# Thiyl Radical-Catalyzed Isomerization of Oils: An Entry to the *trans* Lipid Library

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**ABSTRACT:** The unsaturated fatty acyl moieties of TAG present in natural oils of borage, olive, and rice were converted to their corresponding geometrical *trans* isomers by thiyl radical-catalyzed isomerization. Thiyl radicals were generated from 2-mercaptoethanol under photolytic or thermal conditions. A relevant feature of this method is the absence of double-bond shifts, so that no positional *trans* isomers or conjugated polyenes are formed. Oils obtained after the isomerization were winterized to further increase their *trans* fatty acid content. Methanolysis and hydrolysis of the *trans* oil mixtures using an enzymatic method (lipase B from *Candida antarctica*) gave good conversions to the corresponding *trans* FAME and fatty acids, respectively. These results are relevant for the studies of lipid isomerism and *trans* fatty acid recognition, which is a growing concern in biochemistry and nutrition, and open new perspectives for the synthesis of glycerides and studies of their structure–activity relationships.

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**KEY WORDS:** Geometrical isomerization, lipase, thiyl radicals, *trans* fatty acids, *trans* oils.

During partial hydrogenation of oils, a fraction of the unsaturated fatty acyl (FA) moieties of the oils is converted to *trans* isomers. In the case of edible oils, this conversion is of concern owing to the purported adverse health effects of *trans* FA (1). Partial hydrogenation affords mixtures of positional and geometrical isomers, with shifted and unshifted *trans* double bonds, and their analysis becomes a complicated task, especially in the case of oils with PUFA content (2). For analytical purposes, synthesis of TAG is achieved through the esterification of glycerol with the appropriate acyl chloride. In this manner, pure (*E*)-ethylenic TAG can be prepared, depending on the availability of the *trans* FA derivatives (3,4).

As a result of the early work of Sgoutas and Kummerow (5), thiyl radicals are known to induce *cis/trans* isomerization of unsaturated fatty acids without the double-bond shift. Only recently has the thiyl radical-catalyzed isomerization process been applied to phospholipids in solution and in organized systems such as liposomes, thus allowing assessment of their biological significance (6–8). In this paper we extended the thiyl radical-catalyzed isomerization to natural oils, such as borage, olive, and rice. We anticipated the formation of oils containing geometrical *trans* FA moieties without any contamination by positional isomers or conjugated polyenes. We have also reported the results of enzymatic hydrolysis and methanolysis carried out on *trans* FA-containing oils using immobilized *Candida antarctica* lipase B (9). The transformation proposed represents a convenient access to geometrical *trans* lipids and provides an economical and flexible entry to the *trans* lipid library, which is potentially important for biotechnological (10), industrial (11), and biochemical (12,13) applications.

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## MATERIALS AND METHODS

*Materials.* Borage oil was from Sigma-Aldrich Co., rice oil was purchased from a local grocery store, and olive oil was from Douar Beni Kallad (Tangeri, Morocco). Oils were purified before use on a silica gel column by using *n*-hexane/EtOAc (94:6) as the eluent. 2-Mercaptoethanol, di-*tert*-butyl ketone, *tert*-butanol, 2,2′-azo-bis-isobutyronitrile (AIBN), immobilized *C. antarctica* lipase (Novozyme 435), and reference samples of *cis* and *trans* FAME were purchased from Sigma-Aldrich (Milan, Italy) Analytical TLC was carried out on silica gel 60 $F_{254}$ plates (Merck 5744; Merck, Darmstadt, Germany), and lipids were visualized with cerium ammonium sulfate/ammonium molybdate reagent.

