Preparation of Geometrical Isomers of Linolenic Acid

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Two methods were developed to prepare important quantities of 18:3Δ9,12,15 geometrical isomers. The first method was based on the isolation of 18:3Δ9*c*,12*c*,15*c* from a linseed oil by a combination of urea inclusion and preparative high performance liquid chromatography (HPLC). The isolated 18:3 was isomerized using nitrous acid.

The second method was based on the isolation of 18:3Δ9,12,15 geometrical isomers which usually are formed during the heat treatment of vegetable oils. For that purpose a linseed oil was heated at 275 C for 12 hr under nitrogen. The geometrical isomers were isolated using a combination of column chromatography on silicic acid, urea inclusion and preparative HPLC.

Several years ago, minor quantities of geometrical isomers of linolenic acid were detected by Ackman et. al. (1) in deodorized oils. The same compounds (18.3Δ9*c*, 12*c*,15*t*; 18:3Δ9*t*,12*c*, 15*c* and 18:3Δ9*t*,12*c*,15*t*) were identified recently as being the major isomers of linolenic acid formed during heat treatment of vegetable oils in the laboratory (2). Minor amounts of 18:3Δ9*c*, 12*t*,15*c*: 18:3Δ9*c*,12*t*,15*t* and 18:3Δ9*t*,12*t*, 15*c* also were identified (2). All these components have also been isolated in some frying oils obtained from restaurants (3).

Little is known about the metabolism and the physiological effects of these geometrical isomers of linolenic acid. However, the 20:5Δ5*c*,8*c*,11*c*,14*c*,17*t*, an isomer of 25:0 ω3, was identified as probably being a metabolite of the ingested 18:3Δ9*c*,12*c*,15*t* (4) in the liver of rats fed a heated linseed oil. The 22:6Δ4*c*, 7*c*,10*c*,13*c*,16*c*,19*t* was also identified recently when the same diet was used (5). Other C20 and C22 polyunsaturated fatty acids are still under investigation. These probably are isomers of the usual C20 and C22 ω3 fatty acids. These nutritional studies on the isomeric 18:3 acids were carried out using heated oils. It is now well established that heated oils are complex mixtures of components such as polymers, oxidized compounds and cyclic monomers (6-8). In order to gain a better understanding of the metabolism of 18:3 geometrical isomers, it is necessary to carry out nutritional studies on pure fractions.

Knowing that great quantities of linolenic acid isomers are needed for nutritional experiments, it did not seem feasible to use the synthesis procedure described by Rakoff and Emken (9). Therefore, we have developed two methods. The first one was based on the isolation of linolenic acid followed by isomerization by nitrous acid. In the second method, the linolenic acid isomers were isolated from a heated linseed oil (10). The two methods involve combinations of column chromatography on silicic acid, preparative high performance liquid chromatography and urea crystallization.

MATERIALS AND METHODS

All solvents were redistilled before use. Hexane was stirred with sulfuric acid (25 ml acid/1 hexane) for two days prior to distillation.

Gas liquid chromatography (GLC). Each preparatory step was monitored by GLC of the fatty acid methyl esters (FAME). The GLC analyses were carried out on a Becker-Packard 417 or 420, or on a Girdel 3000 chromatograph fitted with a flame ionization detector (FID) and a solid injector (11). Glass capillary columns coated with Silar 10C (50 m long and 0.25 mm i.d.) or Carbowax 20M AT (40 m long and 0.35 mm i.d.) or a fused silica column (CPSil 88, Chrompack, 50 m long and 0.32 mm i.d.) were used. Helium was the carrier gas. The temperature of both the injector and the FID detector was 240 C, while the oven temperature was 170 C. The quantitative analyses were performed using a Vista CDS 401 (Varian) or an Autolab System 4 (Spectraphysics) data acquisition systems.

Urea fractionation of fatty acids of linseed oil. Refined but undeodorized linseed oil (Robbe-Hyfran, France) containing about 54.6% or 18:3 ω3 was saponified (300 g) and the unsaponifiables removed according to AOCS procedure (Ca-6a-40). A portion (200 g) of the resulting fatty acids was mixed with a hot solution of urea in methanol (300 g urea/1.2 l methanol). After cooling under nitrogen, the flask was placed at 4 C overnight. The urea adduct and non-adduct fractions were then separated by filtration. The crystals were washed with refrigerated hexane. The filtrate was transferred into a separatory funnel, and the fatty acids were extracted with hexane after addition of two 1 water and 30 ml HCl (6N). The resulting fatty acids were then esterified by refluxing for six hr with a solution of 1% sulfuric acid in methanol.

