Kinetics of Geometrical Isomerization of Unsaturated FA in Soybean Oil

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ABSTRACT: Laboratory treatments of soybean oil were carried out at the following conditions: atmospheric pressure in the presence of air or nitrogen at different temperatures ranging from 160 to 250ºC for 12 to 72 h. These conditions were used to study geometric isomerization of *cis,cis*-linoleic and *cis,cis,cis*-linolenic acid in the presence or in the absence of oxidative degradation reactions. Based on these experiments, a model of consecutive, parallel reactions was developed to describe the reaction steps occurring in the soybean oil during heating at constant temperature. For both *cis,cis*-linoleic and *cis,cis,cis*-linolenic acid, the reaction of formation isomers followed a first-order reaction, and the rate constant of isomerization varied according to the Arrhenius law. The isomerization rate constant for linoleic acid was $9.57 \times 10^{-3} \pm 0.50\ h^{-1}$ in the presence of oxygen and $7.39 \times 10^{-3} \pm 0.39\ h^{-1}$ in its absence, and the isomerization rate constant for linolenic acid was $1.18 \times 10^{-1} \pm 0.10\ h^{-1}$ in the presence of oxygen and $0.87 \times 10^{-1} \pm 0.07\ h^{-1}$ in its absence (all obtained at 250ºC).

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KEY WORDS: Isomerization, kinetics, linoleic acid, linolenic acid, modeling, oxidation, soybean oil.

Since 1974 when Ackman and Hooper (1) detected some minor geometrical isomers in some commercial vegetable oils, these isomers (described later as nutritionally undesirable) have received a great deal of attention. Ackman and Hooper proved that the isomerization of linoleic and linolenic acid is a result of the deodorization process under regular industrial conditions. Ten years later, Grandgirard *et al*. (2) identified the same compounds in laboratory heat-treated vegetable oils. These components were also isolated from some frying oils obtained from restaurants (3). It became obvious that considerable amounts of *trans* and positional isomers of FA are formed during heat treatment in various vegetable oils at severe conditions, particularly at temperatures greater than 230°C. However, much larger amounts of *trans* and positional isomer FA are formed during hydrogenation of vegetable oils (4); the theoretical basis for this reaction was described by Hoffman (5). Wolff (6) and O'Keefe *et al*. (7) studied the effect of temperature and heating time on the extent of *trans* isomer formation. Kinetic measurements showed that isomerization reactions follow first-order kinetics and that the isomerization rate constants of individual FA exhibit an Arrhenius relationship with temperature. Precht *et al.* (8) also registered the changes of oleic and linoleic acid content, including CLA in milk fat. They made comparisons between summer and winter feeding depending on heating temperature. Hénon *et al*. (9) and Hénon (10) compared the kinetics of isomerization of linoleic and linolenic acid during deodorization and developed a mathematical model to predict the operational conditions for selective isomerization. Recently, Léon-Camacho *et al.* (11) also described the *cis-trans* isomerization undergone by linoleic acid during industrial deodorization and/or the physical refining of edible fats. The article compared sunflower, olive, and soybean oils. In their experiments, nitrogen was used as stripping gas instead of steam. They determined the kinetic constants for the formation of linoleic acid geometrical isomers (*trans*-9,*trans*-12-, *cis*-9,*trans*-12-, and *trans*-9,*cis*-12-linoleic acid).

Deep-fat frying is a complicated thermochemical process in which oil is continuously or repeatedly subjected to temperatures from 170 to 220ºC or higher in the presence of air and moisture. Under these conditions many chemical reactions occur in the oil. These reactions may be grouped into oxidation, isomerization, polymerization, and hydrolysis (12). The purpose of the present investigation was to determine the *cis-trans* isomerization kinetics of linoleic and linolenic acid in the presence of air or in the presence of pure nitrogen.

EXPERIMENTAL PROCEDURES

Materials. The soybean oil was produced by GEA-Slovenska Bistrica (Slovenska Bistrica, Slovenia).

Linoleic acid methyl ester isomer mix (Sigma-Aldrich Chemie Gmbh, Steinheim, Germany) and linolenic acid methyl ester isomer mix (Sigma-Aldrich Chemie Gmbh) were used as standards for detection and quantification of FA and their isomers.

Laboratory-scale treatments. The influence of high temperature on geometric isomerization was studied in two series of experiments. In the first series, 100 mL of soybean oil in a 250-mL glass vessel (diameter: 67 mm; atmospheric pressure: $1.013 \cdot 10^5$ Pa) was heated at constant temperature (160, 190, 210, and 230ºC, controlled with a digital thermometer) for 72 h. The final temperature was reached within approximately 20 min. Some overheating occurred before the temperature could be stabilized, but it did not exceed 5ºC and lasted less than 5 min. Operating temperatures were kept within ±1ºC. At least two replicates of each sample of heated oil were taken every 9 h.