*Isomerization protocols.* Oil isomerization was carried out using one of the following two methods. (i) Thermal method: A solution containing the oil (260 mg; 0.29 mmol) in ethanol (4.7 mL) was placed in a 20-mL vial equipped with an open-top screw cap and a Teflon-faced septum. The solution was degassed by bubbling argon for 20 min. Then 2-mercaptoethanol (27 mg; 0.35 mmol; *ca.* 50 mol% of the total FA content) and AIBN (37.7 mg; 0.23 mmol) were added, and the reaction mixture was stirred for 6 h at 60°C under an argon atmosphere. (ii) Photolytic method: A solution containing the oil (2.60 g; 2.93 mmol) in *tert*-butanol (47 mL) was placed in a quartz photochemical reactor and bubbled with argon for 20 min. 2-Mercaptoethanol (270 mg; 3.5 mmol; *ca*. 50 mol% of the total FA content) and di-*tert*-butyl ketone (133.4 mg; 0.94 mmol) were added, and the solution was irradiated by a 5.5-W low-pressure mercury lamp. The temperature was maintained at 22°C by means of a thermostated bath, composed of $NiSO_4 \cdot 7H_2O$ and $CoSO_4 \cdot 7H_2O$ at pH 1, which allows the UV light (240–350 nm) to pass through.

After evaporation of the solvent under vacuum, the crude reaction mixture was dissolved in $CHCl_3$/MeOH (2:1, vol/vol) and washed with cold 0.1 M NaOH to eliminate the thiol. The organic phase was separated, washed with brine until neutral,

and evaporated to dryness to afford the *trans* oil (250 mg; *ca.* 0.28 mmol; 97% yield by both methods). The *trans* FA content was increased by winterization, which was performed using *n*-hexane as solvent: 2.5 g of *trans* oil was dissolved in solvent (5 mL) and stored for 48–72 h at −20°C. The solvent was decanted and the white solid was collected and analyzed.

*NMR analysis of* trans *oils*. $^1$H and $^{13}$C NMR spectra were recorded on a Varian VXR 400, using $CDCl_3$ as the solvent and reference peaks at 7.26 and 77.0 ppm for $^1$H and $^{13}$C, respectively.

*(i)* Trans *rice oil.* Winterization yield 56%; m.p. 28–29°C; $^1$H NMR ($CDCl_3$, 400 MHz) δ 0.86 (*t, J* = 7 Hz, methyl protons), 1.25 (*m*, methylene protons), 1.60 (*m*, methylene protons in position 3 of acyl moieties), 1.90–2.06 (*m*, allylic protons), 2.26 (*t, J* = 7.2 Hz, methylene protons in position 2 of acyl moieties), 2.62–2.78 (*m*, bisallylic protons), 4.11 (*dd, J* = 11.6, 6 Hz, H1 or H3 in the glycerol moiety), 4.26 (*dd, J* = 11.6, 6 Hz, H1 or H3 in the glycerol moiety), 5.23 (*m*, H2 in the glycerol moiety), 5.28–5.45 (*m*, olefinic protons); $^{13}$C NMR ($CDCl_3$, 100 MHz) δ 14.1 ($CH_3$), 22.8, 23.0 (C17), 25.1, (C3), 29.2–29.9 [$(CH_2)_n$], 31.6 (C16), 32.1, 32.2, 32.8 (C allylic and bisallylic), 34.3, 34.4, 35.8 (C2), 62.2 (C1 and C3 in the glycerol moiety), 68.9 (C2 in the glycerol moiety), 128.3 $C_{LE}12$, 128.4 $C_{LE}10$, 129.9 $C_E9$, 130.2 $C_{LE}9$ and $C_E10$, 130.8 $C_{LE}13$ (olefinic carbons), 172.3 and 172.7 (carbonyl group in the glycerol moiety).

*(ii)* Trans *olive oil.* Winterization yield 72%; m.p. 32–33°C; $^1$H NMR ($CDCl_3$, 400 MHz) δ 0.86 (*t, J* = 6.8 Hz, methyl protons), 1.25 (*m*, methylene protons), 1.58 (*m*, methylene protons in position 3 of acyl moieties), 1.94 (*m*, allylic protons), 2.29 (*td, J* = 7.4, 2.3 Hz, methylene protons in position 2 of acyl moieties), 2.65–2.87 (*m*, bisallylic protons), 4.13 (*dd, J* = 11.7, 6 Hz, H1 or H3 in the glycerol moiety), 4.28 (*dd, J* = 11.7, 4.4 Hz, H1 or H3 in the glycerol moiety), 5.26 (*m*, H2 in the glycerol moiety), 5.32–5.41 (*m*, olefinic protons), $^{13}$C NMR ($CDCl_3$, 100 MHz) δ 14.1 ($CH_3$), 22.7 (C17), 24.8 (C3), 28.9–29.7 [$(CH_2)_n$], 31.9 (C16), 32.5, 32.6 (C allylic), 34.0, 34.2 (C2), 62.0 (C1 and C3 in the glycerol moiety), 68.8 (C2 in the glycerol moiety), 130.1 $C_E9$, 130.4 $C_E10$ (olefinic carbons), 172.9 and 173.3 (carbonyl group in the glycerol moiety).

