JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Kinetics of Photoirradiation-Induced Synthesis of Soy Oil-Conjugated Linoleic Acid Isomers

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Photoirradiation of soy oil with UV/visible light has been shown to produce significant amounts of *trans*,*trans* conjugated linoleic acid (CLA) isomers through conversion of various synthesized intermediate *cis*,*trans* isomers. The objective of this study was to determine the kinetics of CLA isomers synthesis to better understand the production of various isomers. Soy oil was irradiated with UV/visible light for 144 h in the presence of an iodine catalyst and CLA isomers analyzed by gas chromatography (GC). Arrhenius plots were developed for the conversion of soy oil linoleic acid (A) to form *cis*-, *trans*/*trans*-, *cis*-CLA (B), conversion of *cis*-, *trans*/*trans*-, *cis*-CLA to form *trans*,*trans*-CLA (C) with respect to B, and formation of *trans*,*trans*-CLA isomers with respect to C. The kinetics of consumption of linoleic acid (LA) to form *cis*-, *trans*/*trans*-, *cis*-CLA was found to be of second-order with a rate constant of 9.01×10^{-7} L/mol s. The rate of formation of *cis*-, *trans*/*trans*-, *cis*-CLA isomers depends on the rate of formation from LA and its rate of consumption to form *trans*,*trans*-CLA isomers. The conversion of *cis*-, *trans*/*trans*-, *cis*-CLA isomers to *trans*,*trans*-CLA isomers was found to be of first-order with a rate constant of 2.75×10^{-6} s^{-1}. However, the formation of thermodynamically stable *trans*,*trans*-CLA isomers (C) with respect to C was found to be a zero-order reaction with a rate constant of 10.66×10^{-7} mol/L s. The consumption of LA was found to be the rate-determining step in the CLA isomers formation reaction mechanism. The findings provide a better understanding of the mechanism of CLA isomers synthesis by photoirradiation and the factors controlling the ratio of various isomers.

KEYWORDS: Conjugated linoleic acid (CLA); soy oil; photoirradiation; kinetics; Arrhenius plots

INTRODUCTION

Animal studies have shown conjugated linoleic acid (CLA) to be an effective anticarcinogenic (*1*, *2*), antiartherosclerotic (*3*), antimutagenic, and antioxidant (*4*). However, the amount of CLA in recommended dietary servings of beef and dairy products would not be sufficient to provide the desired effects in humans, as determined by preliminary studies (*5*). A CLA-rich oil was produced, containing 24% CLA by photoisomerization of soy oil linoleic acid (LA), which would provide CLA in nutritionally significant quantities (*6*).

CLA is comprised of various geometric and positional isomers of conjugated C18:2 fatty acids and can be synthesized chemically by alkali isomerization of unsaturated fatty acids (*7*) or photoisomerization of soy oil LA (*8*) or biochemically by enzymatic interesterification of fatty acids (*9*). Recent studies describe the relative concentrations of the CLA isomers. Jung and Ha (*10*) reported only *trans*,*trans*-CLA isomers at higher reaction times during nonselective hydrogenation of soy oil. Selective hydrogenation is reported to be more favorable than nonselective hydrogenation, predominantly yielding *trans*,*trans* isomers (*10*). Ju and Jung hydrogenated soy oil in the presence of a nickel catalyst and sulfur and observed that, although *cis*,*trans*-CLA isomers predominated initially over other CLA isomers, higher concentrations of *trans*,*trans*-CLA isomers were formed when soy oil was hydrogenated with increasing time (*11*).

Although Jain and Proctor (*6*) synthesized CLA in soy oil in much higher yields than the previously reported method (*8*), approximately 75% of the total CLA content was found to be *trans*,*trans* isomers, mainly composed of *trans*-8,*trans*-10-CLA, *trans*-9,*trans*-11-CLA, and *trans*-10,*trans*-12-CLA. The formation of *trans*,*trans*-CLA was the result of a series of reactions starting with *cis*-9,*cis*-12-octadecadienoic acid that yielded *cis*-, *trans*/*trans*-, and *cis*-CLA as intermediate compounds. These isomers were then converted to thermodynamically stable *trans*,*trans*-CLA isomers. Nevertheless, *trans*,*trans*-CLA isomers also have the health benefits of the other CLA isomers (*12*). A kinetic study of these reactions would provide a better understanding of isomer formation and the isomer ratios produced. **Figure 1** shows a proposed scheme whereby soy oil CLA isomers are formed. *cis*-9,*cis*-12-Octadecadienoic acid (LA) in soy oil is converted to *cis*-9,*trans*-11-CLA, and *trans*-10,*cis*-12-CLA isomers are formed. A portion of LA is also