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Similarly, one experiment was performed by heating and stirring 100 mL of oil in a glass vessel at a constant temperature of 250ºC for 12 h in the presence of air. Another experiment was performed in which 100 mL of oil was heated at 250ºC in nitrogen for 12 h. At least two replicates of each sample of heated oil were taken every 1.5 h. The hot samples were quickly cooled and stored at 4ºC in a tightly closed amber vial until analysis.

All samples were protected from light during heating.

Preparation of FAME. FA contents were determined as FAME. They were prepared as follows: Anhydrous sodium sulfate and 1 mL of a 4 mg/mL heptadecanoic acid solution in *n*-hexane were added as an internal standard (purity: ~99%; Fluka, Buchs, Switzerland) to 30 to 40 mg of cooled oil in a tightly closed 4-mL amber vial. The mixture was manually stirred and 1 mL of a boron trifluoride/methanol (~10% boron trifluoride in methanol; Fluka) complex solution was added. After repeated stirring of the mixture, the sample was heated at 90ºC for 1 h.

GLC. A quantity of 0.4 µL of FAME in *n*-hexane was injected into a Varian 3400 gas chromatograph (Walnut Creek, CA) equipped with an all-glass splitter system and an FID. The injection temperature was 210ºC, and the FID temperature was 250ºC. The separation was performed on a CP-Sil 88 WCOT fused-silica capillary column coated with 100% cyanopropyl polysiloxane (50 m × 0.25 mm i.d., 0.20 µm film thickness; Chrompack, Folsom, CA). Helium was used as the carrier gas at an inlet pressure of $1.103 \cdot 10^5$ Pa. The column was operated in a temperature-programmed mode. The oven temperature was held at 180ºC for 7 min, then increased to 190ºC at the rate of 5ºC/min and held at this temperature for 5 min. The temperature was again increased at the rate of 6ºC/min up to 230ºC and held at this temperature for 10 min.

RESULTS AND DISCUSSION

Evaluation of remaining cis,cis-*linoleic (*cis-*L) and* cis,cis,cis-*linolenic acid (*cis-*Ln) contents.* The FA composition of the soybean oil is given in Table 1. The starting soybean oil contained no measurable amounts of *trans* isomers of oleic, linoleic, and linolenic acid.

TABLE 1
FA Composition (%) of Soybean Oil Used for the Tests

FA	Soybean oil (%)
C14:0	0.04–0.11
C16:0	9.23–9.94
C16:1	0.01–0.09
C18:0	3.03–4.15
C18:1	21.59–34.22
All *trans*-C18:2	—
cis,cis-C18:2	46.25–58.01
C20:0	0.16–0.37
All *trans*-C18:3	—
cis,cis,cis-C18:3	6.38–7.60

In the present study we were interested in the rate of isomerization during heating in the presence of air. Soybean oil was heated at different temperatures: 160, 190, 210, and 230ºC. All experiments were conducted in the presence of air except one, which was performed in nitrogen atmosphere at 250ºC. As mentioned earlier, many reactions occur during oil heating, and some of them could be even more important than isomerization. Therefore, the following kinetic model, which includes isomerization and some other reactions (mostly oxidation) in simplified mode, was proposed:

$$\begin{tikzcd}
cis\text{-FA} \arrow[rr, "k_1"] \arrow[dr, "k_3"'] & & iso\text{-FA} \arrow[dl, "k_2"] \\
& ox\text{-FA} &
\end{tikzcd} \tag{1}$$

This reaction scheme is described with the following set of equations (13).

Related to the conversion of FA (linoleic or linolenic acid),

$$\left(-r_{cis\text{-FA}}\right) = -\frac{dC_{cis\text{-FA}}}{dt} = k_1 C_{cis\text{-FA}} + k_3 C_{cis\text{-FA}} = \left(k_1 + k_3\right) C_{cis\text{-FA}} \tag{2}$$

expressed as conversion,

$$X_{cis\text{-FA}}(\%) = \left(1 - \frac{C_{cis\text{-FA}}}{C_{cis\text{-FA}_o}}\right) \cdot 100 \tag{3}$$

the final equation is

$$X_{cis\text{-FA}}(\%) = 100 \cdot \left(1 - e^{-\left(k_1 + k_3\right) \cdot t}\right) \tag{4}$$

Related to the isomer forms of FA (linoleic or linolenic acid),

$$\left(r_{iso\text{-FA}}\right) = \frac{dC_{iso\text{-FA}}}{dt} = k_1 C_{cis\text{-FA}} - k_2 C_{iso\text{-FA}} \tag{5}$$

expressed as conversion,

$$X_{iso\text{-FA}}(\%) = \left(\frac{C_{iso\text{-FA}}}{C_{cis\text{-FA}_o}}\right) \cdot 100 \tag{6}$$

the final equation is

$$X_{iso\text{-FA}}(\%) = 100 \cdot \frac{k_1}{k_2 - \left(k_1 + k_3\right)} \cdot \left(e^{-\left(k_1 + k_3\right) \cdot t} - e^{-k_2 \cdot t}\right) \tag{7}$$

