Heat-Induced Geometrical Isomerization of α-Linolenic Acid: Effect of Temperature and Heating Time on the Appearance of Individual Isomers

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The formation of linolenic acid geometrical isomers (LAGIs) was studied in linseed oil that was heated under vacuum in sealed ampoules at different temperatures (190–260°C) for several durations (2–16 h). A temperature of about 190°C seems to be necessary to induce the formation of LAGIs. At higher temperatures, disappearance of linolenic acid follows a first-order kinetic. The formation of LAGIs increases with both heating time and temperature, degrees of isomerization of linolenic acid higher than 50–60% could easily be obtained by simply heating the oil under vacuum. Side reactions remain at a low level. The mean probabilities of isomerization of individual ethylenic bonds are similar to those determined in linolenic acid-containing oils marketed in European countries, 41.9, 4.7 and 53.3% for double bonds in positions 9, 12 and 15, respectively. The di-*trans t,c,t (trans,cis,trans)* isomer is formed *via* the mono-*trans c,c,t* and *t,c,c* isomers by a two-step reaction. The proportions of the *c,c,t* and *t,c,c* isomers (relative to total LAGIs) decrease linearly with the heating time. The proportion of the *c,t,c* isomer is only slightly affected by this parameter; however, it increases with temperature. The proportion of the *t,c,t* isomer increases linearly with heating time at each tested temperature, at the expense of the *c,c,t* and *t,c,c* isomers. However, there is no simple relationship linking the disappearance of each of the mono-*trans* isomers and the formation of the di-*trans* isomer.

KEY WORDS: Deodorization, geometrical isomers, heated oil, linolenic acid, linseed oil, *trans* fatty acids.

Linolenic acid geometrical isomers (LAGIs) have been detected in edible soybean and rapeseed oils marketed in North America (1), France (2), some other European countries (Belgium, Germany, Great Britain) (3), Poland (2) and Israel (4). They may also occur in foods containing such oils (2,5) and in refined walnut oils (6). Although LAGIs can be generated in soybean and rapeseed oils that are overheated for prolonged periods in the laboratory (7) or in restaurants (8), it is now evident that the main dietary source resides in the "fresh" commercial oils themselves.

The appearance of LAGIs in oils is linked to the deodorization step (1,9). This operation is generally conducted at high temperatures (230 to 250°C) (10,11), under vacuum and in the presence of steam, for periods ranging from a few minutes to several hours. The resulting LAGIs have the structure *cis*-9,*cis*-12,*trans*-15, *trans*-9,*cis*-12,*cis*-15 (85–90% of total LAGIs), *cis*-9,*trans*-12,*cis*-15, *trans*-9, *cis*-12,*trans*-15 (10–15%) and *trans*-9,*trans*-9,*cis*-15 and *cis*-9,*trans*-12,*trans*-15 (trace amounts, generally not detected without preliminary concentration) (1,5).

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In Europe, about 9 samples out of 10 contain geometrical isomers of linolenic acid (3). It is thus difficult to consume pure α-linolenic acid without ingesting its geometrical isomers at the same time. However, little is known about the biochemical and physiological effects of LAGIs. They are readily incorporated in rat tissues, either as such or after elongation and desaturation to longer polyunsaturated fatty acids (12–14), showing peculiar biochemical properties (15). Apparently, LAGIs have not yet been detected in human tissues (4,16,17).

A few experiments have been devoted to the study of the physical conditions that lead to linolenic acid isomerization (1,9). Their conclusions are that isomerization begins between 180 and 200°C and that it increases with temperature and the time of heating. However, no (9) or only partial (1) data were given for individual isomers. It is now possible to study almost all of the individual LAGIs with highly efficient capillary columns coated with cyanoalkyl polysiloxane stationary phases and operated under optimal conditions (2,18,19). Identification of all LAGIs, based on literature and experimental chromatographic data, has been achieved recently (18). In the present study we have used linseed oil as a model with the assumption that linolenic acid will isomerize in this oil as in soybean or rapeseed oils. Its high linolenic acid content facilitates the observation of the isomerization reaction. This oil was heated at different temperatures for different periods in glass ampoules that were sealed under vacuum. The fatty acid composition of the oil was then determined by gas-liquid chromatography (GLC), and each LAGI was quantitated.