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10.1021/jf061695b CCC: $37.00
Published on Web 01/17/2007

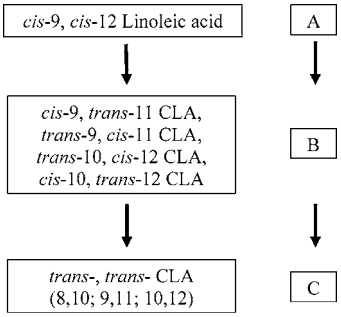

Figure 1. Scheme of proposed soy oil CLA isomer formation by photoirradiation.

converted to *trans*-9,*cis*-12-CLA and *cis*-10,*trans*-12-CLA, respectively. These isomers would then form the more stable *trans*-8,*trans*-10-CLA, *trans*-9,*trans*-11-CLA, and *trans*-10,*trans*-12-CLA isomers.

The goal of this study was to elucidate the formation of various CLA isomers in soy oil photoirradiation, which may provide a rationale of how to better control the reaction and manipulate isomer ratios. The objectives of this research were to (i) determine the kinetics of LA consumption and CLA isomers formation and (ii) determine the kinetics of formation of stable *trans*,*trans*-CLA isomers.

MATERIALS AND METHODS

Materials. Refined, bleached, and deodorized soy oil (Wesson; ConAgra, Irvine, CA) was obtained from a local grocery store (Fayetteville, AR). Resublimed iodine crystals were used as the catalyst (EM Science, Cherry Hill, NJ). Commercial CLA methyl esters (Sigma Aldrich, St. Louis, MO), which were formed of a mixture of *cis*-9,*trans*-11-CLA, *trans*-10,*cis*-12-CLA, and *trans*,*trans*-CLA isomers, were used as standards, and heptadecanoic acid methyl ester (17:0; Sigma Aldrich) was used as the internal standard.

CLA Synthesis by Photoirradiation. A customized photoirradiation system as described by Jain and Proctor (*6*) was used to synthesize CLA in soy oil in the presence of ultraviolet (UV)/visible light with iodine as a catalyst. The reaction system 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 between the reaction vessel and the immersion well held the oil for irradiation. The immersion well supported a 100 W UV/visible medium-pressure, quartz, mercury vapor lamp (Ace Glass Inc.). The lamp operated at 120 V, 60 Hz, and 15 Amp power supply. 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 the oil to light. The oil was heated to 70 °C while flushing with nitrogen to avoid oxidation. Then, 0.15% iodine was added to the oil, and the contents in the beaker were stirred until the iodine was completely dissolved (*6*). The 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 144 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 performed in duplicate.

Methyl Ester Preparation. Methyl esters were prepared from the photoisomerized oil by base-catalyzed method to reduce the formation of conjugated *trans*,*trans* isomers during analysis (*13*). 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 the 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 milliliters 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 Gas Chromatography (GC). 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) (*14*) with a flame ionization detector (FID) (model 3800, Varian, Walton Creek, CA). The samples, prepared in hexane, were injected in duplicates 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:

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

Statistical Analysis. A 2 × 2 experiment was designed to obtain the CLA isomer concentrations in irradiated oil. Soy oil irradiation and CLA isomer concentration analysis were each run in duplicate. Statistical software JMP 5.0.1 (SAS Institute, Cary, NC) was used to conduct analysis of variance data. Least significant differences were calculated to compare mean values, with significance defined at $P < 0.05$.

Kinetics of CLA Isomer Synthesis. The reaction scheme shown in **Figure 1** was used to develop the kinetic studies of LA and CLA isomers during photoirradiation. Arrhenius plots for the reactions (i) conversion of LA to *cis*,*trans*- and *trans*,*cis*-CLA isomers (A to B), (ii) conversion of *cis*,*trans*- and *trans*,*cis*-CLA isomers to *trans*,*trans*-CLA isomers (B to C with respect to B), and (iii) formation of *trans*,*trans*-CLA isomers (B to C with respect to C) were developed using the experimental data on the basis of zero-, first-, and second-order kinetics, to see which order best explained the data. The plot with the best R^2 value was considered to best describe the kinetics. Corresponding rate constants were also obtained. To validate the order of reaction using the R^2 values from the Arrhenius plots, residuals were calculated from each plot for every reaction step. A comparison of the residuals for each assumed order of reaction confirmed the order for the reaction step.