Related to the degradation products of FA (linoleic or linolenic acid),

$$\left(r_{ox\text{-FA}}\right) = \frac{dC_{ox\text{-FA}}}{dt} = k_3 C_{cis\text{-FA}} + k_2 C_{iso\text{-FA}} \tag{8}$$

$$X_{ox\text{-FA}}(\%) = \left(\frac{C_{ox\text{-FA}}}{C_{cis\text{-FA}_o}}\right) \cdot 100 \tag{9}$$

the final equation is

$$X_{ox\text{-FA}}(\%) = 100 \cdot \left(1 - e^{-\left(k_1 + k_3\right) \cdot t} - \frac{k_1}{k_2 - \left(k_1 + k_3\right)} \cdot \left[e^{-\left(k_1 + k_3\right) \cdot t} - e^{-k_2 \cdot t}\right]\right) \tag{10}$$

where $C_{cis\text{-FA}}$ = *cis*-isomers of linoleic or linolenic acid content in oil at time *t*, $C_{iso\text{-FA}}$ = amount of all *trans* isomers of linoleic or linolenic acid isomers formed during heating from the beginning until time t, $C_{cis\text{-FA}_o}$ = initial content of *cis*-linoleic or *cis*-linolenic acid in oil at time t_o, $C_{ox\text{-FA}}$ = content of all degradation products of linoleic or linolenic acid in heated oil at time t, $X_{cis\text{-FA}}(X_{cis\text{-L}},X_{cis\text{-Ln}})$ = conversion of *cis*-L or *cis*-Ln at time t, $X_{iso\text{-FA}}(X_{iso\text{-L}}, X_{iso\text{-Ln}})$ = conversion of isomers of linoleic or linolenic acid at time t, $X_{ox\text{-FA}}(X_{ox\text{-L}}, X_{ox\text{-Ln}})$ = ratio between the content of all degradation products of linoleic or linolenic acid in heated oil at time t, and the initial content of *cis*-linoleic or *cis*-linolenic acid in oil at time t_o, k_1 = rate constant for isomerization of *cis* to formed *trans* isomers, k_2 = rate constant for degradation of formed *trans* isomers, k_3 = rate constant for degradation of primary *cis*-isomers (*cis*-L and/or *cis*-Ln) in the soybean oil.

These equations are applicable in this specific instance, i.e., with the geometry of the equipment used and atmospheric pressure. These equations are of very limited utility and are applicable only for this specific instance. In these equations, for the more general conditions the effect of oxygen concentration have to be included.

The standard integral method was used to calculate the rate constants for individual FA for each reaction in the proposed mathematical model. Good agreement between the experimental data and Equations 4 and 7 confirms the utility of these equations for calculations of the rate constants for appropriate FA (Figs. 1–3). We established that after 72 h of heating at 230ºC, *cis*-L reaches about 70% of conversion (Fig. 1A), whereas *cis*-Ln converts almost completely (Fig. 1B). Simultaneously, we investigated the formation of *trans* isomers. The conversion of isomers of linoleic acid after 72 h at 230ºC was 8.3% (Fig. 2A), and the isomer conversion of linolenic acid reached the value of approximately 30% after 54 h (Fig. 2B). We presumed that at higher temperature (250°C), the time needed for the conversion would be significantly shorter; therefore, all experiments at that temperature lasted only 12 h. The percentage of isomers of linoleic acid $X_{iso\text{-L}}$ after 12 h of heating at 250ºC was 6.8% (Fig. 2A), whereas the isomer content of linolenic acid reached approximately 35% after 10.5 h (Fig. 2B). The comparison of the results of the experiments done in the presence of different atmospheres is presented in Figures 3A and 3B. The conversion values for *cis*-L are 35.8% in the presence of air and 9.3% in nitrogen (Fig. 3A). The conversion values for *cis*-Ln are 89.5% in the presence of air and 84.9% in nitrogen (Fig. 3B). The relatively high conversion value of *cis*-Ln in nitrogen atmosphere compared to *cis*-Ln in the presence of air and to *cis*-L is the consequence of a high sensitivity of *cis*-Ln based on the number of double bonds. This is also confirmed by the much higher rate constants for