*(iii)* Trans *borage oil.* Winterization yield 40%; m.p. 25–26°C; $^1$H NMR ($CDCl_3$, 400 MHz) δ 0.82 (*t, J* = 7.2 Hz, methyl protons), 1.22 (*m*, methylene protons), 1.58 (*m*, methylene protons in position 3 of acyl moieties), 1.86–2.03 (*m*, allylic protons), 2.23 (*dd, J* = 7.2, 7.6 Hz, methylene protons in position 2 of acyl moieties), 2.56–2.75 (*m*, bisallylic protons), 4.09 (*dd, J* = 11.6, 6 Hz, H1 or H3 in the glycerol moiety), 4.22 (*dd, J* = 11.6, 6 Hz, H1 or H3 in the glycerol moiety), 5.19 (*m*, H2 in the glycerol moiety), 5.24–5.32 (*m*, olefinic protons); $^{13}$C NMR ($CDCl_3$, 100 MHz) δ 13.9 ($CH_3$), 22.4, 22.5, 22.6 (C17), 24.7 (C3), 28.9–29.6 [$(CH_2)_n$], 31.3 (C16), 31.5, 31.8, 31.9, 32.4, 32.5 (C allylic and bisallylic), 33.8–34.0, 35.5 (C2), 61.9 (C1 and C3 in the glycerol moiety), 68.8 (C2 in the glycerol moiety), 127.5, 127.7, 128.2, 128.3, 128.4, 128.6, 128.9, 129.3, 129.5, 129.7, 129.8, 130.0, 130.2, 130.3, 130.6, 130.8, 131.0 (olefinic carbons), 172.4 and 172.8 (carbonyl group in the glycerol moiety).

*GC and FTIR analyses*. The oils were transesterified (14) to FAME, and GC analysis was performed on a Varian CP-3800 gas chromatograph equipped with an FID and a 90% biscyanopropyl/10% phenylcyanopropyl polysiloxane capillary column (60 m, 0.25 mm i.d., 0.20 μm film thickness; (Rtx-2330; Restek Co., State College, PA) (6,15). The oven temperature program started at 160°C with a hold for 55 min, followed by an increase of 10°C/min to 250°C. This method included a constant pressure mode at 29 psi. *Cis* and *trans* FAME were identified using commercial standards. Aliquots of the reaction mixtures (200 μL) at different times were evaporated under vacuum and analyzed by GC or dissolved in $CCl_4$ and analyzed by FTIR on a Perkin-Elmer BX FTIR system for total *trans* FA content (16). The control experiments in the absence of 2-mercaptoethanol gave quantitative recovery of the starting material without detectable *trans* isomers by GC and FTIR analyses.

*Enzymatic conversion of* trans *oils*. Hydrolysis and methanolysis of *trans* oils were carried out with immobilized *C. antarctica* lipase. The enzymatic reactions were performed in 20-mL screw-capped vessels using a thermostated orbital shaker at 47°C and 150 oscillations per min.

*(i) Methanolysis.* Immobilized *C. antarctica* lipase (4 wt%) was added to 1 g of oil, and three portions of MeOH (1/3 molar equiv) were each added to the mixture successively (9). One cycle of reaction was carried out and, after 24–48 h, depending on the oil, the reaction was stopped by adding $CH_2Cl_2$ and the solution was filtered to separate the enzyme. The organic solution was dried over anhydrous $Na_2SO_4$ and evaporated under reduced pressure, and the residue was separated by flash chromatography (*n*-hexane/EtOAc 96:4) to obtain the mixture of FAME in quantitative yield, based on the starting material recovered by further elution (*n*-hexane/EtOAc 80:20).

