# Linoleic Acid Isomers in Heat Treated Sunflower Oils[1]

**J.L. Sebedio, A. Grandgirard** and **J. Prevost**
I.N.R.A.—Station de Recherches sur la Qualité des Aliments de l'Homme, 17 rue Sully, B.V. 1540, 21034 Dijon Cédex, France

**Heat treatment of sunflower oil resulted in the formation of linoleic geometrical and positional isomers. These isomers were isolated using a combination of column chromatography, urea fractionation, high performance liquid chromatography (HPLC) on a C18 reverse phase column and silver nitrate thin layer chromatography (TLC). Each component was submitted to hydrazine reduction and the resulting monoenes to $AgNO_3$-TLC. The resulting *cis* and *trans* fractions were submitted to ozonolysis in $BF_3$-MeOH in order to determine the position of the ethylenic bonds. The major isomers were the *cis, trans* and *trans, cis* 18:2 Δ9, 12, the *trans, trans* 18:2 Δ9, 12 and some *cis, trans, trans, cis* and *trans, trans* 18:2 conjugated dienes. The *cis, trans* and *trans, cis* conjugated dienes were the Δ9,11, Δ10,12, Δ11,13 and Δ12,14 while the *trans, trans* isomers were the Δ9,11, Δ10,12 and Δ11,13. These C18:2 isomers also were detected in oils collected from restaurants and market vendors.**

Geometrical fatty acid isomers of unsaturated fatty acids have been found mainly in food products such as margarine and shortening (1-4) as a result of partial hydrogenation (5). Geometrical isomers of linolenic acid (18:3 Δ9, 12, 15) were also found as a result of the deodorization process (6). Three major isomers (18:3 Δ9*c*, 12*c*, 15*t*, 18:3 Δ9*t*, 12*c*, 15*c* and 18:3 Δ9*t*, 12*c*, 15*t*) recently were identified in oils heated in the laboratory under severe conditions (7) as well as in samples collected from commercial frying operations (8,9). However, only few data were available on the isomerization of linoleic acid during heat or frying treatment (6,10,11). For example, the 18:2 Δ9*t*, 12*c* was identified as a result of the deodorization process. Furthermore, the gas liquid chromatographic (GLC) analysis of the heated rapeseed oil used by Grandgirard et al. (7) to identify the 18:3 geometrical isomers revealed two unknown components having retention times on Carbowax-20M close to that of 18:2 ω6 and several other components having retention times close to 20:0 and 20:1. The unknown components were also observed in oils containing a large amount of 18:2 ω6 (~65%, sunflower), heated in the laboratory. Considering the GLC retention data, these components could be anticipated to be cyclic fatty acid monomers which are known to be formed during the heat treatment (12-16) or, most probably, positional and/or geometrical isomers of linoleic acid (17).

The purpose of this study was to characterize these unknown components. These were identified in a heated sunflower oil as being a mixture of 18:2 Δ9*t*, 12*t*, 18:2 Δ9*c*, 12*t*, 18:2 Δ9*t*, 12*c*, and some *cis, trans* and *trans, trans* conjugated 18:2 isomers.

## MATERIALS AND METHODS

*Purification of solvents.* All the solvents were redistilled before used.

*Heating conditions.* A refined sunflower oil was purchased from Lesieur Cotelle (France). This oil was heated at 275 C for 12 hr under nitrogen in a 1-l round-bottomed flask. It was also heated in a commercial fryer (Calor 08) at 200 C for 48 hr using a 2-hr daily cycle. The volume of the oil in the aluminum-coated tank was 3.0 l.

*Gas liquid chromatography (GLC).* All the GLC analyses were carried out on a Becker-Packard 420 chromatograph fitted with a flame ionization detector (FID). The analyses were effected on capillary columns coated with Silar-10C (50 m long and 0.50 mm i.d.) or CP SIL-88 (50 m long and 0.32 mm i.d.). All quantitative analyses were effected using a Vista CDS 401 (Varian).

*High performance liquid chromatography [HPLC].* The semi-preparative HPLC was carried out on a reverse phase column (Merck, Lichrosorb, 7 μ, 7 mm i.d., 25 cm). The sample (~40 mg) was dissolved in acetone. Pure methanol was used at 4 ml/min.