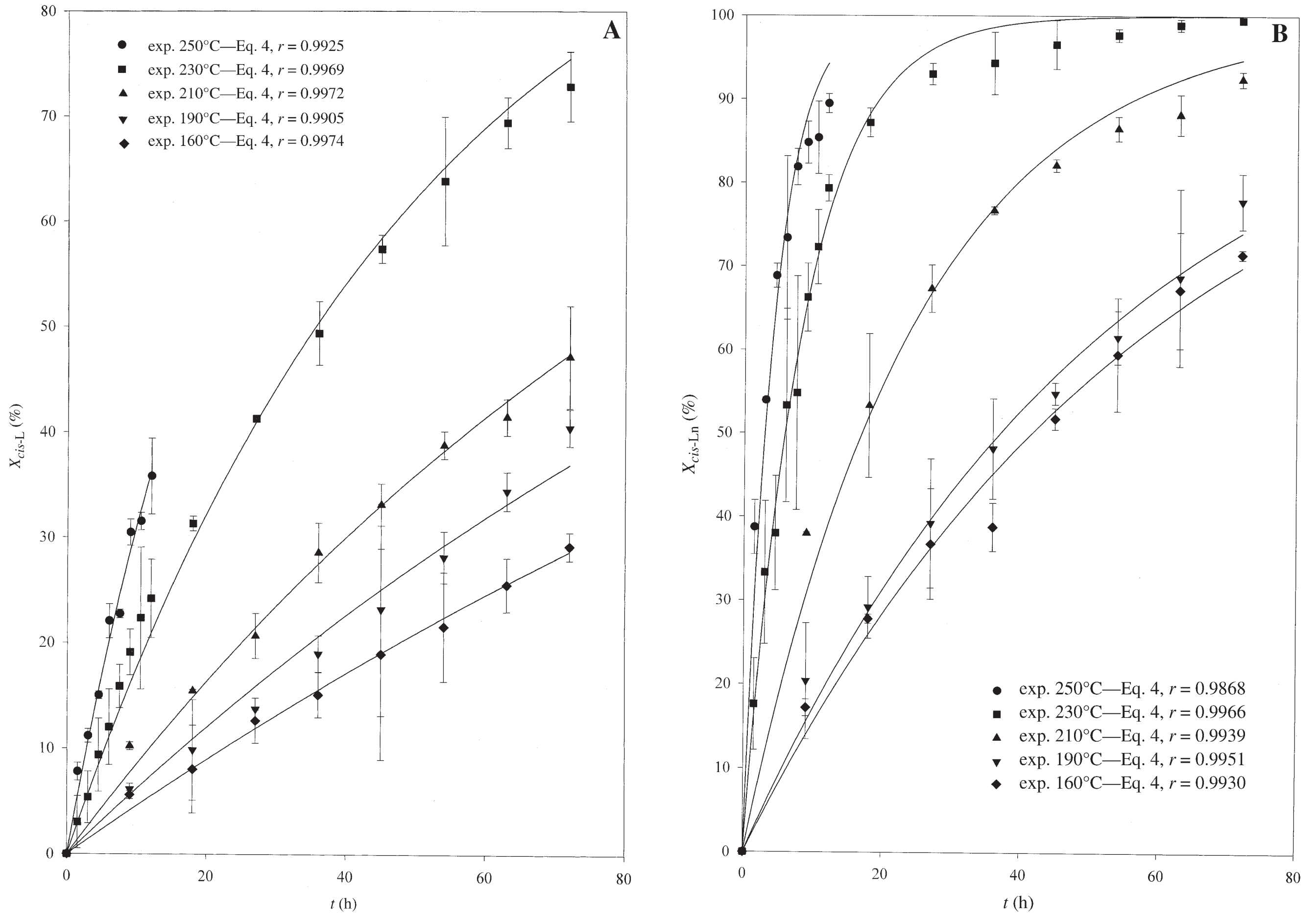

FIG. 1. Conversion curves of *cis*-linoleic (A) and *cis*-linolenic acid (B) at different temperatures, with prediction according to Equation 4 compared to experimental data. Error bars represent 95% confidence limits (n = 3).

