Influence of Heat and Refining on Formation of CLA Isomers in Sunflower Oil

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ABSTRACT: The aims of this study were to determine whether CLA are formed during refining of vegetable oils and to study the level and composition of CLA during heating. The effects of three refining steps (neutralization, bleaching, and deodorization) were analyzed with respect to their effect on CLA content. Two temperatures (180 and 220°C) were used for heating; CLA appeared only after deodorization. The level of CLA was positively influenced by temperature. More CLA were present after treatment at 220°C than at 180°C (1.3 and 0.2% of total FA, respectively). The high temperature modified the relative proportions of the CLA isomers. The main CLA isomers in fresh or heated oils were the *trans,trans* ones (mainly 9,11 and 10,12 isomers).

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KEY WORDS: CLA, deodorization, heat treatment, isomers, polar compounds, refining, sunflower oil.

CLA is a generic term to describe isomers of linoleic acid (9*cis*,12*cis*-18:2), which contains two conjugated double bonds in positions Δ7 to Δ15 and can be in *cis*,*cis*, *cis*,*trans*, *trans*,*cis,* or *trans*,*trans* configurations. Multiple biological effects attributed to CLA isomers were recently reviewed by Pariza *et al.* (1). CLA are naturally present in dairy products, but they also are produced by chemical isomerizations. In dairy products, 9*c,*11*t*-18:2 represents about 80–85% of the CLA isomers (2). Commercial CLA mixtures obtained by chemical processes contain mainly 9*c,*11*t*-, 10*t,*12*c*-, 8*t,*10*c*-, and 11*c,*13*t*-18:2, accompanied by smaller quantities of 8-10, 9-11, 10-12, and 11-13 isomers in *cis*,*cis* and *trans*,*trans* configurations (3). Sébédio *et al.* (4) detected some CLA in sunflower oil heated under harsh conditions. CLA are also present in margarine and in partially hydrogenated vegetable oils (1) as a result of catalytic hydrogenation. More recently, the levels and structures of CLA isomers in vegetable oils collected from restaurants have been described (5). But no information indicates whether CLA are naturally present in crude oil and what the influence is of heating on the structures and the levels of CLA isomers.

The aims of this study were to determine whether CLA are present in crude sunflower oil and whether any step of the refining process (neutralization, bleaching, and deodorization) induces formation of CLA isomers, and to understand the effects of heating on the level and the composition of CLA.

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MATERIAL AND METHODS

Chemicals. All solvents were distilled before use. For HPLC, acetonitrile was of UV grade (SDS, Peypin, France). The chemical reagents were obtained from Sigma (L'Isle d'Abeau, France).

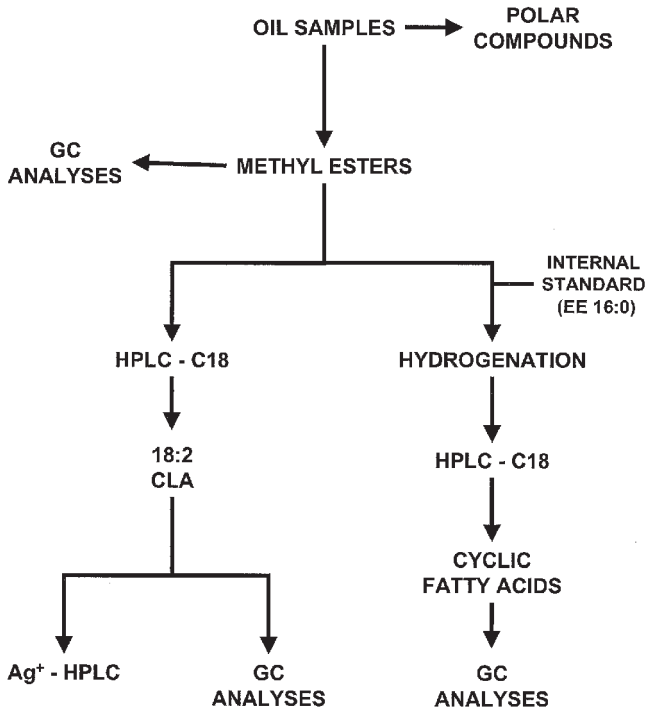
Samples and heating conditions. Crude and processed sunflower oil samples (neutralized, bleached, and deodorized) were obtained from a European manufacturer. Each sample was collected during the industrial processing of a single batch.