*Preparation of fatty acid methyl esters.* Oil samples were saponified with KOH, and unsaponifiables were removed by AOCS procedure Ca-6a-40. The recovered fatty acids were converted to methyl esters by refluxing for 5 hr with 4 volumes of 1% $H_2SO_4$ in MeOH (18). The esters were recovered with hexane and washed free of acids.

*Fractionation of methyl esters by column chromatography.* The method used by Perrin et al. (19) was slightly modified as follows: Silicic acid (70-200 mesh) was dehydrated at 160 C for 6 hr and further rehydrated at 5%. The column used was 55 cm long and 4 cm i.d. and 40 g of methyl esters were fractionated at once (20). The nonpolar fraction was eluted with 1 l of a mixture of petroleum ether: diethyl ether (95:5).

*Urea fractionation of fatty acid methyl esters.* A saturated solution of urea in methanol was heated until complete dissolution of urea, and the methyl esters were added (~140 g). The solution was cooled under nitrogen and remained at 4 C overnight. The adducts (crystals) were separated from the nonadduct fraction by filtration. Two l of $H_2O$ and 30 ml of HCl (6N) were added to the crystals, and the fatty acid methyl esters were extracted with petroleum ether.

*Silver nitrate thin layer chromatography (TLC).* Fatty acid geometrical isomers were fractionated by $AgNO_3$-TLC using Merck's plates (5721, 0.25 mm thickness) previously dipped in a 10% solution of $AgNO_3$ in acetonitrile as described elsewhere (18).

*Hydrazine reduction.* The hydrazine reduction of the C18 diunsaturated fatty acids followed the procedure published for the more highly unsaturated fatty acids (21).

*Ozonolysis in $BF_3$-MeOH.* The ozonolysis in $BF_3$-MeOH followed the method described by Ackman et al. (22), slightly modified for the Supelco micro-ozonizer (7). The analyses of the resulting mono- and diesters were carried out on a Silar-10C column.

## RESULTS AND DISCUSSION

Heat treatment of a refined sunflower oil resulted in a

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slight decrease in oleic acid, a large decrease in linoleic acid and the formation of unknown components (1, 4 and 5, Fig. 1 and Table 1). Components 2 and 3 already had been detected in the refined oil (Fig. 1). Considering their retention times, these could be anticipated to be geometrical isomers of 18:2 Δ9*c*, 12*c*, probably formed during the deodorization process (6, 11). If we consider that the saturated fatty acid could be only slightly modified under these heating conditions (23, 24), it is obvious that the quantities of components 2 and 3 increased during the heat treatment. The ratio 18:0/2+3 decreased from 13.88 in the fresh oil to 3.85 in the sample heated at 200 C for 48 hr.

All these unknown components were isolated using the method described in Figure 2. This method was

**TABLE 1**

**Evolution of the Quantities of Some C16 and C18 Fatty Acids in Sunflower Oil After Heat Treatment**

| Peak area ratios (Fig. 1) | Sunflower oil | Sunflower oil 200 C/48 hr |
|---|---|---|
| 16:0/18:0 | 1.33 | 1.33 |
| 18:1*c*/18:0 | 4.04 | 3.66 |
| 18:2*cc*/18:0 | 14.32 | 8.14 |
| 18:0/1 | — | 13.89 |
| 18:0/2+3 | 13.88 | 3.85 |
| 18:0/4 | — | 45.36 |
| 18:0/5 | — | 13.51 |