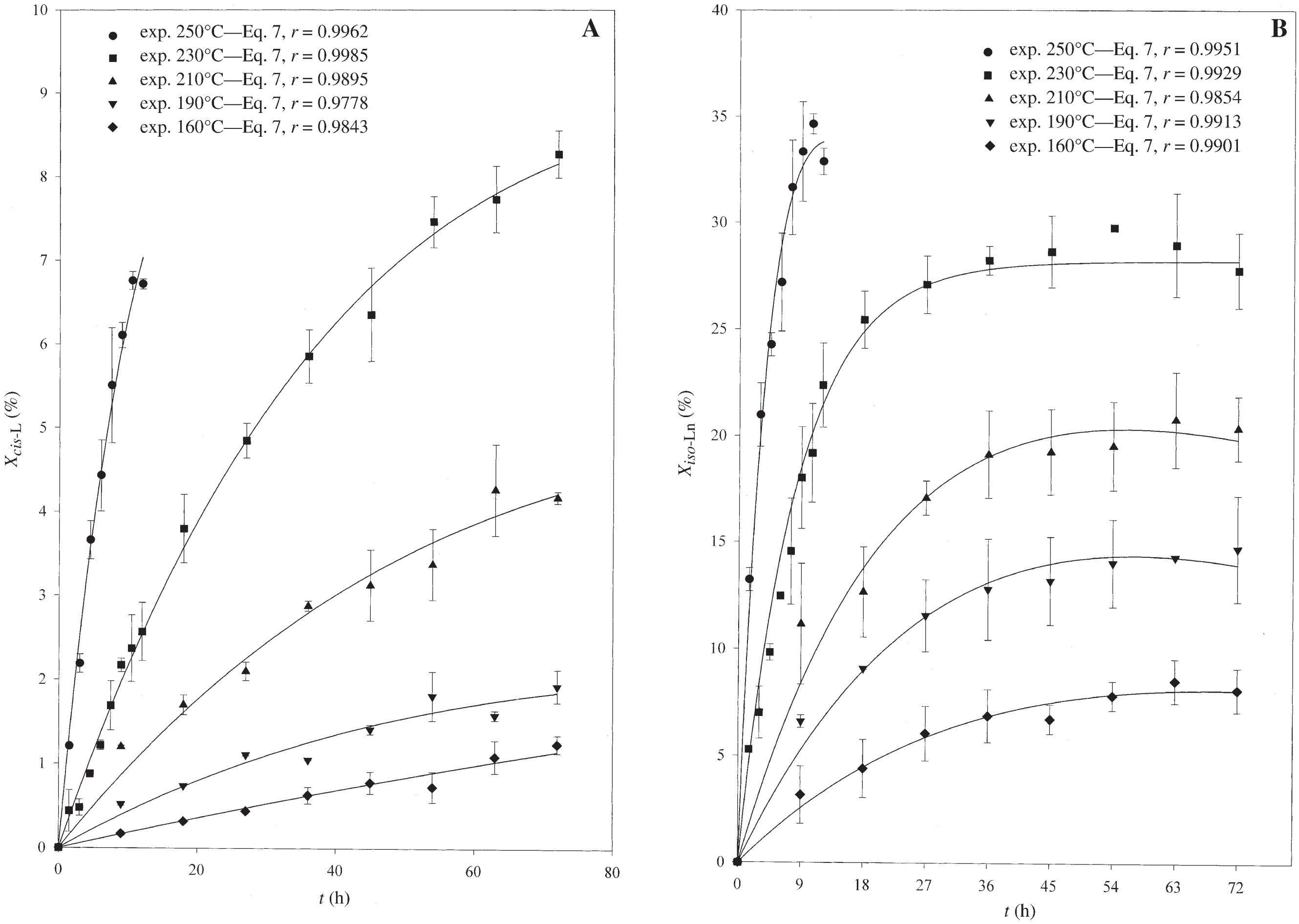

FIG. 2. Conversion curves of *iso*-linoleic (A) and *iso*-linolenic acid (B) at different temperatures, with prediction according to Equation 7 compared to experimental data. Error bars represent 95% confidence limits ($n = 3$).

cis-Ln acid (k_{Ln1} and k_{Ln3}) compared to those for *cis*-L acid (k_{L1} and k_{L3}) (Tables 2 and 3).

Next, the isomerization reaction of *cis*-L and *cis*-Ln was isolated from oxidative reactions. Therefore, in the last experiment a nitrogen atmosphere was used to eliminate all reactions initiated by the oxygen in the air (Figs. 3A,B). In this case, consecutive, parallel reactions could be simplified into the following reaction:

$$cis\text{-FA} \xrightarrow{k} iso\text{-FA} \qquad [11]$$

Besides isomerization, numerous other reactions appear (polymerization, hydrolysis, oxidation, etc.) with emphasis on oxidation. It is easier to examine the *cis-trans* isomerization when oxidation is eliminated. Calculations of isomerization rate constants (±SD) according to Equation 11 are $k_{L1} = (7.39 \pm 0.39) \times 10^{-3}\ \text{h}^{-1}$ and $k_{Ln1} = (0.87 \pm 0.07) \times 10^{-1}\ \text{h}^{-1}$, and isomerization rate constants according to Equation 1 are $k_{L1} = (7.07 \pm 0.33) \times 10^{-3}\ \text{h}^{-1}$ and $k_{Ln1} = (0.76 \pm 0.03) \times 10^{-1}\ \text{h}^{-1}$.

The logarithms of rate constants for reactions in a hypothetical model (Eq. 1) of *cis*-L, k_L, and *cis*-Ln acid, k_{Ln} (Tables 2 and 3) follow the reciprocal absolute temperature according to the Arrhenius law (Eq. 12). From calculated activation energies (Figs. 4 and 5), it is clear that for both unsaturated FA activation energies for the isomerization reac-

TABLE 2
Reaction Rate Constants (h^{-1}) of Linoleic Acid k_{L1}, k_{L2}, and k_{L3}