Sunflower oil also was purchased in a local supermarket. Sunflower oil (1 L) was heated while exposed to air in a glass flask with a temperature control system. Two temperatures were used: 180°C, which corresponds to what is usually recommended for frying foods (6), and 220°C, which corresponds to a temperature giving rise to isomerization products (7). Ten heating cycles were carried out. The temperature was increased from ambient to 180 or 220°C. This final temperature was maintained for 30 min, then the heating was stopped. While cooling, an oil sample (10 mL) was collected at 40°C. Each heating cycle was carried out for 30 min after the heated oil had reached ambient temperature. The general procedures for oil analyses are shown in Scheme 1.

Polar compounds. The amount of polar compounds was determined by HPLC on two ChromSpher Si (100 × 3 mm i.d., 5 µm, Varian, les Ulis, France) and an ELSD IIA (Varex, France). Calibration was performed with standard solutions prepared with heated rapeseed oils on which the polar compounds were carefully determined using the ISO 8420 method (7).

FA analysis. All the samples were converted to methyl esters using sodium methoxide (0.5 N) (8). The FAME were analyzed by GC using a Hewlett-Packard HP5890 series II chromatograph (Palo Alto, CA) fitted with a split-splitless injector (250°C) and an FID (280°C), coupled with a Borwin integrating system (JMBS, Grenoble, France). Two different capillary columns were used: a CP-Sil 88 (100 m × 0.25 mm i.d., 0.2 µm film thickness; Varian SA, Les Ulis, France) and a BPX70 (120 m × 0.25 mm i.d., 0.25 µm film thickness; SGE, Melbourne, Australia). The oven temperature was programmed from 60 to 170°C at 20°C/min, and the final temperature was maintained for 50 min. Hydrogen was used as carrier gas (0.7 mL/min at 60°C).

CLA analyses. To complete the characterization of CLA, the total FAME were fractionated by RP-HPLC (8) using a Nucleosil-C18 column and a refractometer detector as previously described (8). The isolated CLA fraction was analyzed by GC as described above and by silver-ion HPLC.



SCHEME 1

For silver-ion HPLC, two ChromSpher 5 Lipids columns (250 × 4.6 mm i.d., 5 µm; Varian) were used in series (8). The mobile phase was a mixture of hexane/acetonitrile (99.9:0.1, vol/vol) of 1 mL/min flow rate. A diode array detector (Model MD 1510; JASCO, Nantes, France) was used, and UV spectroscopy detection from 190 to 300 nm was applied to detect the conjugated FA (234 nm) and the nonconjugated FA (200 nm). The quantitative analyses were performed using a JASCO-PDA integrating system (JMBS).

CLA were also converted into 4-methyl-1,2,4-triazoline-3,5-dione (MTAD) adducts, as previously described (8), for GC–MS analysis, as already reported (9)

Cyclic FA monomer analyses. The cyclic FA monomers were quantified according to the procedure described by Sébédio *et al.* (10). The methyl esters were weighed and a 16:0 ethyl ester (EE) was added as internal standard. The samples were hydrogenated using PtO_2 as catalyst and fractionated using RP-HPLC. The internal standard and the cyclic FA monomers were collected and analyzed by GC as previously described.

RESULTS AND DISCUSSION

FA and CLA profile modification during refining. The FA composition of the sample collected after each refining step is given in Table 1. Refining carried out under industrial conditions did not change the levels of the saturates, monoenes, and PUFA of sunflower oil. After deodorization, the nonconjugated *trans* isomers of linoleic acid (9*cis*,12*trans*-18:2 and 9*trans*,12*cis*-18:2) increased slightly from 0.1 to 0.3% and 0.0 to 0.2% of total FAME, respectively. Some CLA also formed at a level of 0.1% of total FAME.

TABLE 1
FA Composition (wt% of total FA) of Crude Sunflower Oil and the Same Oil After Different Refining Steps (*n* = 1)

FA	Crude	Neutralization	Bleaching	Deodorization
14:0	0.1	0.1	0.1	0.1
16:0	6.2	6.1	6.1	6.2
16:1n-9	Trace[a]	Trace	Trace	Trace
16:1n-7	0.1	0.1	0.1	0.1
17:0	0.1	Trace	0.1	Trace
18:0	4.0	4.2	4.2	4.2
18:1n-9	22.5	22.7	22.8	22.1
18:1n-7	0.6	0.6	0.6	0.6
18:2-*c,t*	0.1	0.1	0.1	0.3
18:2-*t,c*	0.0	0.0	0.0	0.2
18:2n-6	65.8	65.5	65.5	65.5
20:0	0.3	0.2	0.2	0.3
18:3n-3	0.3	0.3	0.3	0.3
CLA	ND[b]	ND	ND	0.1

[a]Trace: <0.1%.
[b]ND, not detected under analytical conditions.