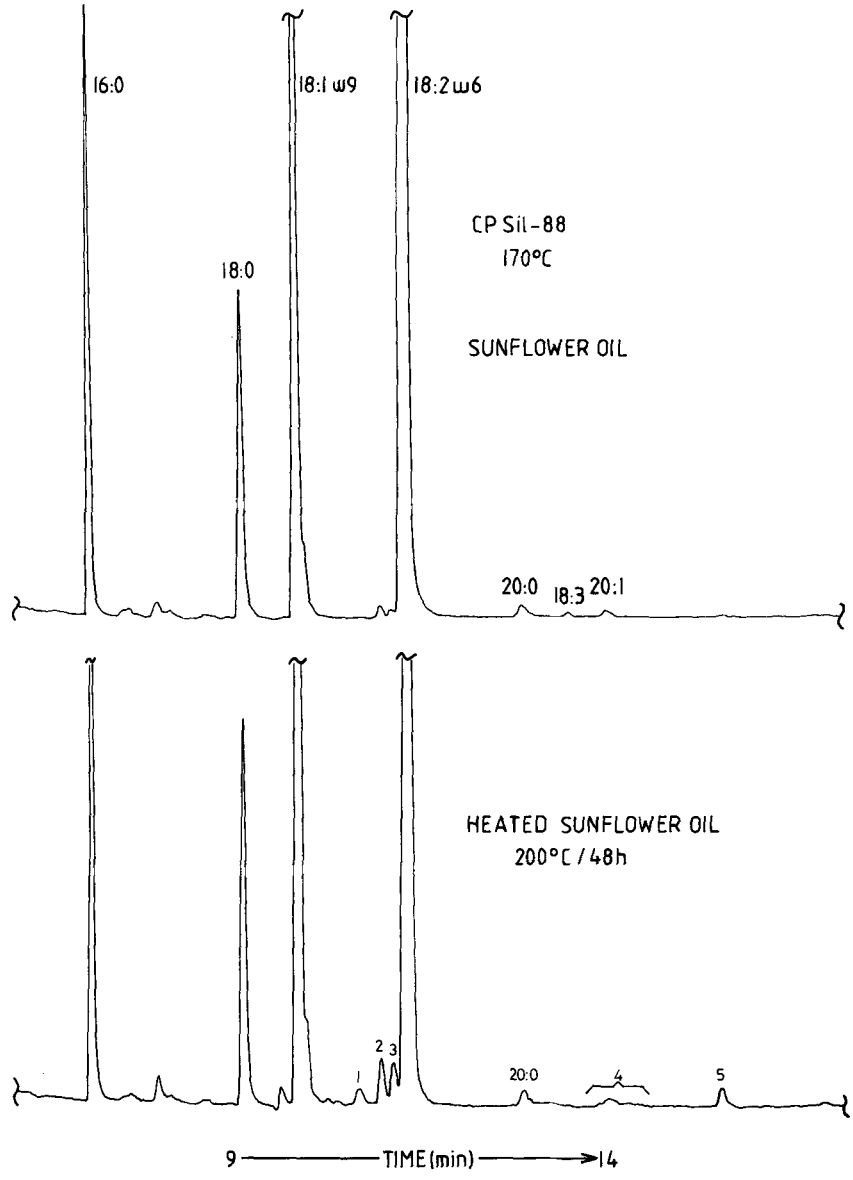


**FIG. 1. Gas liquid chromatographic analyses on a glass capillary column coated with Silar-10C (50 m long and 0.50 mm i.d.) of a refined sunflower oil and a sunflower oil heated at 200 C for 48 hr.**