EXPERIMENTAL PROCEDURES

Samples and chemicals. Industrial linseed oil (not deodorized, practically devoid of LAGI) was a kind gift from Dr. J.-L. Sebedio (INRA, Dijon, France). Linolenic and linoleic acid geometrical isomers were prepared by elaidination of linseed oil (18) essentially as described by Grandgirard *et al.* (20).

Heating of linseed oil. An aliquot of linseed oil (2.5 mL) was introduced in a ready-to-seal glass ampoule. The ampoule was connected to a water vacuum aspirator with a stopcock in the line. The ampoule was immersed in a water bath at 50°C and left under vacuum for 15 min with occasional shaking. The stopcock was then closed and disconnected together with the ampoule from the aspirator before sealing the ampoule. Each sealed ampoule was heated in an oven at the appropriate temperature and for the desired time of heating (plus 10 min to allow equilibration).

Fatty acid methyl esters (FAME) preparation. A 1.5 mL of a 12% (wt/vol) solution of BF_3 in methanol (Fluka, Buchs, Switzerland) was added to 2 drops of oil, and the resulting suspension was homogenized with benzene (21). The reaction was performed in an oil bath at 100°C for

1 h (21). After cooling the tube, hexane (2.5 mL) and water (1.5 mL) were added successively. FAME were extracted a second time with hexane (2.5 mL). The pooled extracts were stored at −20°C.

GLC. Analyses of FAME by GLC were carried out on a Carlo Erba 4130 chromatograph equipped with a flame ionization detector and a split injector (Carlo Erba, Milano, Italy). A fused-silica capillary column coated with a 100% cyanopropyl polysiloxane stationary phase (CP Sil 88, 50 m × 0.33 mm i.d., 0.25 μm film; Chrompack, Middleburg, Holland) was used with helium as carrier gas at an inlet pressure of either 0.8 or 1.0 kg/cm^2. In the first case, the column was operated isothermally at 150°C for 50 min. The temperature was then increased at a rate of 10°C/min up to 195°C and held at this temperature until completion of the analysis. In the second case, the column was operated isothermally at 175°C for 18 min. The temperature was then increased at a rate of 10°C/min up to 195°C and held at this temperature until completion of the analysis. In both cases, the injection port and the detector were maintained at 250°C. Quantitative analyses were performed with an SP 4290 integrator (Spectra Physics, San Jose, CA). Identification of individual isomers in heated linseed oil was realized (18) with elaidinized linseed oil FAME as reference compounds.

RESULTS AND DISCUSSION

A partial chromatogram of FAME prepared with linseed oil that was heated at 245°C for 16 h is shown in Figure 1. Although LAGIs present in rapeseed and soybean oils are fairly well resolved at low pressure (0.8 kg/cm^2) of the carrier gas and at low temperature (150°C) (18), it was necessary to slightly modify these chromatographic conditions for linseed oil. At the beginning of the reaction, the peak corresponding to the *t,c,c (t = trans, c = cis)* isomer is small and insufficiently resolved from the main *c,c,c* isomer. A better resolution is obtained between these two isomers upon increasing the carrier gas pressure (up to 1 kg/cm^2) and the oven temperature (up to 175°C). However, the *t,c,c* isomer is no more separated from the *c,t,c* isomer, and the peak corresponding to the 20:1 acid (0.21% of total fatty acids) is no longer distinguishable from the trailing edge of the *c,c,c* peak (results not shown). Thus, it was necessary to combine data obtained under both chromatographic conditions to accurately quantitate individual LAGIs. One can note on the chromatogram of Figure 1 the presence of small unidentified peaks that were not present in the fresh oil—these peaks may correspond to cyclic FAME.

The fatty acid compositions of fresh and heated linseed oil are given in Table 1. Summing up saturated fatty acids, monoenes and even dienes indicates that there is a slight but definite relative increase of these fatty acids with heating time and temperature. Any reaction of α-linolenic acid and/or of its *trans* isomers that produces unchromatographiable components [dimers and polymers, unlikely to occur under our experimental conditions (9); oxidation products, limited by removal of most oxygen from the ampoule; and volatile products] will lead to an apparent relative increase of those fatty acids that do not participate in these reactions (mainly saturated fatty acids). However, the observed increases of saturated fatty acids, monoenes and dienes are small and no corrections were