Kinetics of the Reaction A to B. Conversion of A to the intermediate B isomers is dependent only on consumption of A. LA concentrations were measured every 24 h interval during photoirradiation to study the kinetics and obtain the order and the rate constant for the reaction A to B. The rate of this reaction can be defined with the equation:

$$\frac{dA}{dt} = -k_1 A^n \quad (1)$$

Arrhenius plots were developed by assuming the order of reaction as zero-, first-, and second-order ($n = 0$, 1, and 2). The plot with the best R^2 fit gave the order and the rate constant (k_1) for consumption of LA. The order of the reaction was confirmed by comparing the residuals obtained from plots for each assumed order.

Kinetics of the Reaction B to C with Respect to B. As proposed in **Figure 1**, the intermediate B isomers are formed from A. However, at the same time, some amount of B isomers is converted to C isomers.

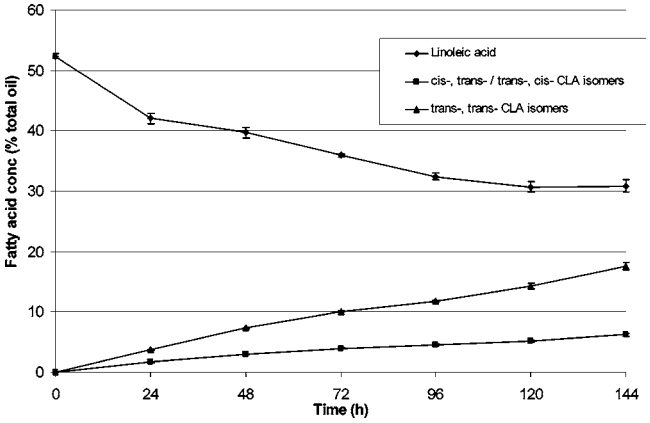

Figure 2. LA and CLA isomers formation in soy oil by photoirradiation with 0.15% iodine. Plots are the means, with standard deviations, of duplicate irradiations, with duplicate isomer analysis for each irradiation.

Thus, the rate of formation of B isomers in the reaction system depends on the rate of formation of B from A and the rate of consumption of B to form C isomers. The rate of reaction can be defined with:

$$\frac{dB}{dt} = k_1 A^n - k_2 B^m \quad (2)$$

For the above equation, k_1 and n are obtained from the reaction A to B. The order of the reaction B to C is assumed ($m = 0$, 1, and 2), and equations were developed. The concentration of intermediate B isomers was measured for the photoirradiation reaction at 24 h intervals, and Arrhenius plots were obtained. The plot with the best R^2 fit gave the order and rate constant (k_2) for the formation of B isomers. The order of the reaction was confirmed by comparing the residuals obtained from plots for each assumed order.

Kinetics of the Reaction B to C with Respect to C. The rate of reaction for the formation of C isomers with respect to C can be defined with:

$$\frac{dC}{dt} = k_2 C^p \quad (3)$$

The order and rate constant for the formation of C isomers from B isomers was determined by measuring the C isomers concentration in the photoirradiation reaction system. The order of the reaction was assumed ($p = 0$, 1, and 2), and Arrhenius plots were developed. The plot with the best R^2 fit would give the order and the rate constant for the formation of C isomers. The order of the reaction was confirmed by comparing the residuals obtained from plots for each assumed order.

Table 1. Correlation Coefficients (R^2 Values) of Arrhenius Plots of (i) Conversion of LA (A) to *cis*,*trans*- and *trans*,*cis*-CLA Isomers (B), (ii) Conversion of *cis*-, *trans*/*trans*-, *cis*-CLA Isomers (B) to *trans*,*trans*-CLA Isomers (C) with Respect to B, and (iii) Formation of *trans*,*trans*-CLA Isomers (C) with Respect to C

	R^2		
reaction	zero-order[a]	first-order	second-order
A to B	0.91	0.95	0.99
B to C w.r.t. B	0.97	0.90	0.77
B to C w.r.t. C	0.99	0.91	0.76

[a] Order of reaction.

