously. However, high concentrations of tocopherols and lutein appear to interact in a way as to increase the CLA yields.

Graphs 2a and 2b show the trend in total CLA yield with varying concentrations of tocopherols with phospholipids. In the presence of 1400 ppm tocopherols, the CLA yields showed a small increase from 14 to 15% as the phospholipid concentrations increased from 500 to 3000 ppm. They decreased from 14 to 10% in the presence of 2000 ppm tocopherols and increased from 9 to 13% in the presence of 2600 ppm tocopherols. This is similar to the finding of the single component study, that is, although tocopherols act as natural antioxidants and reduce the level of peroxides in the oil, they also reverse the first step of CLA synthesis, inhibiting the reaction. Phospholipids may act synergistically with tocopherols and regenerate more tocopherols, thus reducing the CLA yields.

Graphs 3a and 3b show the trend in total CLA yield with varying concentrations of tocopherols with FFA. The trend is similar to that seen in the case of tocopherols with phospholipids. In the presence of 1400 ppm tocopherols, CLA yields showed a small increase from 15 to 16% as FFA concentrations increased from 300 to 1200 ppm. They decreased from 14 to 11% in the presence of 2000 ppm tocopherols and increased from 9 to 14% in the presence of 2600 ppm tocopherols. Unsaturated FFAs are prooxidants and probably reduce CLA yields by increasing the peroxide levels in the oil, as described previously. However, at higher concentrations of tocopherols, their antioxidant effect could be nullifying the prooxidant effect of FFA and, hence, increasing the CLA yields.

Graphs 4a and 4b show the trend in total CLA yield with varying concentrations of lutein with phospholipids. The 500 ppm phospholipids decreased CLA yields from 15 to 11% as the lutein concentrations increased from 10.8 to 40.8 ppm, whereas 3000 ppm showed a much larger decrease in CLA yields from 14 to 7%. Phospholipids regenerate tocopherols in the oil, which inhibit the CLA synthesis reaction and lutein absorbs the UV light; hence, both phospholipids and lutein together reduce CLA yields.

Graphs 5a and 5b show the trend in total CLA yield with varying concentrations of lutein with FFA. The 300 ppm FFA reduced CLA yields from 15 to 11%, while 1200 ppm reduced them from 16 to 8% as lutein concentrations increased from 10.8 to 40.8 ppm. In the presence of 10.8 ppm lutein, the CLA yield showed a small increase from 15 to 16% with increase in FFA concentrations from 300 to 1200 ppm, while in the presence of 40.8 ppm the CLA yields reduced from 11 to 9%. The critical concentration of FFA found from the previous study is 1200 ppm and gives appreciable CLA yields at low lutein concentrations. However, as the lutein concentrations increase, they absorb the UV light and hence reduce the CLA yields.

Graphs 6a and 6b show the trend in total CLA yield with varying concentrations of phospholipids with FFA. In the presence of 300 ppm FFA, the CLA yields decreased slightly from 15 to 14% as the phospholipid concentrations increased from 500 to 3000 ppm, but they declined from 16 to 12% in the presence of 1200 ppm FFA. Also, in the presence of 500 ppm phospholipids, the CLA yields increased from 14 to 16% as FFA increased from 300 to 1200 ppm, but they decreased from 13 to 11% in the

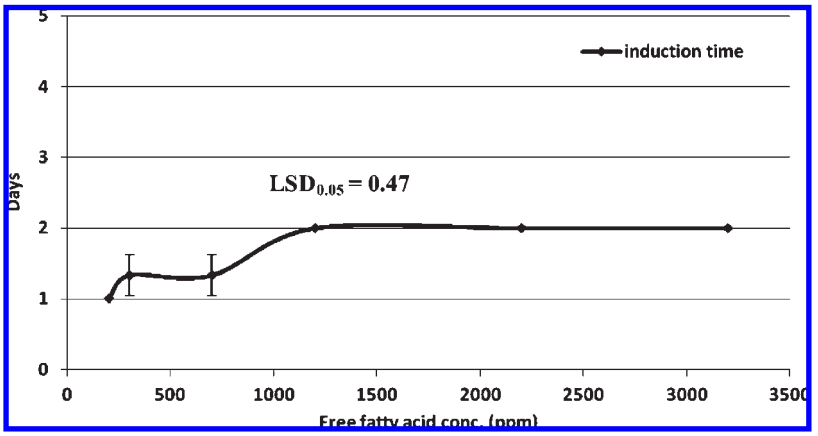

Figure 8. Oxidation induction time of CLA-rich soy oil obtained by photoisomerization of LA with oils of various FFA contents.