*(ii) Hydrolysis.* The oils (1 g) and water (1 mL) were mixed at room temperature, and then immobilized *C. antarctica* lipase (4 wt%) was added. One cycle of reaction was carried out, and after 24–48 h the workup was effected by filtering off the enzyme and by successive washings with $CH_2Cl_2$ and EtOAc. The organic filtrates were collected, dried over anhydrous $Na_2SO_4$, and evaporated under reduced pressure. The residue was transferred to a preparative TLC plate and developed with *n*-hexane/EtOAc/acetic acid (96:4:1) to afford FFA in yields of *ca*. 80%, based on the recovery of the starting material. The fatty acids were transformed to the corresponding FAME by derivatization with $CH_2N_2$ and analyzed by GC.

## RESULTS AND DISCUSSION

The *cis/trans* isomerization was tested on natural oils that have different amounts of *cis* mono- and polyunsaturated fatty acid components. In Table 1 the initial fatty acid compositions of rice, olive, and borage oils are reported together with the final fatty acid composition obtained after thiyl radical-catalyzed isomerization and winterization.

**TABLE 1**
**Fatty Acid Composition of Oils Before and After Isomerization/Winterization Obtained by GC Analysis (% peak area)**

| | Rice | | Olive | | Borage | |
|---|---|---|---|---|---|---|
| Fatty acid | Initial composition | Final composition | Initial composition | Final composition | Initial composition | Final composition |
| 16:0 | 17.0 | 20.6 | 10.5 | 11.5 | 11.8 | 20.0 |
| 18:0 | 1.8 | 4.5 | 2.5 | 3.6 | 3.8 | 6.6 |
| 18:1, *trans*-9 | | 43.0 | | 66.6 | | 20.2 |
| 18:1, *trans*-11 | | | | | | 0.4 |
| 18:1, *cis*-9 | 42.5 | 9.7 | 73.4 | 14.6 | 16.4 | 4.5 |
| 18:1, *cis*-11 | 1.2 | | 1.6 | | 0.6 | |
| 18:2, *trans*-9,12 | | 15.3 | | 3.7 | | 23.3 |
| 18:2, *cis/trans* | | 6.9 | | | | 7.6 |
| 18:2n-6 | 36.8 | | 11.0 | | 39.7 | 1.5 |
| 18:3n-6, *trans* | | | | | | 7 |
| 18:3n-6, *cis/trans* | | | | | | 3.3 |
| 18:3n-6 | 0.7 | | | | 23.6 | |
| 20:1, *trans*-11 | | | | | | 4.8 |
| 20:1, *cis*-11 | | | 1.0 | | 4.1 | 0.8 |

*Radical-based isomerization method*. Radical initiation is obtained either by thermal decomposition of AIBN at 60°C or by photolysis of di-*tert*-butyl ketone at 22°C. The initial carbon-centered radical R˙ reacts instantaneously with the thiol under the experimental conditions to afford thiyl radicals exclusively. The thiyl radical is an effective catalyst for *cis/trans* conversion, which occurs by the addition–elimination mechanism shown in Scheme 1 (6,17). This mechanism has recently been corroborated by a kinetic study (18), and *trans* isomers are expected as the major isomer, both from kinetic and thermodynamic points of view.

*Identification of FAME composition.* Figure 1A shows the GC trace obtained after transesterification of rice oil under basic conditions (14). Figures 1B and 1C show the GC patterns of the transesterification products produced from rice oil that was isomerized (1B) and then winterized (1C). The initial FAME compositions (*cis*) of the three oils are summarized in Table 1. Oils were chromatographed on silica gel prior to isomerization to obtain a TAG fraction free of natural antioxidants that could act as inhibitors of the radical process (19,20). After 30-min photolysis of an alcoholic solution containing oil, thiol, and radical initiator, the GC profiles changed substantially owing to the presence of new peaks assigned to *trans* FA. Figure 1B shows the GC trace of isomerized rice oil. In the absence of thiol, the formation of *trans* isomers was not detected to any extent (data not shown). All reactions underwent a preliminary degassing treatment, which prevented or minimized secondary reactions with molecular oxygen.