Preparative high performance liquid chromatography (HPLC). Preparative HPLC was carried out on a Prep LC/System 500 (Waters) using a Prep-Pak 500 C 18 cartridge (30 cm x 5.7 cm i.d.), refractometric detection and acetonitrile as the solvent at 200 ml/min. In each separation, 12 g of fatty acid methyl esters dissolved in acetone were injected.

Isomerization. The isomerization of linolenic acid methyl esters was effected at 40 C for 10 min using 6.25 ml of 6M nitric acid and 10 ml of an aqueous 2M solution of sodium nitrite/100 g of methyl esters. After addition of water, the methyl esters were extracted with hexane.

These methyl esters were further purified on a silicic acid column. Silicic acid (70-200 mesh) was heated overnight at 160 C. After cooling, 220 g of silicic acid were hydrated at 5% and transferred to a glass column (55 cm long x 4 cm i.d.). About 40 g of methyl esters were fractionated at once with a 1.2-l mixture of petroleum ether-diethyl ether (95/5). The quality of purification was evaluated by the nitrogen content determination of the fatty acid fraction, using the micro-Kjeldahl method.

Identification of geometrical isomers of linoleic and linolenic acids. The determination of the identity of each isomer of linolenic and linoleic acids was effected by GLC (2,9) after silver nitrate TLC. Silicagel 60 precoated plates (Merck 5721) were soaked in a 10% silver nitrate solution in acetonitrile for 30 min. The migration solvent was a mixture of benzene-diethyl ether (90/10). After drying, the bands were revealed under UV light after spraying with a 0.2% solution of 2'7' dichlorofluorescein in ethanol. The bands were scraped off and extracted with a mixture of chloroform-hexane (1/1).

Isolation of linolenic acid isomers from a heated linseed oil. A heated linseed oil (275 C, 12 hr, under nitrogen) was fractionated as described recently (10) by a combination of saponification, esterification, column chromatography and urea inclusion into a nonurea adduct fraction which contained the cyclic fatty acid monomers and a urea adduct fraction.

The urea adduct fraction contained a mixture of saturated acid methyl esters and *cis and trans* isomers of oleic, linoleic and linolenic fatty acid methyl esters. This methyl ester mixture was further submitted to a second urea fractionation as described previously for the fatty acids, with a ratio of urea/fatty acid methyl esters of 1.5. The nonurea adduct fraction was then submitted to a preparative HPLC under the conditions described previously.

RESULTS AND DISCUSSION

Preparation of linolenic acid isomers from fresh linseed oil. The purpose of the first method was to isolate some linolenic acid from a linseed oil and to isomerize it with nitrous acid (Fig. 1). It was, therefore, necessary to test the different parameters of each isolation step in order to obtain a good yield and the optimum purity of the linolenic acid isomers fraction.

After saponification of the linseed oil in large quantities and elimination of the unsaponifiable material, the fatty acids were submitted to urea inclusion. All the urea inclusion tests were effected with 1 g of fatty acid, before carrying out the reaction on large quantities (200 g). The tests have shown (Table 1) that the fractionation carried out at low temperature (4 C) improved the efficiency of the inclusion, as expected from the work of Privett (12). Furthermore, washing out the crystals with refrigerated hexane (+ 4 C) instead of methanol saturated with urea, a method traditionally used (13), improved the yield in nonadducted fatty acids (59.6% instead of 48.5), without modification of the concentration of linolenic acid which in both cases exceeded 70%.