RESULTS AND DISCUSSION

CLA Synthesis. Figure 2 shows the fatty acid isomer concentrations during photoirradiation of soy oil with UV/visible light in presence of iodine. The CLA isomer concentrations increased at the expense of A present in the reaction system. There was an ~23 percentage point decrease in the LA concentration during photoirradiation that corresponds to the sum of CLA isomers concentrations. The *trans*,*trans*-CLA isomers were formed about 17% and the *cis*-, *trans*-, and *trans*,*cis*-CLA isomers about 6% of the total oil present in the reaction system.

Kinetics of CLA Isomer Synthesis. Kinetics of the Reaction A to B. Zero-order and first-order Arrhenius plots for the consumption of A yielded R^2 values of 0.91 and 0.95, respectively. However, a second-order plot gave a R^2 value of 0.99, which best explained the order of the reaction (**Figure 3** and **Table 1**). Although R^2 values for all the plots are high, comparison of residual data confirmed the order for the reaction A to B (**Table 2**). The slope of this second-order plot, k_1, the rate constant for the disappearance of A, was 9.01×10^{-7} L/mol s. From a second-order Arrhenius plot, we get the following equation:

$$A = \frac{A_0}{1 + A_0 k_1 t} \quad (4)$$

Kinetics for the reaction A to B indicates that formation of the intermediate B isomers depends on the presence of A in

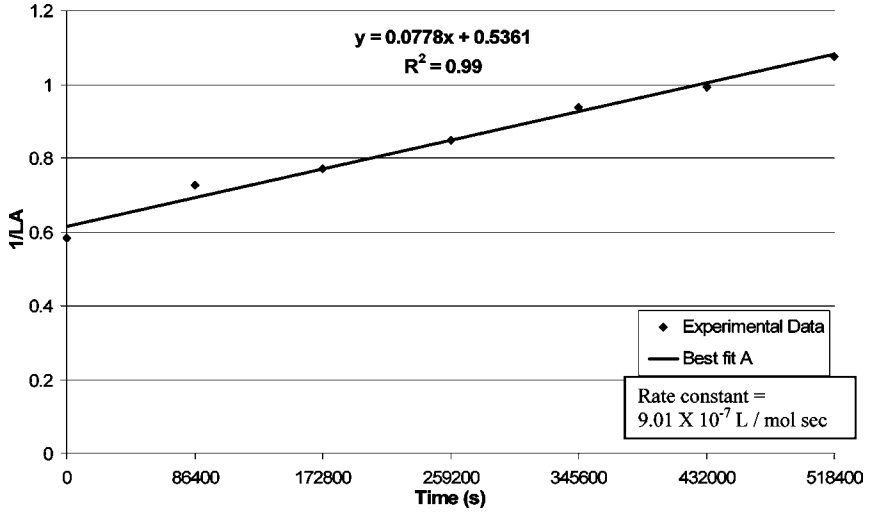

Figure 3. Arrhenius plot showing second-order kinetics for the conversion of LA (A) to *cis*-, *trans*/*trans*-, *cis*-CLA isomers (B) by photoisomerization of soy oil.

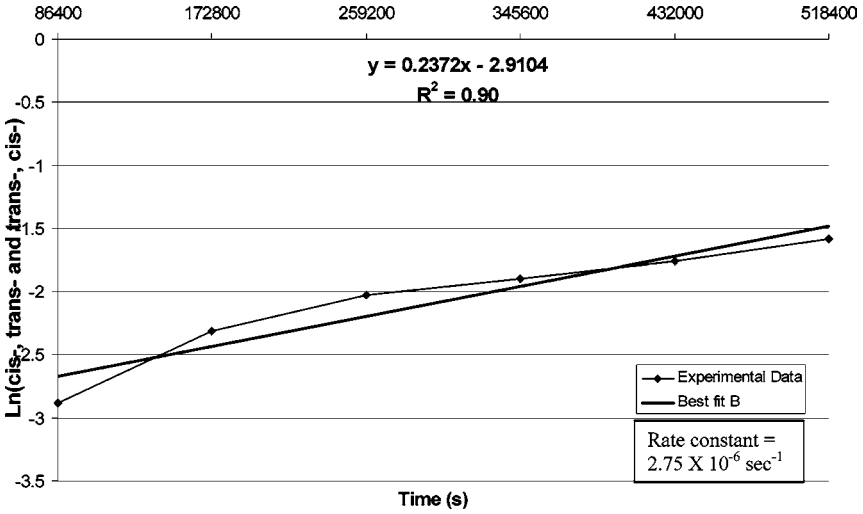

Figure 4. Arrhenius plot showing first-order kinetics for the conversion of *cis,trans*- and *trans,cis*-CLA isomers (B) to *trans,trans*-CLA isomers (C) with respect to B isomers during photoisomerization of soy oil.