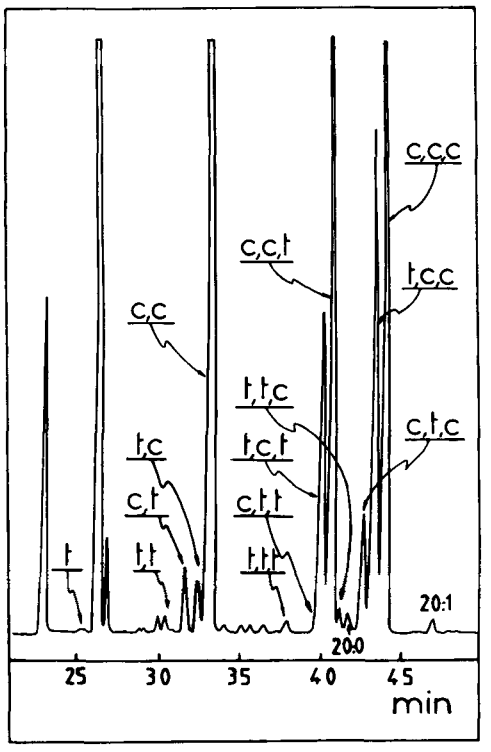

FIG. 1. Partial chromatogram of fatty acid methyl esters (FAME) prepared with linseed oil that was heated under vacuum at 245°C for 16 h. Small unidentified peaks eluting between 34 and 37 min may correspond to cyclic FAME. Analysis on a CP Sil 88 capillary column operated at 150°C with a carrier gas pressure of 0.8 kg/cm^2 (c, *cis;* t, *trans*). Configurations of double bonds are given in the order 9, 12 and 15.

made. Using the sum of saturated fatty acids as an internal standard indicates that the overall loss of chromatographiable components in linseed oil heated at 265°C for 4 h is only 3.9%, of which 3.4% is attributable to linolenic acid-derived unchromatographiable components. Also, fatty acids included in the "Others" section in Table 1 increase with both temperature and heating time. Most of these unknown components are eluted off the column very late. Perhaps these components correspond to conjugated trienes. However, no further analytical work was carried out. Uncorrected values are also necessary to compare data of this study with those previously published for commercial edible linolenic acid-containing oils (2,3), because their true initial linolenic acid content (before deodorization) was not known. As illustrated by Figure 2, the overall time-course evolution of linolenic acid isomers clearly shows that geometrical isomerization is by far the main reaction that occurs during heating; the sum of methylene-interrupted geometrical isomers of linolenic acid decreases only slightly with time. If the late-eluting components (conjugated isomers?) are taken into account together with the preceding isomers, total octadecatrienoic acids remain practically unchanged. Thus, other reactions, such as cyclization, are necessarily low under our conditions.

Heating the oil at 190°C for 2 or 4 h (Table 1) does not lead to appreciable isomerization of linolenic acid (less than 0.5%). Devinat *et al.* (9) have reported that LAGIs represented *ca.* 12 and 2% of total octadecatrienoic acid

TABLE 1

Fatty Acid Composition of Linseed Oil Heated in Sealed Ampoules[a]

		190°C			220°C			240°C	245°C				260°C	
Fatty acids	0 h	2 h	4 h	16 h	4 h	8 h	16 h	2 h	2 h	4 h	8 h	16 h	2.25 h	4 h
Saturates[b]	10.06	10.03	10.16	10.19	10.12	10.18	10.13	10.15	10.14	10.12	10.22	10.41	10.30	10.32
Monoenes	20.54	20.57	20.54	20.62	20.66	20.62	20.65	20.72	20.70	20.86	20.92	21.43	20.94	20.99
t,t-18:2[c]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	trace	0.04	0.12	0.06	0.12
c,t-18:2	0.05	0.05	0.04	0.06	0.07	0.12	0.19	0.07	0.14	0.23	0.42	0.84	0.38	0.70
t,c-18:2	0.00	0.00	0.00	trace[d]	trace	0.06	0.12	0.04	0.09	0.17	0.34	0.79	0.32	0.55
c,c-18:2	23.13	23.14	23.15	23.15	23.20	23.11	22.98	23.25	23.06	22.96	22.67	21.79	22.72	22.30
Dienes	23.18	23.19	23.19	23.21	23.27	23.29	23.29	23.36	23.29	23.36	23.47	23.54	23.48	23.65
t,t,t-18:3	0.00	0.00	0.00	0.00	0.00	0.00	trace	0.00	0.00	0.00	0.06	0.18	trace	0.09
t,c,t-18:3[e]	0.00	0.00	0.00	trace	0.06	0.11	0.41	0.02	0.15	0.54	1.96	6.22	1.16	3.12
c,c,t-18:3	0.15	0.21	0.17	0.73	1.34	2.58	4.80	1.59	2.97	5.13	8.33	10.73	6.76	9.54
t,t,c-18:3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	trace	0.10	0.30	trace	0.08
c,t,c-18:3	0.00	trace	trace	0.05	0.11	0.28	0.55	0.16	0.39	0.71	1.26	2.11	1.17	1.74
t,c,c-18:3	0.00	0.08	0.06	0.51	1.10	2.10	3.74	1.30	2.50	4.22	7.17	9.27	5.83	7.90
c,c,c-18:3	45.70	45.39	45.69	44.17	42.58	39.89	34.96	42.36	38.70	33.51	24.47	12.99	28.24	19.46
Trienes	45.85	45.68	45.92	45.45	45.19	44.96	44.46	45.43	44.71	44.13	43.35	41.80	43.16	41.93
Others	0.37	0.37	0.19	0.87	1.53	2.04	2.82	0.34	1.16	1.53	2.04	2.82	2.12	3.11