*Followup of the isomerization*. Figure 2 shows the isomerization time profile of the oleate (panel A) and linoleate (panel B) residues contained in rice oil. One can clearly see that as oleate disappeared, elaidate formed (open circles, panel A), whereas the two mono-*trans* isomers (open circles, panel B) and linolelaidate (closed squares) replaced linoleate. The two mono-*trans* isomers were formed in equal amounts. Time courses of the reactions were obtained by FAME analyses, withdrawing aliquots of the reaction mixture at successive time intervals. From the reaction profiles we concluded that the percentage of *trans* isomers can be easily planned and may be useful for specific applications.

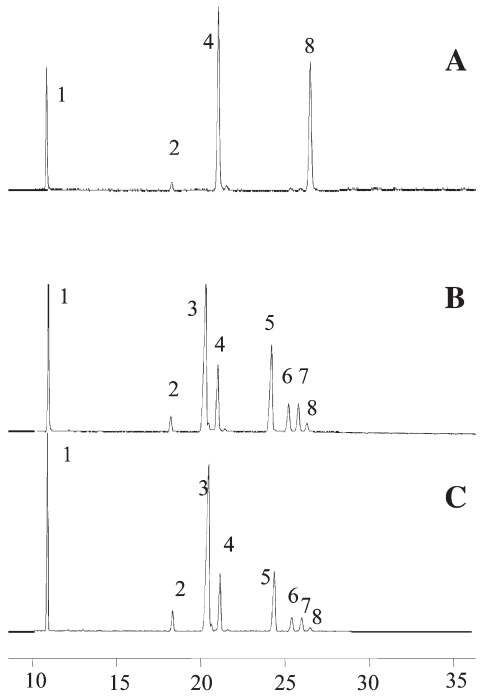
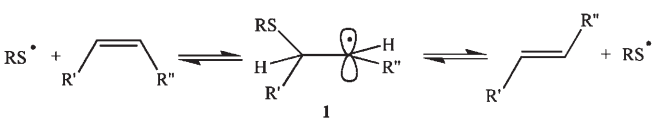



**FIG. 1**. GC traces obtained by using an Rtx-2330 column of (A) the starting fatty acid composition (FAME) of rice oil obtained after transesterification, (B) FAME composition after isomerization (30-min photolysis), and (C) FAME composition after winterization. Peak labels: (1) 16:0, (2) 18:0, (3) 9-*trans*-18:1, (4) 9-*cis*-18:1, (5) 9-*trans*,12-*trans*-18:2, (6) 9-*cis*,12-*trans*-18:2, (7) 9-*trans*,12-*cis*-18:2, (8) 9-*cis*,12-*cis*-18:2.




**SCHEME 1**

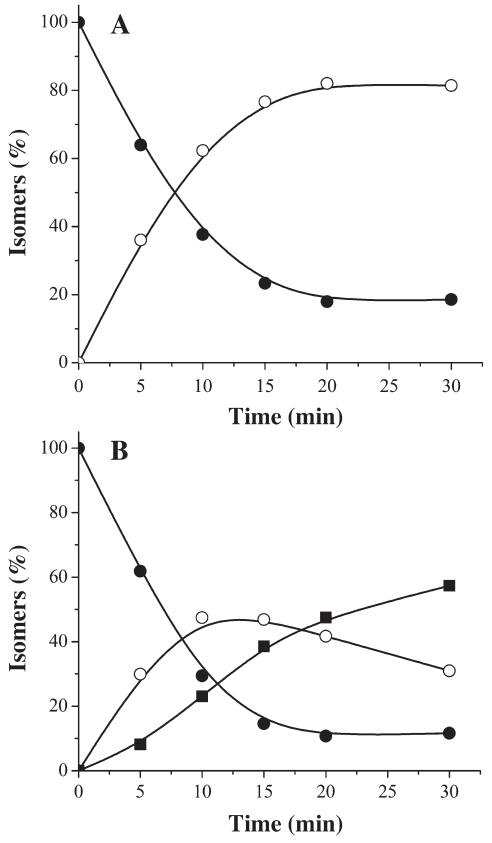



**FIG. 2**. Time profiles for monounsaturated (A) and polyunsaturated (B) fatty acids in the isomerization of rice oil by the photolytic method. (A) (●) 9-*cis*-18:1, (○) 9-*trans*-18:1; (B) (●) 9-*cis*,12-*cis*-18:2, (○) 9-*trans*,12-*trans* and 9-*cis*,12-*trans*-18:2, and (■) 9-*trans*,12-*trans*-18:2.