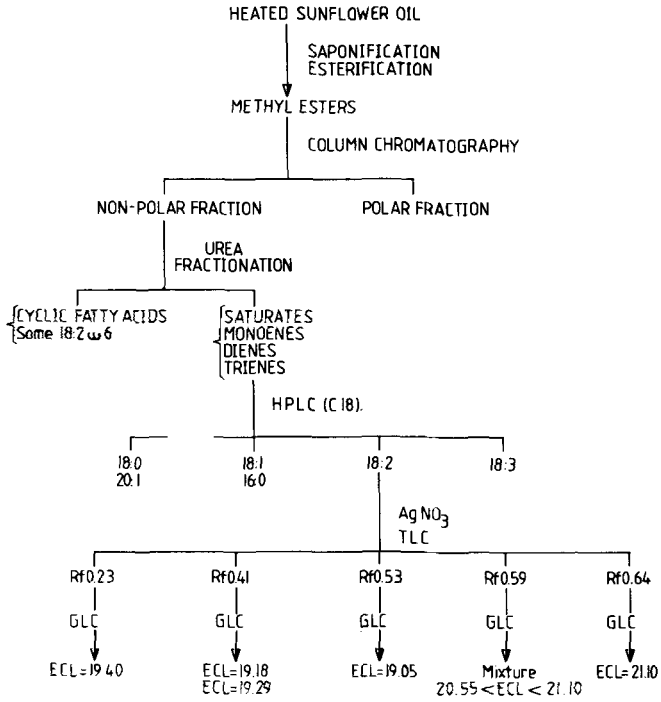


**FIG. 2. Flow chart for the isolation of the 18:2 positional and geometrical isomers in a heated sunflower oil.**

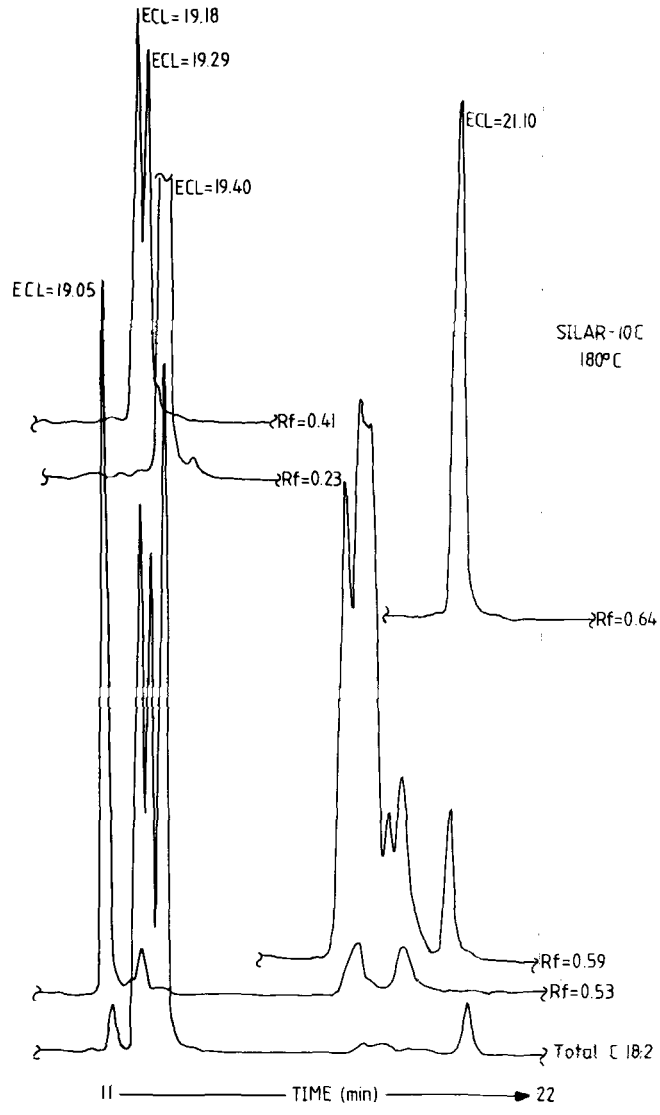


**FIG. 3. Gas liquid chromatographic analyses on a glass capillary column coated with Silar-10C (50 m long and 0.5 mm i.d.) of the total isolated C18:2 and the different $AgNO_3$-TLC bands using the procedure described in Fig. 2.**

slightly different from that used to isolate the geometrical isomers of linolenic acid (7). For the heated sunflower oil, it was necessary as a first step to eliminate the cyclic fatty acid monomers (CFAM) formed during the heat treatment because they could interfere in the HPLC fractionation. This was carried out using urea fractionation. However, in order to eliminate all the CFAM from the urea adduct fraction, it was necessary to use a large methyl ester/urea ratio. All the unknown components were found in the diene fraction isolated by HPLC on a C18 reverse phase column (Fig. 2). These were further fractionated by $AgNO_3$-TLC. Five bands were obtained (Figs. 2 and 3). The band of Rf 0.23 contained one component of equivalent chain length (ECL) value of 19.40 corresponding to the position of 18:2 Δ9*c*, 12*c* (Fig. 3). The following band of Rf 0.41 contained two components of ECL 19.18 and 19.29. The band of Rf 0.53 contained one major component of ECL 19.05 and other minor components of larger ECL values. The band of Rf 0.59 was a complex mixture of components with ECL values ranging from 20.55 to 21.10, while the band of Rf 0.64 contained only one peak of ECL value of 21.10.