[a]Data are given as peak area percentages and are the means of at least two analyses.

[b]Saturates: sum of percentages of 14:0, 16:0, 18:0, 20:0 and 22:0 acids. Monoenes: sum of percentages of 16:1, 18:1n-9, 18:1n-7 and 20:1 acids.

[c]*c, cis; t, trans.* Double bonds are given in the order 9 and 12 for 18:2 acids and in the order 9, 12 and 15 for 18:3 acids.

[d]Trace amounts: peaks visible on the chromatogram but not taken into account by the integrator (<0.04% of total fatty acids).

[e]Includes minor amounts of unresolved *c,t,t*-18:3.

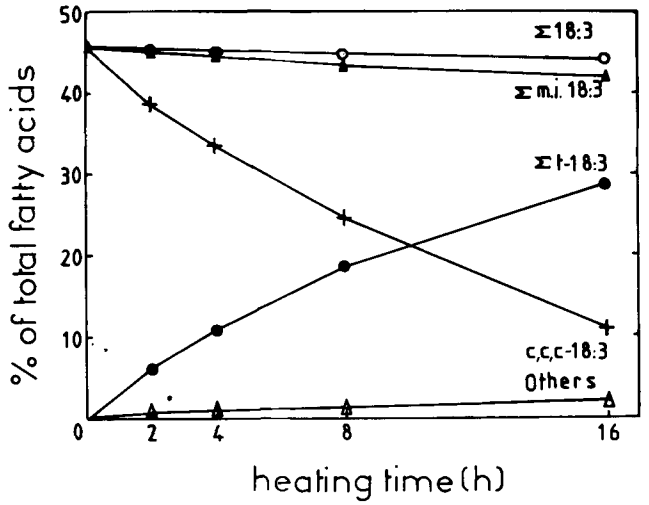

FIG. 2. Time-course evolution of the major classes of linolenic acid geometrical isomers in linseed oil heated at 245°C. "Others" stands for late-eluting components believed to be conjugated octadecatrienoic acids. Total 18:3 includes these components, m.i., methylene-interrupted; c, *cis;* t, *trans.*

in soybean and rapeseed oils, respectively, that were deodorized for 4 h at 200°C. They also reported that a value equal to 12.3% was obtained in soybean oil deodorized at 180°C for 4 h (9). Values given by Grandgirard *et al.* (7) were more homogeneous—7.4 and 7.1% for rapeseed and soybean oils, respectively, after a 10 h period of heating at 200°C. After 16 h at 190°C, the degree of isomerization (DI) of linolenic acid is still low (2.8%) (Table 2). However, if the same rate of isomerization would occur in a rapeseed oil sample initially containing 10% linolenic acid, LAGIs should then represent 0.3% of total fatty acids and should thus be detectable on a chromatogram (2). If LAGIs have to be completely avoided, 190°C appears to be the upper limiting temperature for oil deodorization.