The total *trans* FA content was determined by FTIR on aliquots of the isomerization mixtures at different reaction times. As an example, Figure 3 illustrates the FTIR monitoring of rice oil isomerization under the thermal protocol and the formation of a new absorbance at 966 $cm^{-1}$, relative to nonconjugated *trans* unsaturations (2,16). Quantitative analyses of total *trans* FA contents by FTIR were compared with GC data and found to be in excellent agreement. It is worth noting that the photolytic and thermal methods were similarly effective, although the efficiency of light-induced isomerization was higher owing to the production of higher radical concentrations.

*Winterization*. Quantitative yields of *trans* FA-containing oils were obtained with individual *cis/trans* ratios depending on stability of the two isomers. At room temperature, an equilibrium ratio of 16:84 was observed for oleate/elaidate isomers (14). The *trans* FA content could be further increased by applying a winterization procedure, that is, by dissolving a certain amount of the oil in a solvent and refrigerating the solution for several days. Winterization was usually carried out in *n*-hexane at –20°C, and the resulting solid TAG were analyzed by GC and NMR spectroscopy. Figure 1C shows the GC trace for FAME from rice oil after isomerization and winterization. The final FAME compositions of the solid fractions

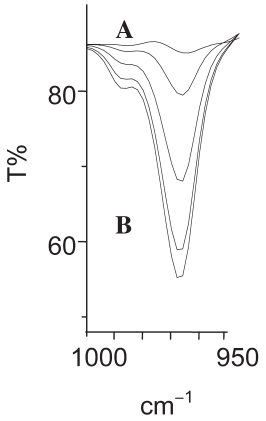



**FIG. 3**. FTIR spectra for rice oil isomerized by the thermal protocol in absolute ethanol at 60°C. (A) All-*cis* and (B) after 200 min of reaction. T, transmission.

for three oils after isomerization and winterization are listed in Table 2. In general, the isomerized/winterized oils had a lower PUFA content compared with the starting oils. This probably was a result of the preference for PUFA-containing TAG species in the isomerized oils to remain in the liquid fraction during the winterization process. Figure 4 is an example of the $^{13}C$ NMR spectral region of the ethylenic carbon atoms of natural rice oil (profile A) and the oil after isomerization and winterization (profile B), which evidences the presence of *trans* isomer resonances.

*Conversion of* trans *FA-containing oils by lipase*. Owing to the importance of vegetable oils in several biotechnological and industrial applications (9–11), we tested the efficiency of lipase-catalyzed hydrolysis and alcoholysis on high *trans* FA-containing oils. Some reports have appeared in the literature on lipase selectivity with *cis* and *trans* fatty acid isomers (21). In this study, we used immobilized lipase from *C. antarctica,* and no selectivity was observed even during the

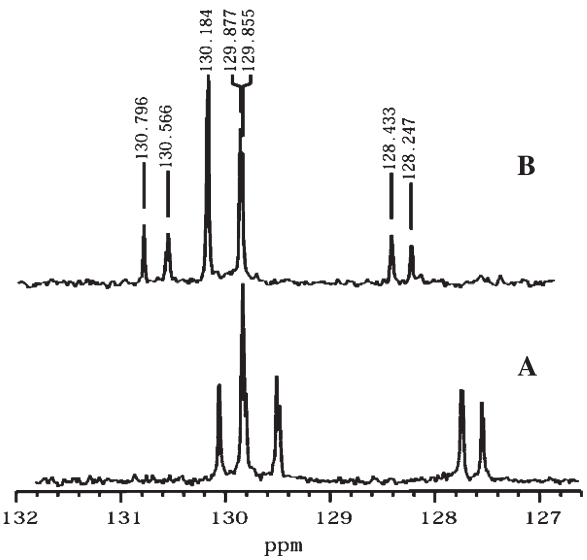



**FIG. 4**. $^{13}C$ NMR spectral region relative to ethylenic carbon atoms. (A) Starting rice oil; (B) rice oil after isomerization and winterization.