Each component was then identified using the method outlined in Figure 4. It is very difficult to determine the position of the ethylenic bonds in a diene using ozonolysis in $BF_3$-MeOH, a technique which was developed to study the monoenes (25), because it is necessary to transform the methyl esters into alcohols prior to ozonolysis (26). Each determination was therefore effected on the corresponding monoenes obtained after hydrazine reduction of the isolated C18:2 unknown component. The hydrazine reduction takes place without modification of either the geometry or the position of the ethylenic bond on the carbon chain (27, 28) so that the ethylenic bonds in the monoenes represent those of the parent molecule. The position of the ethylenic bonds was therefore elucidated after examination of the mixture of dimethylesters produced by ozonolysis of each monoene band (*cis* and *trans*) after separation on silver nitrate TLC plate. We have presented in Table 2 all the results for each isolated 18:2 band (Rf 0.23, 0.41, 0.53, 0.59 and 0.64) for the hydrazine reduction, the silver nitrate TLC and the ozonolysis. For example, the hydrazine reduction of the band of Rf 0.23 gave a mixture of four components: 18:0, two monoenes and some unreacted diene. After isolation of the monoenes by HPLC (Fig. 4), these were further submitted to $AgNO_3$-TLC. Only one *cis* band containing two monoenes was observed. The ozonolysis gave two dimethylesters with nine and 12 carbons. The component was therefore the 18:2 Δ9*c*, 12*c*. All the other 18:2 unknown components

**TABLE 2.**

**Components Formed After Hydrazine Reduction, Fractionation by $AgNO_3$-TLC and Ozonolysis of 5 TLC Bands Containing Some C18:2 Unknown Fatty Acids[a]**

| Band (Rf) | Hydrazine reduction | $AgNO_3$-TLC | Ozonolysis |
|---|---|---|---|
| 0.23 | 18:0<br>18:1 (2 isomers)<br>18:2 | 2 *cis* 18:1 | DMC9[b]<br>DMC12 |
| 0.41 | 18:0<br>18:1 (4 isomers)<br>18:2 (2 isomers) | 2 *cis* 18:1 | DMC9<br>DMC12 |
| | | 2 *trans* 18:1 | DMC9<br>DMC12 |
| 0.53 | 18:0<br>18:1 (2 isomers)<br>18:2 | 2 *trans* 18:1 | DMC9<br>DMC12 |
| 0.59 | 18:0<br>18:1 mixture<br>18:2 mixture | *cis* 18:1 mixture | DMC9<br>DMC10<br>DMC11<br>DMC12<br>DMC13<br>DMC14 |
| | | *trans* 18:1 mixture | DMC9<br>DMC10<br>DMC11<br>DMC12<br>DMC13<br>DMC14 |
| 0.64 | 18:0<br>18:1 mixture<br>18:2 (1 peak) | *trans* 18:1 mixture | DMC9<br>DMC10<br>DMC11<br>DMC12<br>DMC13 |

[a]Following the procedure outlined in Fig. 4.
[b]DMC9, Dimethyl ester with 9 carbon atoms (dimethyl nonanoate).

were identified using the same method as well as their position on $AgNO_3$-TLC (Fig. 2) and their GLC retention times (Fig. 1). Considering the results reported in Table 2, the band of Rf 0.41 contained two unknowns which were the 18:2 Δ9*c*, 12*t* and the 18:2 Δ9*t*, 12*c*. These were the peaks 2 and 3 in Figure 1. In order to establish which component corresponded to peak 2 or 3, a portion of the band of Rf 0.41 was further submitted to another $AgNO_3$-TLC and the resulting band cut into two parts to obtain respective enrichment in one of the components in the higher part (Fig. 1, peak 2 of ECL value of 19.18). It was then possible by looking at the proportion of DMC9 and DMC12 from both the *cis* and the *trans* 18:1 for the higher and for the lower part of the $AgNO_3$-TLC band to attribute peak 2 to the 18:2 Δ9*c*, 12*t* and peak 3 to 18:2 Δ9*t*, 12*c*.