This increase of geometrical isomers could be due to the high temperature that may have been used during deodorization (7). However, the absence of di-*trans* isomers (Δ9*t*,12*t*) seems to indicate that a moderate temperature, probably lower than 220°C, was used (7). During the bleaching step, a temperature of 110°C is used, but this is not sufficient to produce isomerization of PUFA.

The CLA composition after deodorization is given in Table 2. The 9*t*,11*t*- and 10*t*,12*t*-18:2 were the major CLA isomers (about 50% of total CLA). *Cis*,*trans*, *trans*,*cis,* and *cis*,*cis* geometrical isomers of 9,11 and 10,12 were present. Some 8,10 and 11,13 isomers were also detected. A high level of *trans-trans* CLA isomers already has been reported in frying oils collected from restaurants (5) and in heated oil samples (11). Taken together, these previous data and the present ones indicate that this CLA composition seems to be characteristic of what can be found in vegetable oils. CLA are not likely to be naturally present in sunflower seed but were formed during the deodorization step in the refining process. The data also indicate a high proportion of *trans*,*trans* isomers (9*t*,11*t* and 10*t*,12*t*).

FA and CLA compositions in heated oils. Table 3 shows the level of polar compounds and FA composition of fresh sunflower oil and of the samples after 10 heating cycles. Polar com-

TABLE 2
Composition (wt% of total CLA) of the CLA Fraction of the Deodorized Sunflower Oil Sample (*n* = 1)

Isomer	wt%	Isomer	wt%
9*c*,11*t*	12.7	8*t*,10*c*	3.9
9*t*,11*c*	6.5	8*c*,10*c*	0.8
9*c*,11*c*	4.6	8*t*,10*t*	1.2
9*t*,11*t*	23.1		
		11*c*,13*t*	3.4
10*c*,12*t*	6.6	11*c*,13*c*	—
10*t*,12*c*	11.8	11*t*,13*t*	0.1
10*c*,12*c*	2.5		
10*t*,12*t*	22.8		

TABLE 3
Polar compounds (%) and FA Composition (% of total FA) in Fresh Sunflower Oil and of Sunflower Oil After 10 Heating Cycles (n = 1)

	Fresh	180°C	220°C
Polar compounds (%)	2.6	31.2	45.2
FA (% of total FA)			
14:0	0.1	0.1	0.1
16:0	6.1	7.0	7.5
16:1n-9	Trace[a]	Trace	Trace
16:1n-7	0.1	0.1	0.1
17:0	Trace	0.1	0.1
18:0	4.3	4.7	5.2
18:1n-9	22.4	24.2	25.6
18:1n-7	0.7	0.8	0.7
18:2-*c,t*	0.3	0.3	1.4
18:2-*t,c*	0.2	0.2	1.3
18:2n-6	65.0	61.3	55.5
18:3n-3	0.3	0.3	0.2
CLA	0.1	0.2	1.3
20:0	0.2	0.2	0.2

[a]Trace: <0.1%.

pounds were detected at the level of 2.6% in the fresh oil, as previously reported by Sébédio *et al.* (12). The quantity of polar compounds increased with the number of heating cycles, from 2.6 to 31.3% at 180°C and from 2.6 to 45.2% at 220°C (Fig. 1). The quantities of polar compounds recovered in the present study were similar to levels observed in used frying oils (13).

Heat treatment changed the FA composition of the original oils. The saturates increased as well as the monoenes. At the same time, the level of linoleic acid was reduced from 65.0 (fresh oil) to 55.5% (oil heated at 220°C). The 9*cis*,12*trans* and 9*trans*,12*cis* isomers of linoleic acid did not change at 180°C, but increased at 220°C as already reported (13).

The percentage of total CLA was also modified by heating. After 10 cycles at 180°C the CLA content was twice as high (0.2% of total lipids) as that in fresh oil (0.1%), and it increased by 13 times at 220°C (1.3% of total lipids).