At 220°C, which appears to be the mean temperature value recommended for industrial deodorization of oils in France (9), the DI increases from 5.8% after 4 h to 21% after 16 h (Table 2). As it is unlikely that deodorization periods of 8 h or more are commonly used in the oil industry, it would seem that most soybean and rapeseed oils produced in Europe are deodorized at temperatures higher than 220°C. At 245°C, a temperature considered as optimal in the United States (245–250°C) (8), linolenic acid isomerization is moderately fast. To obtain oils with a DI of 15–30%, it is therefore necessary to heat the oils from *ca.* 2.5 h to about 5 h. Small amounts of the *t,t,c* (and also probably of the *c,t,t*) and of the *t,t,t* isomers appear at that temperature when the oil is heated for periods equal to or longer than 8 h.

Finally, we have also tested a higher temperature (260°C) that is probably not frequently used in industry, except perhaps for the so-called physical refining. However, some elements in deodorizers may undergo overheating, and part of the oil in contact with these parts will also be overheated. As indicated in Table 2, isomerization of linolenic acid is then very fast (more than 30% in only 2 h).

Plotting the logarithm of the remaining fraction of unreacted all-*cis* linolenic acid (percentage of the initial quantity) as a function of heating times and temperatures (Fig. 3) gives straight lines characteristic of a first-order chemical reaction. The rate constants of the isomerization reaction are given in Table 3. Plotting the percentages of

TABLE 2

Relative Percentages of Linolenic Acid Geometrical Isomers and Degrees of Isomerization (DI) of Linolenic Acid in Linseed Oil Heated Under Vacuum in Sealed Ampoules

	190°C	220°C			240°C	245°C				260°C	
Fatty acid	16 h	4 h	8 h	16 h	2 h	2 h	4 h	8 h	16 h	2.25 h	4 h
Total *trans*-18:3	1.29	2.60	5.07	9.50	3.08	6.01	10.62	18.88	28.81	14.92	22.47
All-*cis* 18:3	44.17	42.58	39.89	34.96	42.25	38.70	33.51	24.47	12.99	28.24	19.46
Total m.i. 18:3[a]	45.45	45.19	44.96	44.46	45.33	44.71	44.13	43.35	41.80	43.16	41.93
DI 18:3	2.82	5.78	11.28	21.37	6.79	13.44	24.07	43.55	68.92	34.57	53.59
t,t,t[b]	—	—	—	trace	—	—	—	0.3	0.6	trace	0.4
t,c,t	trace	1.9	2.2	4.3	1.0	2.5	5.1	10.4	21.6	7.8	13.9
c,c,t	57.0	51.5	50.9	50.5	51.6	49.4	48.4	44.1	37.2	45.3	42.5
t,t,c	—	—	—	—	—	—	trace	0.5	1.0	trace	0.4
c,t,c	3.9	4.2	5.5	5.8	5.2	6.5	6.7	6.7	7.3	7.8	7.7
t,c,c	39.8	42.3	41.4	39.4	42.2	41.6	39.8	38.0	32.2	39.1	35.2

[a]Total methylene-interrupted 18:3n-3 acids.

[b]*c, cis; t, trans;* the configurations of the ethylenic bonds are given in the order 9, 12 and 15.

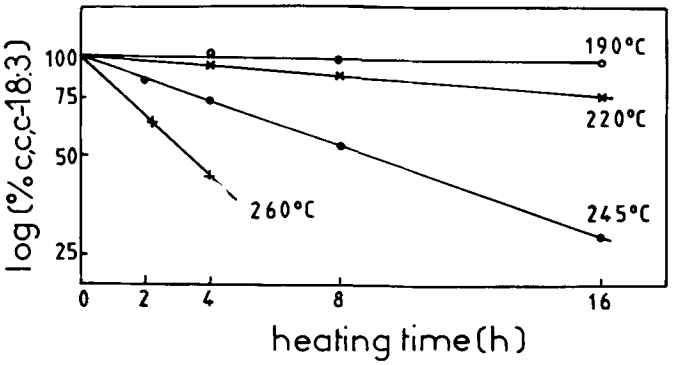

FIG. 3. Logarithm of the quantities of residual all-*cis* linolenic acid (expressed as percentages relative to the initial 18:3 acid content) as a function of temperature and heating time. c, *cis*.