The band of Rf 0.53 contained peak 1 (Fig. 1) which was identified as 18:2 Δ9*t*, 12*t* from the ozonolysis fragments. The band of Rf 0.59 contained the components 4. Considering the ozonolysis results as well as their GLC retention times and their Rf values, these components were identified as a mixture of *cis, trans* and *trans, cis* conjugated 18:2 Δ9,11, 18:2 Δ10,12, 18:2 Δ11,13 and 18:2 Δ12, 14. Both the *cis* and *trans* bands gave the same dimethyl esters; this could not be due to a cross contamination, considering the results published by Gunstone et al. (29). Similarly, the band of Rf 0.64 contained a mixture of components (peak 5) which were identified as *trans, trans* conjugated 18:2 Δ9, 11, 18:2 Δ10, 12 and 18:2 Δ11, 13. These three components have the same retention time on polar columns (Silar 10C or CP-SIL 88). This agrees with the results published by Scholfield (30) on Silar 10C where *cis, trans* and *trans, cis* conjugated dienes such as 18:2 Δ9*c*, 11*t* (ECL = 20.72) and 18:2 Δ10*t*, 12*c* (ECL = 20.86) would be separated while the *trans, trans* conjugated ones such as 18:2 Δ 9*t*, 11*t* (ECL = 21.22), 18:2 Δ10*t*, 12*t* (ECL = 21.23) and 18:2 Δ11*t*, 13*t* (ECL = 21.19) would not.

These unknown components are found not only in oils heated in the laboratory under severe conditions but also in oils collected from restaurants and market vendors (oils A and B in Table 3). For example, similarities exist between the used frying oil B (a sunflower oil) and the oil heated in the laboratory at 200 C for

**TABLE 3.**

**Linoleic Acid Isomers (% of chromatographed esters) in Heated Oils**

| Fatty acids | Sunflower oils heated in the laboratory | | Used frying oils | |
|---|---|---|---|---|
| | 200 C (24 × 2 hr) | 275 C 12 hr, $N_2$ | A | B |
| 18:2 Δ9*t*, 12*t* | 0.2 | 1.0 | 0.3 | tr. |
| 18:2 Δ9*c*, 12*t* | 0.4 | 10.5 | 0.7 | 0.6 |
| 18:2 Δ9*t*, 12*c* | 0.3 | 13.1 | 0.5 | 0.5 |
| 18:2 Δ9*c*, 12*c* | 51.0 | 30.9 | 44.1 | 51.0 |
| 18:2 conj (*ct*+*tc*) | 0.1 | 1.3 | 0.2 | 0.1 |
| 18:2 conj (*tt*) | 0.6 | 2.0 | 0.6 | 0.3 |

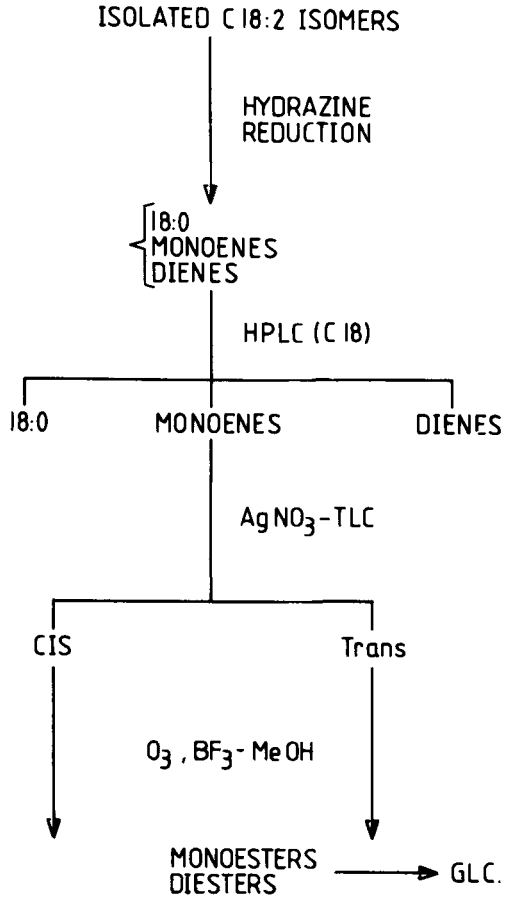


**FIG. 4. Flow chart for the identification of the isolated C18:2 isomers from a heated sunflower oil.**

24 hr using 2-hr daily cycles. For the used frying oil B which contained 39.5% polymeric triglycerides and 54.6% polar components, the 18:2 Δ9*c*, 12*c* still represented 51% of the chromatographed esters while all the 18:2 isomers (*ct, tc, tt* and conjugated) represented about 2.8% of the total C18 dienes. For sample A, these 18:2 isomers (*ct, tc, tt* and conjugated) were about 4.9% of the total C18 dienes. These results indicate that the isomerization of linoleic acid during frying operations is not negligible even if the amount of isomers produced is much smaller compared to that formed from linolenic acid during commercial frying operations (8).

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