Table 2. Residuals Obtained for Zero-, First-, and Second-Order Arrhenius Plots for the (i) Conversion of LA (A) to *cis,trans*- and *trans,cis*-CLA Isomers (B), (ii) Conversion of *cis*-, *trans*/*trans*-, *cis*-CLA Isomers (B) to *trans,trans*-CLA Isomers (C) with Respect to B, and (iii) Formation of *trans,trans*-CLA Isomers (C) with Respect to C

	reaction	residuals at time, *t* (h)						
reaction	order of	0	24	48	72	96	120	144
A to B	zero	0.131	–0.063	–0.005	0.000	0.006	0.019	0.070
	first	0.121	–0.063	–0.028	–0.020	–0.024	0.019	0.030
	second	0.092	–0.053	–0.004	–0.001	–0.004	0.009	0.004
B to C[a]	zero	0.020	0.055	0.080	0.120	0.150	0.180	0.210
	first	0.000	–0.011	0.008	0.026	0.007	–0.002	–0.018
	second	0.000	–0.013	0.015	0.031	0.016	0.010	0.103
B to C[b]	zero	–0.030	0.002	–0.001	0.002	–0.001	–0.009	0.001
	First	0.000	–0.035	0.037	0.060	0.021	–0.026	–0.093
	Second	0.000	–0.034	0.054	0.100	0.066	0.036	–0.534

[a] Formation of C isomers with respect to B isomers. [b] Formation of C isomers with respect to C isomers.

the reaction system. The higher the concentration of A in the system is, the faster would be the rate of formation of B isomers.

Kinetics of the Reaction B to C with Respect to B. Intermediate CLA B isomers are formed from A, and they undergo further photoirradiation to form C. From eq 2 and kinetics of reaction A to B, we can modify eq 2 as:

$$\frac{dB}{dt} = k_1 A^2 - k_2 B^m \tag{5}$$

integrating the above equation from time $t = 0$ to $t = t$,

$$dB_t = \int_0^t k_1 A^2 \, dt - \int_0^t k_2 B^m \, dt \tag{6}$$

Substituting eq 4 in eq 6 we get

$$dB_t = \int_0^t k_1 \left(\frac{A_0}{1 + A_0 k_1 t}\right)^2 dt - \int_0^t k_2 B^m \, dt \tag{7}$$

$$B_t = A_0\left(1 - \frac{1}{1 + A_0 k_1 t}\right) - \int_0^t k_2 B^m \, dt \tag{8}$$

Assuming a zero-order reaction for the formation of *trans,trans*-CLA isomers, we would have $B^n = B^0 = 1$. Thus, eq 6 gives

$$B_t = A_0\left(1 - \frac{1}{1 + A_0 k_1 t}\right) - k_2 t \tag{9}$$

However, as obtained from the reaction A to B, $k_1 = 9.01 \times 10^{-7} \ll 1$, so eq 7 reduces down to

$$B_t = -k_2 t \tag{10}$$

The plot of B_t vs t gave the zero-order plot for the reaction B to C.

Assuming a first-order reaction for the reaction B to C, $B = B_0 e^{-k_2 t}$; therefore, eq 8 would give

$$\ln B_t = -\ln B_0 + k_2 t \tag{11}$$

The plot of $\ln B_t$ vs t gave the first-order plot for the reaction B to C.

Assuming a second-order reaction, $B = B_0/(1 + B_0 k_2 t)$ and eq 6 gives us

$$\frac{1}{B_t} = \frac{1}{B_0} + k_2 t \tag{12}$$

A plot of $1/B_t$ vs t gave the second-order plot for the reaction B to C.

Table 1 shows that the R^2 value for a zero-order plot was found to be higher at 0.97 as compared to the first-order plot (0.90) and the second-order plot (0.77). However, a comparison of the residuals from the plots indicates that the reaction B to C is a first-order reaction with respect to B isomers (**Table 2**). The rate constant for the reaction was obtained from the slope of the first-order plot (**Figure 4**) and equals 2.75×10^{-6} s^{-1}. Thus, the kinetics for the reaction B to C with respect to B suggests that the reaction is dependent on the overall rate of formation of B isomers.