Figure 2A shows the separation of the CLA methyl esters on a BPX70 column. The resolution between the 8*t*,10*c*/9*c*,11*t* isomers was improved with regard to the CP Sil-88 column separation (8). However, the resolution between 10*c*,12*t*/9*t*,11*c*, 11*c*,13*t*/10*t*,12*c*, and 11*t*,13*t*/other *trans*,*trans* isomers was better with the CP Sil-88 column than with the BPX70 (8). Considering that a complete detailed analysis of the *trans*,*trans* CLA could not be obtained by GC, silver ion-HPLC separation (Fig. 2B) using two ChromSpher Lipids columns was performed as previously reported (5). The structures of the CLA isomers were confirmed using GC–MS on MTDA adducts of the collected peaks as previously described (5). The CLA composition (% of total CLA) is given in Table 4. A similar CLA profile was obtained for the fresh oil and the sample heated at 180°C, only the 9*cis*,11*cis* decreased at 180°C. An important modification in a CLA isomer profile occurred after heating at 220°C. compared to the fresh oil, the 9*t*,11*t*, the 9*t*,11*c*, the 10*t*,12*t,* and the 10*c*,12*t* isomers increased. The 9*c*,11*t* and the 10*t*,12*c* was reduced by about half. At the same time, the levels of 8*t,*10*c* and 11*c*,13*t* increased. The results agree with the data already reported by Destaillats *et al.* (11), who showed that part of the 9*cis*,11*trans* could be isomerized to 8*t*,10*c* at high temperature, and, similarly, part of the 10*t*,12*c* could be changed to 11*c*,13*t*. The respective proportions of the 9,11 and 10,12 isomers changed after the first heating at 220°C and remained stable during the nine heating cycles. For the 9,11- and the

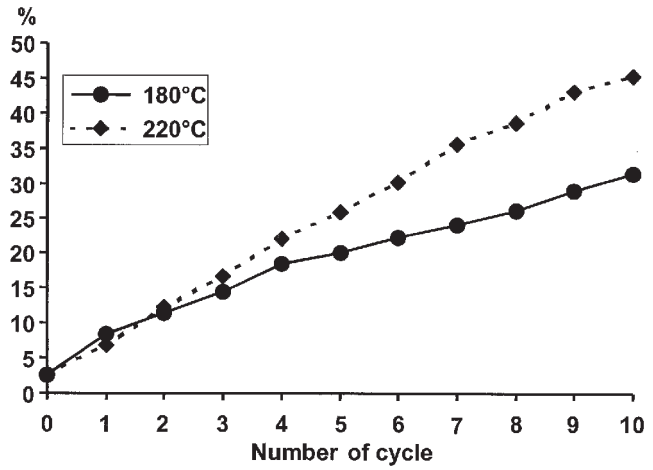


FIG. 1. Evolution of the percentage of polar compounds as a function of the number of the cycle at the two temperatures: ● 180°C, ◆ 220°C.

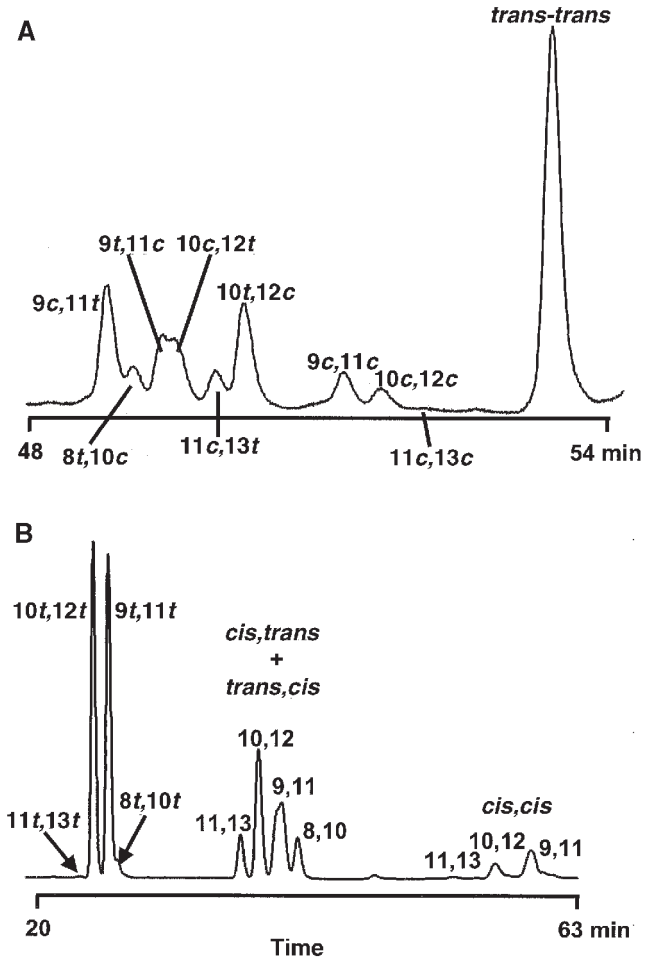


FIG. 2. Chromatograms of the CLA fraction as methyl esters isolated from fresh sunflower oil. (A) GC analyses on a CP Sil-88 column (Varian SA, Les Ulis, France): (B) silver-ion HPLC on two ChromSpher Lipids columns (Varian SA).