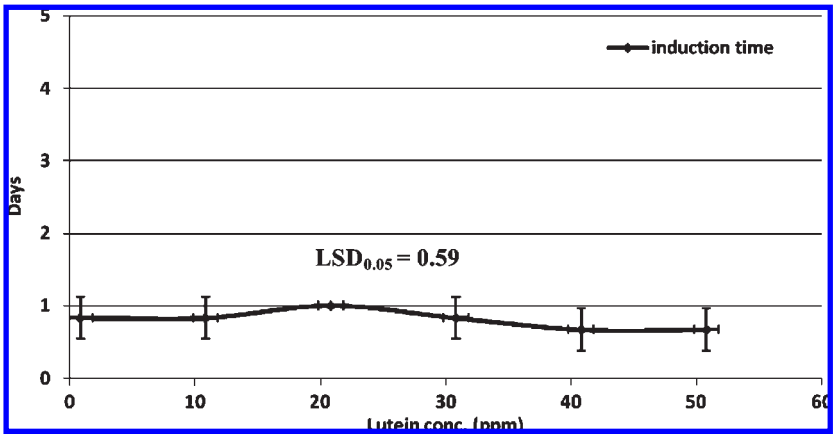

Figure 9. Oxidation induction time of CLA-rich soy oil obtained by photoisomerization of LA with oils of various lutein contents.

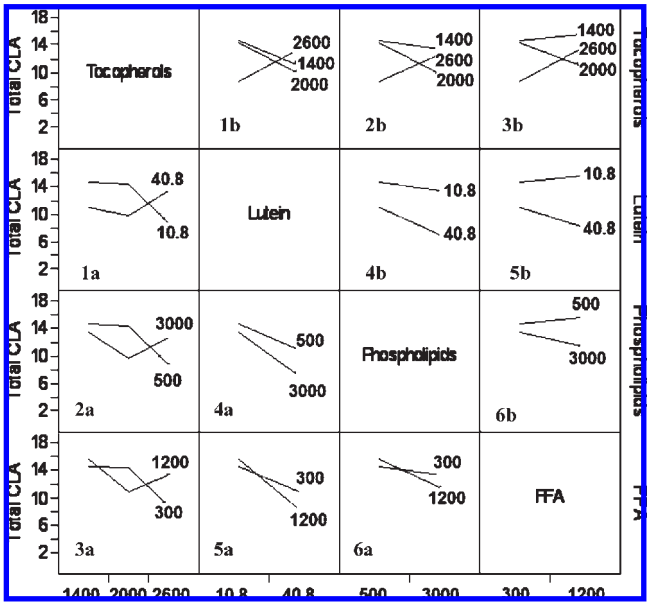

Figure 10. Effects of minor soy oil component interactions on CLA yields obtained by photoisomerization of soy oil LA with varying concentrations of the minor components.

presence of 3000 ppm phospholipids. The critical concentration of phospholipids found from the previous study is 500 ppm and gives appreciable CLA yields probably by opposing the pro-oxidant effect of FFA. However, higher concentrations of phospholipids could be helping tocopherols in the oil to inhibit the CLA synthesis reactions and hence reduce the CLA yields. The *cis*,*trans*-CLA isomer yields also showed similar trends in the effect of interactions between minor components (figures not shown).

Oxidative Stability. The full factorial model for the oxidation induction times gave an R^2 value of 0.90 and a maximum desirability of 9 ± 1.2 days in the presence of 1400 ppm tocopherols, 10.8 ppm lutein, 3000 ppm phospholipids, and 30 ppm FFA. **Figure 11** shows the interaction plots of oxidation induction time vs the minor components obtained from the full factorial model. Graphs 1A and 1B in **Figure 11** show the trend in the oxidation induction time of CLA-rich soy oil with varying concentrations of tocopherols with lutein. The 1400 ppm tocopherols reduced the induction time from 6.5 to 5 days, 2000 ppm tocopherols increased them from 6 to 6.5 days, and 2600 ppm tocopherols reduced them from 5.5 to 4.5 days, as lutein concentrations increased from 10.8 to 40.8 ppm.

Graphs 2A and 2B show the trend in the oxidation induction time of CLA-rich soy oil with varying concentrations of tocopherols with phospholipids. All of the three tocopherol concentrations increased the induction time from about 6 to 9 days, exhibiting the synergistic antioxidant effect of tocopherols and phospholipids.

Graphs 3A and 3B show the trend in the oxidation induction time of CLA-rich soy oil with varying concentrations of tocopherols with FFA. At different tocopherol concentrations, the induction time remained the same irrespective of the FFA