Temperature (°C)	Time (h)	k_{L1} (h^{-1})	RSD (%)	k_{L2} (h^{-1})	RSD (%)	k_{L3} (h^{-1})	RSD (%)	r
160	72	1.91×10^{-4}	6.38	3.96×10^{-4}	6.98	4.51×10^{-3}	1.52	0.984
190	72	3.63×10^{-4}	3.86	1.03×10^{-3}	2.91	6.05×10^{-3}	3.78	0.978
210	72	1.03×10^{-3}	4.76	6.91×10^{-3}	2.99	7.89×10^{-3}	4.60	0.990
230*	72	2.99×10^{-3}	4.58	9.84×10^{-3}	4.96	1.66×10^{-2}	3.95	0.999
250	12	9.57×10^{-3}	5.23	4.39×10^{-2}	6.97	2.81×10^{-2}	2.12	0.996

[a] Variability of the reaction rate constants is expressed as a relative standard deviation (RSD) and is calculated from at least duplicate analyses of the experiment. *This experiment was performed three times and variability in this case is calculated as average of RSD of all three experiments.

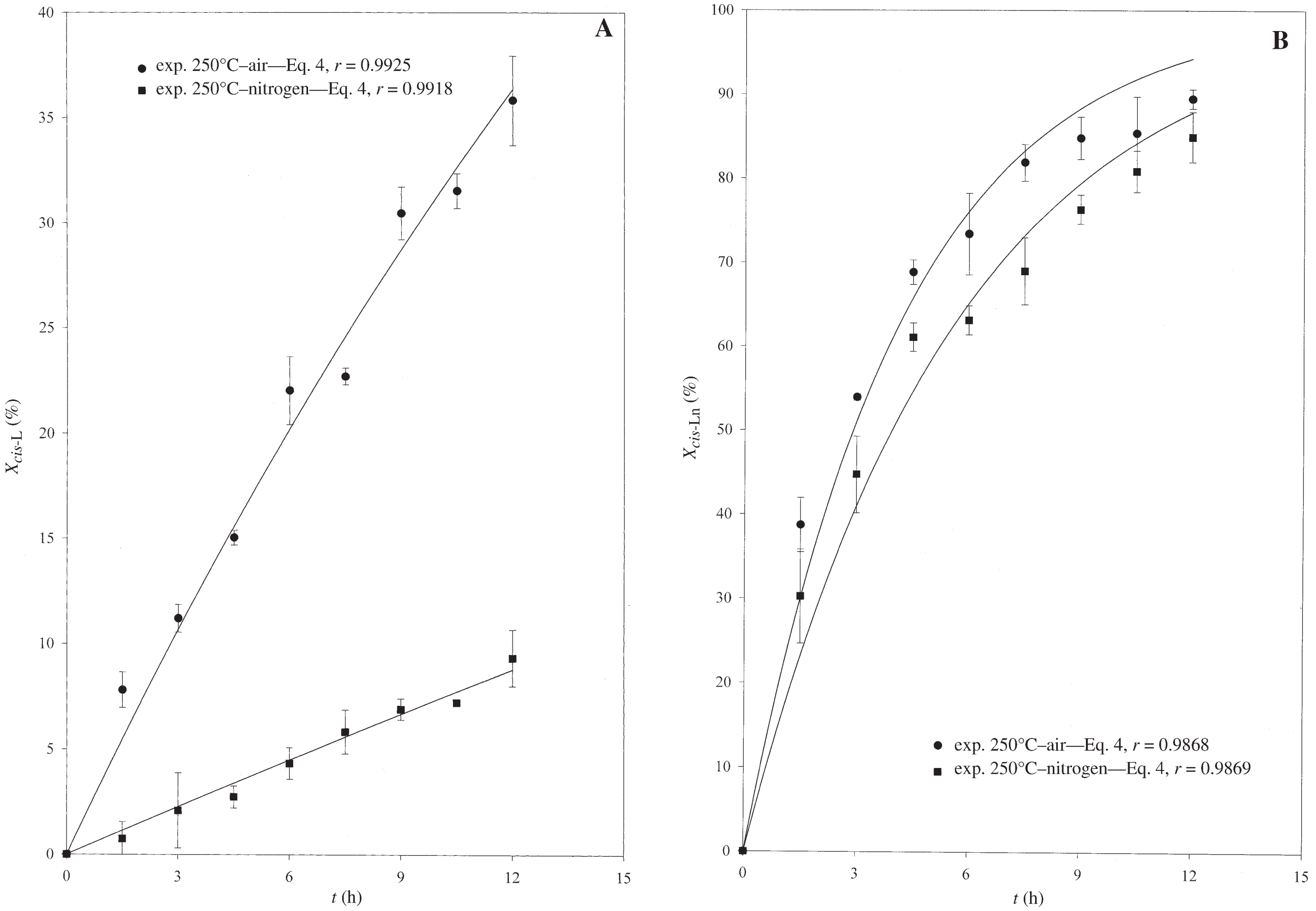

FIG. 3. Comparison of conversion curves of *cis*-linoleic (A) and *cis*-linolenic acid (B) at 250ºC (in the presence of air or nitrogen), with prediction according to Equation 4 compared to experimental data. Error bars represent 95% confidence limits ($n = 3$).