**TABLE 2**
**Free Fatty Acid and FAME Mixtures Obtained by *Candida antarctica* Lipase Hydrolysis or Transesterification of Modified Oils (after isomerization/winterization)**

| | Modified rice | | Modified olive | | Modified borage | |
|---|---|---|---|---|---|---|
| Fatty acid | FAME | Free fatty acid | FAME | Free fatty acid | FAME | Free fatty acid |
| 16:0 | 25.8 | 22.9 | 12.8 | 14.0 | 19.7 | 20.0 |
| 18:0 | 5.7 | 4.1 | 3.2 | 3.6 | 5.6 | 6.5 |
| 18:1, *trans*-9 | 42.0 | 44.7 | 68.4 | 64.7 | 17.5 | 20.1 |
| 18:1, *trans*-11 | | | | | 0.1 | 0.4 |
| 18:1, *cis*-9 | 8.2 | 6.3 | 12.6 | 14.1 | 4.3 | 4.9 |
| 18:2, *trans*-9,12 | 12.5 | 15.5 | 3.0 | 3.6 | 23.7 | 22.8 |
| 18:2, *cis/trans* | 5.8 | 6.5 | | | 9.5 | 8.6 |
| 18:2n-6 | | 2 | | | 1.5 | 1.5 |
| 18:3n-6, *trans* | | | | | 9.3 | 7.1 |
| 18:3n-6, *cis/trans* | | | | | 4.8 | 3.3 |
| 20:1, *trans*-11 | | | | | 4.0 | 4.8 |

earlier stages of the alcoholysis of *cis* and *trans* isomers to oleate and linoleate derivatives. We used a known protocol (9) for one cycle, with a progressive addition of the alcohol equivalents during the reaction. In all cases, the first cycle gave satisfactory results, with an 80% conversion without process optimization. The products were analyzed, and Table 2 lists the FA compositions found after transformation to free acids and methyl esters, which mainly reflect the fatty acid contents of the starting TAG mixtures.

In summary, we found that the thiyl radical-catalyzed conversion of natural oils yields *trans* FA-containing oils as the predominant geometrical isomers. This transformation was achieved in an easy, flexible, and high-yield protocol, which could be coupled with enzymatic methods, thus offering a smooth access to the *trans* lipid library. This study aims at helping lipid researchers in the field of lipid isomerism and *trans* fatty acid recognition, as well as at opening new perspectives in TAG synthesis and structure–activity studies.