Kinetics of the Reaction B to C with Respect to C. Figure 5 shows a zero-order plot for the formation of C isomers based on the appearance of C isomers in the photoirradiation reaction system with a R^2 of 0.99. In contrast, a R^2 value of 0.91 and 0.76 for a first-order and a second-order plot, respectively, was

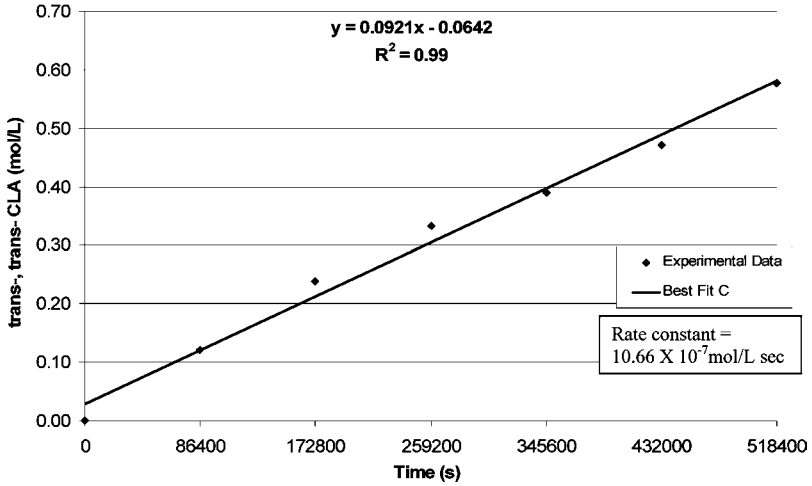

Figure 5. Arrhenius plot showing zero-order kinetics for the formation of *trans*,*trans*-CLA isomers (C) with respect to C isomers during photoisomerization of soy oil.

found (**Table 1**) (plots not shown). A comparison of the residuals from the plot also confirms that the formation of C isomers is a zero-order plot (**Table 2**). This suggests that formation of C isomers is probably predominantly governed by thermodynamics. The *trans*,*trans* configuration is a thermodynamically more stable form than *cis*-, *trans*/*trans*-, *cis* configuration. Thus, intermediate B isomers form stable *trans*,*trans*-CLA isomers, and this conversion is governed by the rate constant for the reaction obtained as 10.66×10^{-7} mol/L s.

Reaction A to B being a second-order reaction (**Figure 3**), the rate of consumption of A depends on the amount of A present at a given time. Thus, the rate of consumption of A is greater at time 0 and it slows with time as observed in **Figure 2**. Reaction B to C with respect to B isomers is a first-order reaction (**Figure 4**) and depends on the B isomers in the reaction system. Thus, the rate of reaction increases steadily with time and hence the amount of C isomers in the system. The rate-controlling step in the formation of CLA isomers from LA was found to be the consumption of LA to form *cis*-, *trans*/*trans*-, *cis*-CLA isomers.

The kinetic study of the CLA isomers formation shows that the greater the concentration of LA is, the greater the concentration of *cis*,*trans*- and *trans*,*cis*-CLA isomers produced is. As the reaction proceeds, the rate of formation of these intermediate isomers slows down. Hence, to maintain a high rate of formation of B isomers, we may need to maintain a large concentration of LA. Thererfore, a continuous reaction system may perform better than a batch reaction system. In contrast, the formation of the stable *trans*,*trans*-CLA isomers is governed by thermodyanamics. However, the kinetics may be changed by increasing the temperature or modifying the catalyst system for the reaction. Therefore, this study provides a better understanding of the CLA isomer synthesis mechanism by photoirradiation.

ABBREVIATIONS USED

CLA, conjugated linoleic acid; UV, ultraviolet; LA, linoleic acid; GC, gas chromatography; FID, flame ionization detector.

ACKNOWLEDGMENT

We thank Dr. Robert Beitle (Department of Chemical Engineering, University of Arkansas), Arjun Dasgupta (University of Arkansas), and Shriram Paranjpe (Department of Food Science, Purdue University, West Lafayette) for their help with the kinetic study.

Supporting Information Available: Arrhenius plots for the disappearance and appearance of LA, first- and second-order plots for the appearance of CLA isomers, and first- and second-order plots for the appearance of *trans*,*trans*-CLA. This material is available free of charge via the Internet at http://pubs.acs.org.

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Received for review June 16, 2006. Revised manuscript received December 1, 2006. Accepted December 1, 2006.

JF061695B