TABLE 3
Reaction Rate Constants (h^{-1}) of Linolenic Acid k_{Ln1}, k_{Ln2}, and k_{Ln3}

Temperature (ºC)	Time (h)	k_{L1} (h^{-1})	RSD (%)	k_{L2} (h^{-1})	RSD (%)	k_{L3} (h^{-1})	RSD (%)	r
160	72	3.24×10^{-3}	8.69	1.28×10^{-2}	7.40	1.34×10^{-2}	4.94	0.990
190	72	6.40×10^{-3}	5.95	1.66×10^{-2}	2.61	1.85×10^{-2}	4.90	0.991
210	72	1.86×10^{-2}	5.11	2.85×10^{-2}	3.07	2.66×10^{-2}	2.24	0.985
230*	72	5.43×10^{-2}	5.46	4.28×10^{-2}	5.25	6.35×10^{-2}	5.43	0.993
250	12	1.18×10^{-1}	8.37	6.02×10^{-2}	7.37	1.13×10^{-1}	8.84	0.995

[a]Variability of the reaction rate constants is expressed as a RSD and is calculated from at least duplicate analyses of the experiment. *This experiment was performed three times and variability in this case is calculated as average of RSD of all three experiments. For abbreviation see Table 2.

tions are quite similar, but activation energy for the oxidation of *trans*-linoleic acids formed is much higher compared to that of linolenic acid isomers. It also is clear that in both unsaturated FA the original *cis* isomer is more easily transformed into degradation or oxidized forms than into isomer forms. This applies to all the following processes: the transformations into *trans* isomers (k_{L1} and k_{Ln1}), into the degradation products from the *trans* isomers (k_{L2} and k_{Ln2}), and for the original reactants (k_{L3} and k_{Ln3}):

$$\ln k = -\frac{E_a}{R}\cdot\frac{1}{T} + \ln k_o \qquad [12]$$

The good linear relationships (linear correlation coefficient $r > 0.95$) found for all the reactions verify that they follow first-order kinetics. Kinetic studies and Arrhenius plot parameters can be a source of valuable information about the course of the consecutive, parallel reactions. Therefore, by applying these kinetic equations it is possible to calculate the FA composition of heated oil at the defined conditions.

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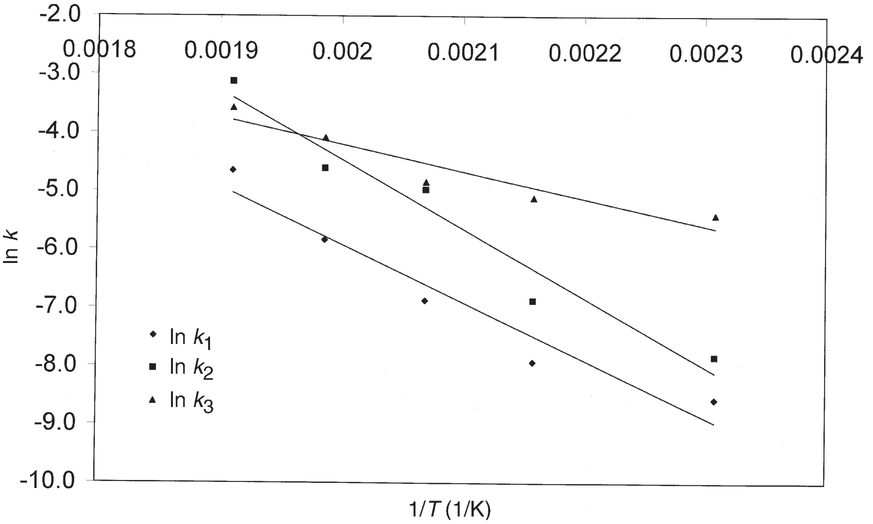

FIG. 4. Temperature dependence of each individual reaction rate constant of *cis,cis*-linoleic acid in a system of consecutive, parallel reactions. E_a, activation energies: $E_{a,L1}$ = 82.3 kJ/mol, $E_{a,L2}$ = 98.3 kJ/mol, and $E_{a,L3}$ = 38.3 kJ/mol; and pre-exponential factors obtained from experimental data: $k_{o,L1} = 1.10 \times 10^6$ h^{-1}, $k_{o,L2} = 2.16 \times 10^8$ h^{-1}, and $k_{o,L3} = 1.51 \times 10^2$ h^{-1}.