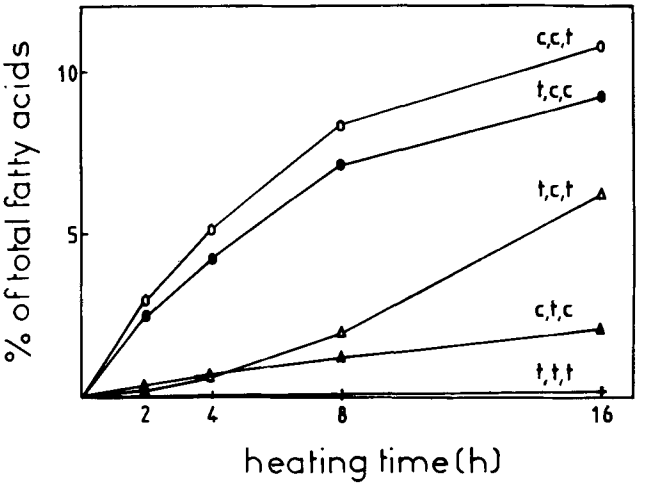

FIG. 4. Time-course evolution of individual linolenic acid geometrical isomers (expressed as percentages of total fatty acid methyl esters) in linseed oil heated at 245°C. Configurations of double bonds are given in the order 9, 12 and 15. c, *cis*; t, *trans*.

TABLE 3

Characteristics of Curves Representative of the Function $\log Q = -kt + \log Q_0$, with Q Standing for the Remaining Quantity of all-*cis* 18:3n-3 Acid as a Percentage of Its Initial Quantity $Q_0 = 100$ (values calculated for curves in Fig. 3)

Temperature (°C)	Slope (k) (h^{-1})	Intercept ($\log Q_0$)	Correlation factor
190	0.00093	2.0007	−0.96
220	0.00724	1.9992	−1.00
245	0.03504	1.9990	−1.00
260	0.09270	1.9999	−1.00

individual geometrical isomers as a function of time (Fig. 4) clearly shows that the appearance of the di-*trans* *t,c,t* isomer follows a two-step reaction. The di-*trans* isomer seems to be issued from both the *c,c,t* and *t,c,c* isomers.

If one expresses the relative percentages of individual isomers as a function of linolenic acid DIs, straight lines with good correlation factors can be constructed (Fig. 5). Such straight lines were previously obtained with data relative to commercial samples of soybean, rapeseed and walnut oils (3). However, curves for these oils were limited to DI values of 30% instead of about 70% in the present study. As the DI increases, the relative proportions of the *c,c,t* and *t,c,c* isomers decrease linearly, the former at a relatively higher rate than the latter. Adjusted values for intercepts with the axis of ordinates are similar to those previously calculated for commercial oils from European countries (2): 53.3 *vs.* 52.7 for the *c,c,t* isomer, 41.9 *vs.* 43.0 for the *t,c,c* isomer and 4.7 *vs.* 4.9 for the *c,t,c* isomer. These values correspond to the relative probabilities of isomerization of each individual double bond. As they are identical in linseed oil and in other commercial linolenic acid-containing oils, this means that this characteristic does not depend on the initial linolenic acid content. This would in turn imply that the isomerization of the ethylenic bonds in linolenic acid is an intramolecular reaction rather than an intermolecular reaction. The similarities between linseed oil on the one hand, and commercial rapeseed, soybean and walnut oils on the other hand, clearly show that