TABLE 4
Composition (% of total CLA) of the CLA Fraction in Fresh Sunflower Oil and of Sunflower Oil After 10 Heating Cycles (n = 1)

	Fresh	180°C	220°C
9*c*,11*t*	12.6	14.6	6.7
9*t*,11*c*	6.6	6.4	7.4
9*c*,11*c*	4.5	2.8	2.5
9*t*,11*t*	22.9	24.3	26.7
10*c*,12*t*	6.7	5.8	7.3
10*t*,12*c*	11.6	11.9	6.2
10*c*,12*c*	2.6	1.7	2.2
10*t*,12*t*	22.6	24.2	26.7
8*t*,10*c*	3.8	3.4	5.1
8*c*,10*c*	0.9	0.4	0.8
8*t*,10*t*	1.2	1.5	1.4
11*c*,13*t*	3.5	2.8	5.3
11*c*,13*c*	—	—	0.5
11*t*,13*t*	0.1	0.1	1.0

10,12-18:2, the percentage of the *trans*,*trans* isomer was about 60%, the *cis*,*trans* and *trans*,*cis* isomers represented 15 and 17%, respectively, and the *cis*,*cis* isomers represented only 5% of the total CLA. This proportion could be a characteristic of the equilibrium of the four geometrical isomers at high temperature, as shown with heated linoleate at 200°C and with heated oils (5,11).

Figure 3 shows the evolution of the modified FA (*trans*-18:2, CLA, and cyclic FA) as a function of the percentage of polar compounds. Temperature has been shown to be the critical parameter for the formation of cyclic FA (13) and *trans* isomers of linoleic acids (7). Above 200°C, cyclic FA appeared, and one of the *cis* double bonds of linoleic acid (9*cis*,12*cis*-18:2) changed to *trans*, i.e., 9*cis*,12*trans*- and 9*trans*,12*cis*-18:2 (*trans*-18:2 in Fig. 3). As shown in Figure 3, the modified FA, formed at 220°C, increased linearly until 30% of the polar compounds as the cyclic FA. After six heating cycles at 220°C, the level of the 18:2 isomers (*trans*-18:2 and CLA) increased much more, whereas the cyclic FA progressed with the same slope from the start. The level of CLA did not change in comparison to the fresh oil after 10 heat treatments at 180°C.

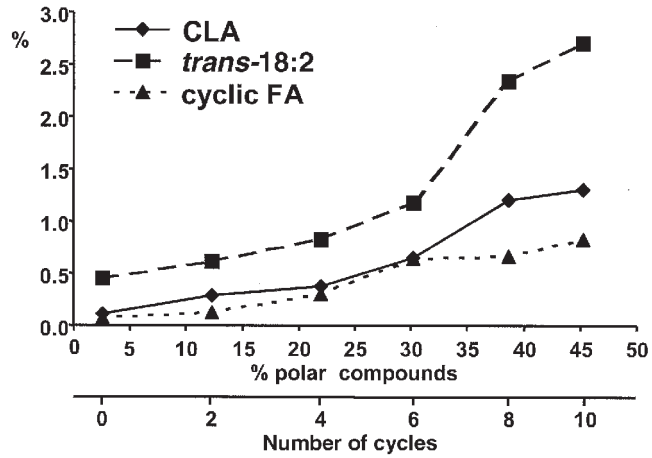


FIG. 3. Plot of the percentage of *trans*-18:2, cyclic FA and CLA (Y) as a function of the percentage of polar compounds (X) and the number of heating cycles at 220°C.

As for the *trans* methylene-interrupted dienes, the quantity of total CLA formed depended greatly on the temperature used for heating. Although the quantity formed at 180°C is low, these conjugated FA represent about 1.3% of the total FA at 220°C. In this case, the major isomers are the 9*t*,11*t* and the 10*t*,12*t*. These minor FA isomers are found either in dairy product isomers (2) or in synthetic mixtures (3).

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