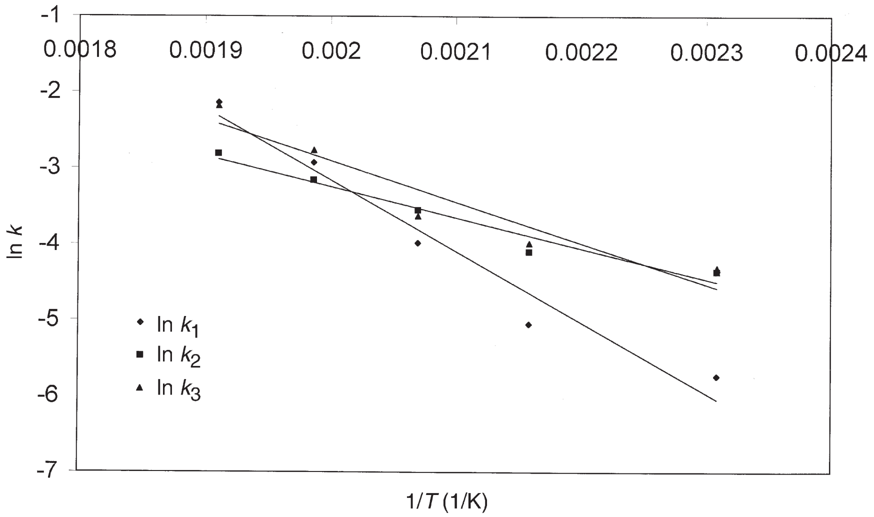

FIG. 5. Temperature dependence of each individual reaction rate constant of *cis,cis,cis*-linolenic acid in a system of consecutive, parallel reactions. $E_{a,Ln1}$ = 77.9 kJ/mol, $E_{a,Ln2}$ = 33.8 kJ/mol, and $E_{a,Ln3}$ = 45.1 kJ/mol; and pre-exponential factor obtained from experimental data: $k_{o,Ln1} = 5.86 \times 10^6$ h^{-1}, $k_{o,Ln2} = 1.32 \times 10^2$ h^{-1}, and $k_{o,Ln3} = 2.84 \times 10^3$ h^{-1}.

REFERENCES

1. Ackman, R.G., and S.N. Hooper, Linolenic Acid Artifacts from the Deodorization of Oils, *J. Am. Oil Chem. Soc. 51*:42–49 (1974).
2. Grandgirard, A., J.L. Sebedio, and J. Fleury, Geometrical Isomerization of Linolenic Acid During Heat-Treatment of Vegetable Oils, *Ibid. 61*:1563–1568 (1984).
3. Sebedio, J.L., A. Grandgirard, Ch. Septier, and J. Prevost, Chemical Analyses of Some Frying Oils Obtained from Restaurants, *Rev. Fr. Corps Gras 54*:15–18 (1987).
4. Swern, D., Reactions of Fats and Fatty Acids, in *Bailey's Industrial Oil and Fat Products,* 3rd edn., edited by D. Swern, John Wiley & Sons, 1964, pp. 55–95.
5. Hoffmann, G., *The Chemistry and Technology of Edible Oils and Fats and Their High Fat Products,* Academic Press, New York, 1989, pp. 201–216.
6. Wolff, R.L., Heat-Induced Geometrical Isomerization of α-Linolenic Acid: Effect of Temperature and Heating Time on the Appearance of Individual Isomers, *J. Am. Oil Chem. Soc. 70*: 425–430 (1993).
7. O'Keefe, S.F., V.A. Wiley, and D. Wright, Effect of Temperature on Linolenic Acid Loss and 18:3 Δ9-*cis*, Δ12-*cis*, Δ15-*trans* Formation of Soybean Oil, *Ibid. 70*:915–917 (1993).
8. Precht, D., J. Molkentin, and M. Vahlendieck, Influence of the Heating Temperature on the Fat Composition of Milk Fat with Emphasis on *cis-/trans*-Isomerization, *Nahrung 43*:25–33 (1999).
9. Hénon, G., Zs. Kemény, K. Recseg, F. Zwobada, and K. Kovari, Deodorization of Vegetable Oils. Part I: Modeling the Geometrical Isomerization of Polyunsaturated Fatty Acids, *J. Am. Oil Chem. Soc. 76*:73–81 (1999).
10. Hénon, G., Modèle prédictif de l'isomérisation *trans* des acides gras polyinsaturés au cours la désodorisation industrielle des huiles végétales, *OCL–Oleagineux Corps Gras Lipides 7*: 91–94 (2000).
11. Léon-Camacho, M., M.V. Ruiz-Méndez, M. Graciani-Constante, and E. Graciani-Constante, Kinetics of the *cis-trans* Isomerization of Linoleic Acid in the Deodorization and/or Physical Refining of Edible Fats, *Eur. J. Lipid Sci. Technol. 103*:85–92 (2001).
12. Mattil, K.F., Hydrogenation, in *Bailey's Industrial Oil and Fat Products,* 3rd edn., edited by D. Swern, John Wiley & Sons, New York, 1964, pp. 793–896.
13. Levenspiel, O., *Chemical Reaction Engineering,* John Wiley & Sons, New York, 1999, pp. 52–56, 178–183, 194–197, 217–218, and 235–237.

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