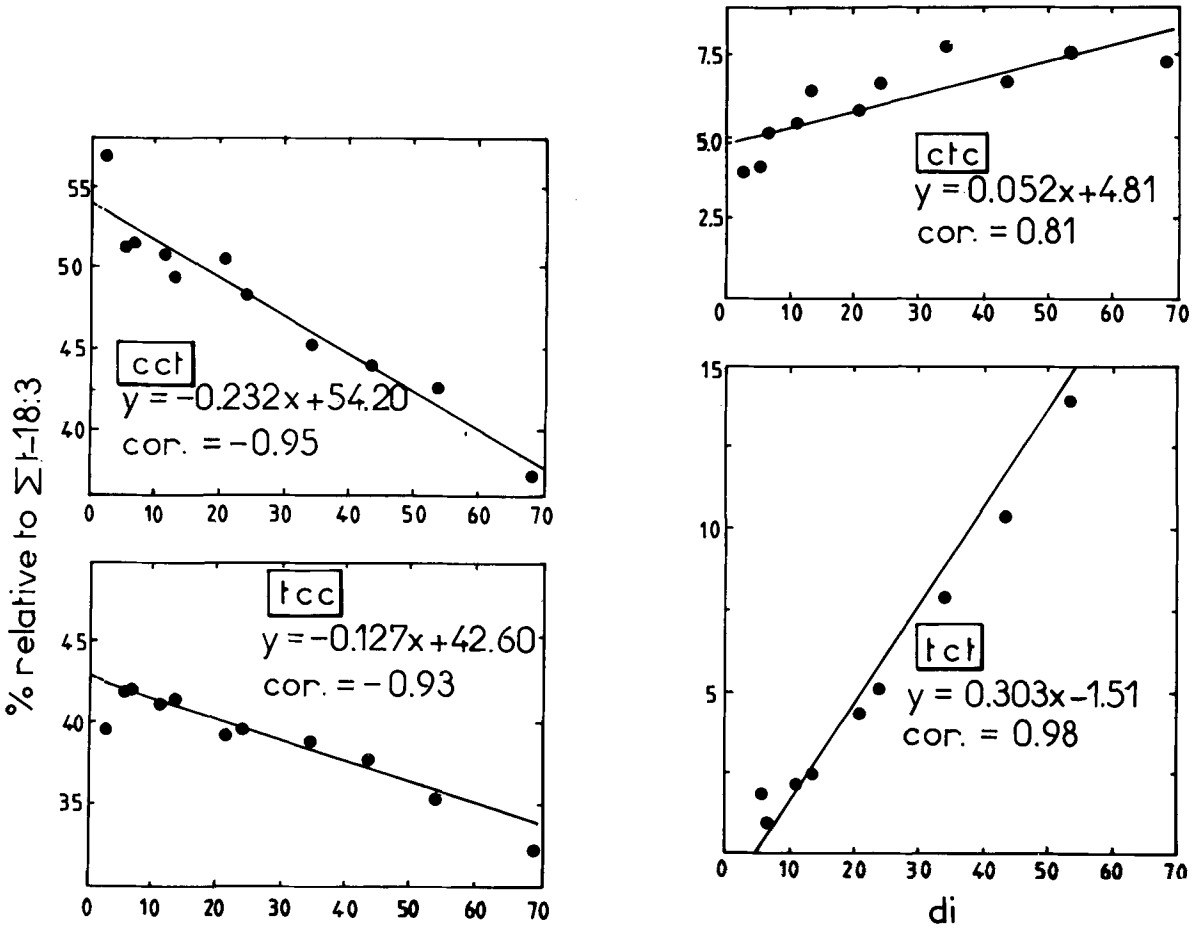

FIG. 5. Plots of the percentages of individual linolenic acid geometrical isomers relative to their sum as a function of linolenic acid degrees of isomerization. From top to bottom and from left to right: *cis*-9,*cis*-12,*trans*-15 18:3, *trans*-9,*cis*-12,*cis*-15 18:3, *cis*-9,*trans*-12,*cis*-15 18:3, *trans*-9,*cis*-12,*trans*-15 18:3 acids. Cor., correlation factor; c, *cis*; t, *trans*.

TABLE 4

Characteristics of Curves in Figure 6

Temperature (°C) (number of samples)	Isomer[a]	Slope	Intercept	Correlation factor
220 (n = 3)	*c,c,t*	−0.079	51.7	−0.95
	t,c,c	−0.242	43.3	−1
	c,t,c	0.120	4.1	0.86
	t,c,t	0.209	0.9	0.98
245 (n = 4)	*c,c,t*	−0.894	51.5	−1
	t,c,c	−0.656	42.8	−1
	c,t,c	0.054	6.4	0.96
	t,c,t	1.367	−0.35	1
260 (n = 2)	*c,c,t*	−1.600	48.9	—
	t,c,c	−2.229	44.1	—
	c,t,c	−0.057	7.9	—
	t,c,t	3.486	−0.05	—

[a]*c, cis; t, trans.*

heating the oils under vacuum is a good laboratory model for deodorization-induced linolenic acid geometrical isomerization. As a corollary, isomerization of linolenic acid during deodorization mainly, if not only, depends on heating.

Plotting the percentages of individual LAGIs (relative to their total) as a function of heating time gives straight lines for all isomers at each temperature (Fig. 6 and Table 4). The relative percentages of the *c,c,t* and *t,c,c* isomers decrease with increasing heating times while the *t,c,t* isomer increases. However, we could not find a simple correlation for the conversion of the mono-*trans* isomers into the di-*trans* one (Table 4). We do not know from these curves whether one of the two isomers is preferentially isomerized more than the other one or not. The relative percentage of the *c,t,c* isomer is only slightly affected by the heating time. However, its probability of isomerization is practically doubled (7.9 *vs.* 4.1%) between 220 and 260°C (Fig. 6 and Table 4).