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## REFERENCES

1. Aro, A., Epidemiological Studies of *trans* Fatty Acids and Coronary Heart Disease, in Trans *Fatty Acids in Human Nutrition*, edited by J.L. Sébédio and W.W. Christie, The Oily Press, Dundee, 1998, pp. 235–260.
2. McDonald, R.E., and M.M. Mossoba, Methods for *trans* Fatty Acid Analysis, in *Food Lipids: Chemistry, Nutrition, and Biotechnology*, edited by C.C. Akoh and D.B. Min, Marcel Dekker, New York, 2002, pp. 169–204.
3. Lie Ken Jie, M.S.F., and C.C. Lam, $^1$H-Nuclear Magnetic Resonance Spectroscopic Studies of Saturated, Acetylenic and Ethylenic Triacylglycerols, *Chem. Phys. Lipids 77*:155–171 (1995).
4. Lie Ken Jie, M.S.F., and J. Mustafa, High-Resolution Nuclear Magnetic Resonance Spectroscopy—Applications to Fatty Acids and Triacylglycerols, *Lipids 32*:1019–1035 (1997).
5. Sgoutas, D.S., and F.A. Kummerow, *Cis-trans* Isomerization of Unsaturated Fatty Acid Methyl Esters Without Double Bond Migration, *Ibid. 4*:283–287 (1969).
6. Ferreri, C., C. Costantino, L. Perrotta, L. Landi, Q.G. Mulazzani, and C. Chatgilialoglu, *Cis-trans* Isomerization of Polyunsaturated Fatty Acid Residues in Phospholipids Catalyzed by Thiyl Radicals, *J. Am. Chem. Soc. 123*:4459–4468 (2001).
7. Ferreri, C., A. Samadi, F. Sassatelli, L. Landi, and C. Chatgilialoglu, Regioselective *cis-trans* Isomerization of Arachidonic Double Bonds by Thiyl Radicals: The Influence of Phospholipid Supramolecular Organization, *Ibid. 126*:1063–1072 (2004).
8. Ferreri, C., M.R. Faraone-Mennella, C. Formisano, L. Landi, and C. Chatgilialoglu, Arachidonate Geometrical Isomers Generated by Thiyl Radicals: The Relationship with *trans* Lipids Detected in Biological Samples, *Free Radic. Biol. Med. 33*:1516–1526 (2002).
9. Shimada, Y., Y. Watanabe, T. Samukawa, A. Sugihara, H. Noda, H. Fukuda, and A. Tominaga, Conversion of Vegetable Oil to Biodiesel Using Immobilized *Candida antarctica* Lipase, *J. Am. Oil Chem. Soc. 76*:789–793 (1999).
10. Borgdorf, R., and S. Warwel, Substrate Selectivity of Various Lipases in the Esterification of *cis*- and *trans*-9-Octadecenoic Acid, *Appl. Microb. Biotechnol. 51*:480-485 (1999).
11. Jaworski, J., and E.B. Cahoon, Industrial Oils from Transgenic Plants, *Curr. Opin. Plant Biol. 6*:178-184 (2003).
12. Grandgirard, A., A. Piconneaux, J.L. Sébédio, and F. Julliard, *Trans* Isomers of Long-Chain n-3 Polyunsaturated Fatty Acids in Tissue Lipid Classes of Rats Fed with Heated Linseed Oil, *Reprod. Nutr. Dev. 38*:17–29 (1998).
13. Huang, X., and C. Fang, Dietary *trans* Fatty Acids Increase Hepatic Acyl-CoA:Cholesterol Acyltransferase Activity in Hamsters, *Nutr. Res. 20*:547–558 (2000).
14. Kramer, J.K.C., V. Fellner, M.E.R. Dugan, F.D. Sauer, M.M. Mossoba, and M.P. Yurawecz, Evaluating Acid and Base Catalysis in the Methylation of Milk and Rumen Fatty Acids with Special Emphasis on Conjugated Dienes and Total *trans* Fatty Acids, *Lipids 32*:1219–1228 (1997).
15. Wolff, R.L., and D. Precht, A Critique of 50-m CP-Sil 88 Capillary Columns Used Alone to Assess *trans*-Unsaturated FA in Foods: The Case of the TRANSFAIR Study, *Ibid. 37*:627–629 (2002).
16. *Official Methods and Recommended Practices of the AOCS*, edited by D. Firestone, 5th edn., AOCS Press, Champaign, 1998.
17. Chatgilialoglu, C., C. Ferreri, M. Ballestri, Q.G. Mulazzani, and L. Landi, *Cis-trans* Isomerization of Monounsaturated Fatty Acid Residues in Phospholipids by Thiyl Radicals. *J. Am. Chem. Soc. 122*:4593–4601 (2000).

18. Chatgilialoglu, C., A. Altieri, and H. Fischer, The Kinetics of Thiyl-Radical Induced Reactions of Monounsaturated Fatty Acid Esters, *Ibid. 124*:12816–12823 (2002).
19. Loliger, J., P. Lambelet, R. Aeschbach, and E. Prior, Natural Antioxidants: From Radical Mechanisms to Food Stabilization, in *Food Lipids and Health*, edited by R.E. McDonald and D.B. Min, Marcel Dekker, New York, 1996, pp. 68–77.
20. Chatgilialoglu, C., L. Zambonin, A. Altieri, C. Ferreri, Q.G. Mulazzani, and L. Landi, Geometrical Isomerism of Monounsaturated Fatty Acids. Thiyl Radical Catalysis and Influence of Antioxidant Vitamins*, Free Radic. Biol. Med. 33*:1143–1147 (2002).
21. Warwel, S., R. Borgdorf, and L. Brühl, Substrate Selectivity of Lipases in the Esterification of Oleic Acid, Linoleic Acid, Linolenic Acid and Their All-*trans*-Isomers and in the Transesterification of *cis/trans*-Isomers of Linoleic Acid Methyl Ester, *Biotechnol. Lett. 21*:431–436 (1999).

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