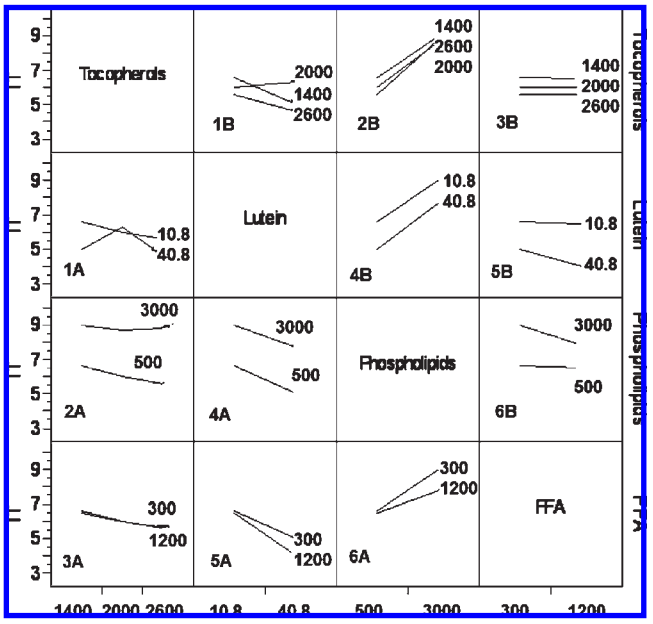

Figure 11. Effects of minor soy oil component interactions on oxidative stability of CLA-rich oil obtained by photoisomerization of soy oil LA with varying concentrations of the minor components.

concentrations. However, at both 300 and 1200 ppm FFA, the induction time decreased from 6.5 to 5.5 days as tocopherol concentrations increased from 1400 to 2600 ppm. Thus, low concentrations of tocopherols act as antioxidants and probably oppose the prooxidant effect of FFA, maintaining the oxidative stability of the CLA-rich oil. However, as the tocopherols increase, they could be acting as prooxidants decreasing the induction time.

Graphs 4A and 4B show the trend in the oxidation induction time of CLA-rich soy oil with varying concentrations of lutein with phospholipids. At a particular lutein concentration, the induction time of CLA-rich oil increased by almost 2.5 days with increases in the phospholipid concentrations from 500 to 3000 ppm. However, at a particular phospholipid concentration, the induction time decreases by 1−1.5 days with an increase in the lutein concentrations from 10.8 to 40.8 ppm. Thus, phospholipids exhibit antioxidant behavior themselves or in synergy with tocopherols, while lutein loses its antioxidant properties probably due to its isomerization on UV irradiation.

Graphs 5A and 5B show the trend in the oxidation induction time of CLA-rich soy oil with varying concentrations of lutein with FFA. In the presence of 10.8 ppm lutein, an increase in FFA did not change the induction time, and it remained constant at 6.5 days. However, in the presence of 40.8 ppm lutein, the induction time reduced slightly from 4.5 to 3.5 days with increases in the FFA concentrations. Both 300 and 1200 ppm FFA reduced the induction times of CLA-rich oil as the lutein concentrations increased from 10.8 to 40.8 ppm, but the declining gradient was greater with 1200 ppm FFA.

Graphs 6A and 6B show the trend in the oxidation induction time of CLA-rich soy oil with varying concentrations of phospholipids with FFA. At 500 ppm phospholipids, increases in FFA concentrations from 300 to 1200 ppm did not change the induction time of CLA-rich oil, which remained constant at 6.5 days, but at 3000 ppm phospholipids, the induction time decreased from 9 to 7.5 days. At both 300 and 1200 ppm of FFA, increases in phospholipid concentrations from 500 to 3000 ppm increased the induction times, but the increasing gradient was greater for 3000 ppm phospholipids.

Table 3. Comparison between RBD Soy Oil and Magnesol-Treated RBD Soy Oil[a]

	RBD soy oil	Magnesol-treated RBD soy oil
phospholipid concn (ppm)	0	0
FFA concn (ppm)	200 a	200 a
lutein concn (ppm)	0.8 a	0.7 a
tocopherol concn (ppm)	1164 a	1127 a
PV	0.8 a	0.4 b
% *cis,trans*-CLA isomers	4.96 b	7.97 a
% total CLA	17.1 b	26.85 a

[a] Values with the same letter in the same row are not statistically different.

Determination of How Magnesol Pretreatment of Soy Oil Affects CLA Yields. Table 3 shows the comparison between RBD soy oil and Magnesol adsorbed RBD soy oil with respect to their composition of minor components and the CLA yields produced. Magnesol reduced peroxides significantly from 0.8 to 0.4. However, there was no significant difference in the concentrations of the other minor components after adsorption. The Magnesol pretreated RBD soy oil produced significantly higher CLA with total CLA yield increasing by almost 10% relative to the control RBD oil. This could be due to significant removal of peroxides, which had a great effect on CLA yield reduction (**Figure 1**).

Low or zero PV oil is essential for optimum CLA yields. Highly refined oils must be used for CLA production as small PVs, tocopherols, phospholipids, FFAs, and lutein have detrimental effects on CLA yields. Only tocopherols up to 1400 ppm promoted CLA yields, but larger amounts must be avoided. Magnesol removed large amounts of peroxides from the oil and greatly enhanced CLA yields. Tocopherols and lutein have human health benefits and may be added to the oil after irradiation.

ABBREVIATIONS USED

CLA, conjugated linoleic acid; LA, linoleic acid; LNA, linolenic acid; RBD, alkali-refined bleached deodorized oil; FFA, free fatty acids; GC, gas chromatography; FID, flame ionization detector; ANOVA, analysis of variance; PV, peroxide value.

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