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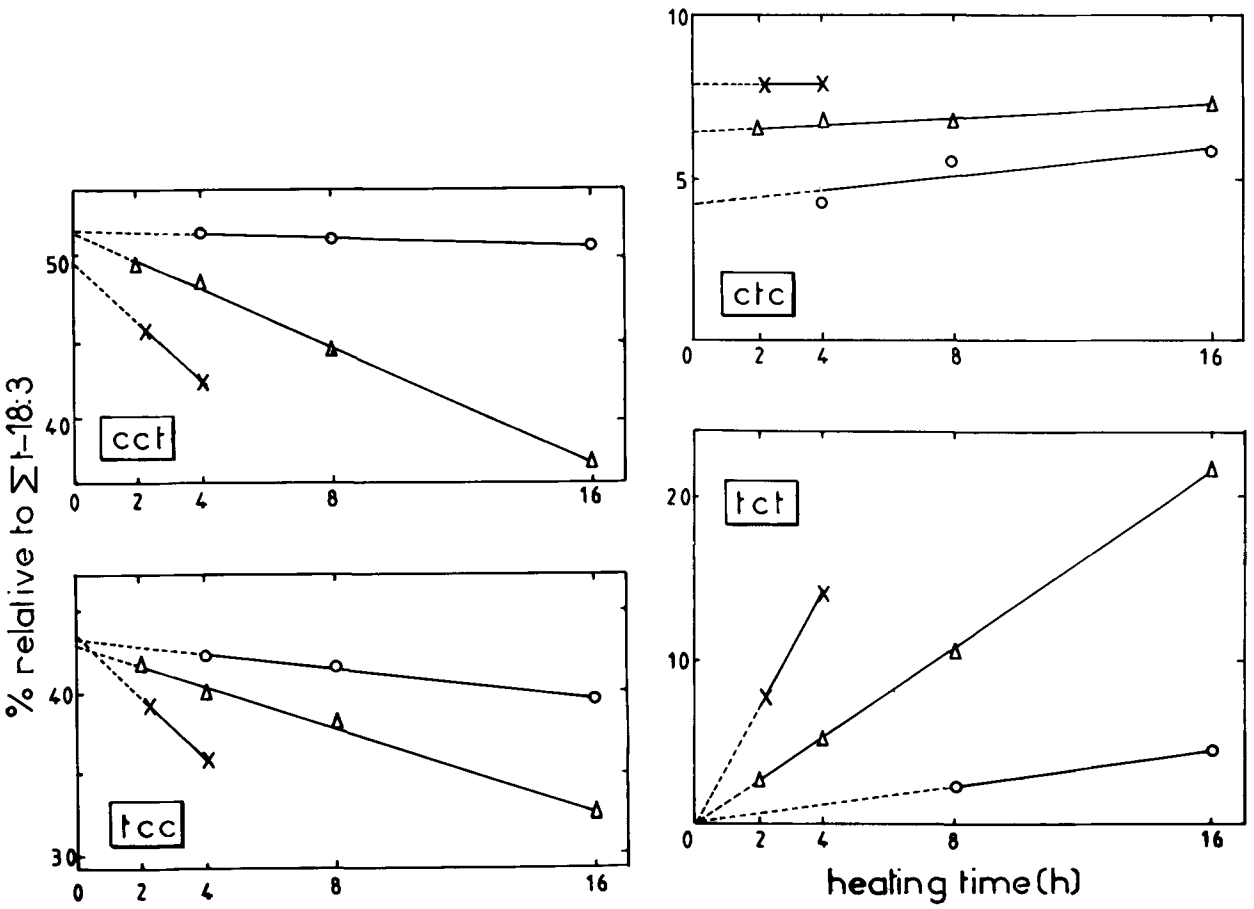

FIG. 6. Time-course evolution of individual linolenic acid geometrical isomers (expressed as percentages of their total) in linseed oil heated at different temperatures: ○, 220°C; △, 245°C; X, 260°C. From top to bottom and left to right: *cis*-9,*cis*-12,*trans*-15 18:3, *trans*-9,*cis*-12,*cis*-15 18:3, *cis*-9,*trans*-12,*cis*-15 18:3, *trans*-9,*cis*-12,*trans*-15 18:3 acids. c, *cis*; t, *trans*.

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