The next step was to find the best ratio between the respective quantities of fatty acids and urea in order to obtain a linolenic acid fraction as pure as possible. Four different ratios of urea/fatty acids were tested (Table 2). As expected, the increase in the urea/fatty acids ratio from 1 to 2 increased the percentage of linolenic acid in the filtrate, while the yield of the reaction was reduced from 73.5% to 48.7%. However, the level of linoleic acid is remarkably constant (24.4% compared to 24.7%). A second urea fractionation of this fraction did not result in any decrease of linoleic

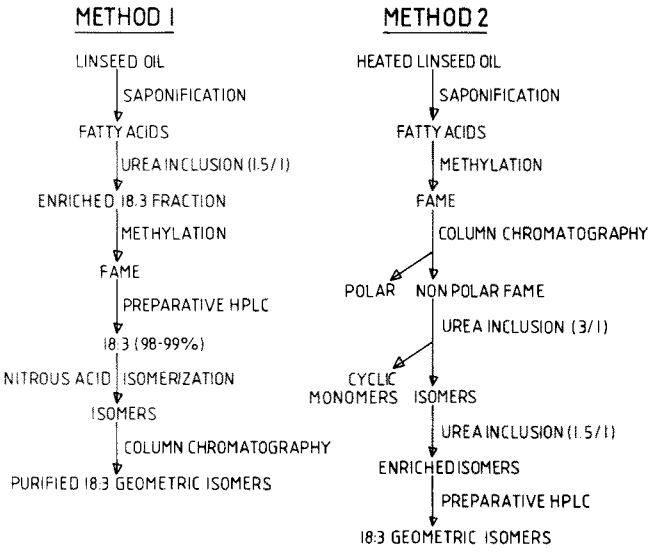

FIG. 1. Flow chart for the preparation of a mixture of 18:3 geometrical isomers.

TABLE 1

Effects of Temperature and Fatty Acid Types on the Yield of Urea Inclusion[a]

Fatty acid	20 C	4 C
16:0	97.2	99.1
16:1 (ω9 + ω7)	25.	62.5
17:0	100	100
17:1	40	75
18:0	99.3	99.4
18:1 (ω9 + ω7)	64.4	89.8
18:2 *ct* + *tc*	50	83
18:2 *cc*	18.9	34.9
18:3 *ccc*	14.8	27.1

[a]% of fatty acid included.

TABLE 2

Influence of the Urea/Fatty Acid Ratio on the Fatty Acid Composition of the Nonurea Adduct Fraction

	Urea (g)/fatty acids (g)			
	1	1.3	1.5	2
% of main fatty acids in the nonadduct fraction				
16:0	0.2	0.1	tr[a]	tr
18:0	0.1	0.1	tr	tr
18:1	8.3	5.4	3.3	2.4
18:2	24.4	24.6	25.5	24.7
18:3	65.5	66.1	70.3	71.2
% of nonadduct fraction	73.5	64.7	59.6	48.7
% of adduct fraction	26.5	35.3	40.4	51.3

[a]tr = trace (<0.1).

acid in the filtrate. It was, therefore, necessary to use another step to obtain a fraction richer in 18:3 ω3. Preparative HPLC seemed to be a suitable method to isolate a pure fraction of 18:3 ω3, according to the results published by Bascetta et al. (14). For this purpose, it was useful to carry out the HPLC fractionation on a fatty acid mixture which does not contain any saturated fatty acids, because these acids have large retention volumes and increase the quantity of solvent used. For that reason, the choice was limited between the urea/fatty acids ratios 1.5 and 2, which gave fractions containing only traces of saturated acids and more than 70% of linolenic acid (Table 2). However, the yield of the nonurea adduct fraction (filtrate) was much smaller with the ratio 2 when compared to the results obtained with the ratio 1.5 (48.7% compared to 59.6%). Therefore, the urea/fatty acids ratio 1.5 was chosen.

It was then necessary to find out the optimum conditions to carry out the HPLC fractionation of the mixture enriched in linolenic acid. The separation was effected after esterification (Fig. 1). Five fractions were collected (Fig. 2). Fraction 1 was almost pure methyl linolenate (99%), while fractions 2 and 3 contained 86% and 48%, respectively, of 18:3 ω3. Fraction 3 was discarded and fraction 2 recycled. The first part of the recycled peak (fraction 4) was also rich in methyl linolenate (99%); it was added to fraction 1. Fraction 5 was discarded. The total analysis took 12 min, and 2.5 l of acetonitrile were used. An injection of 12 g gave about 6 g of methyl linolenate with a purity of 98.5 to 99.5%.

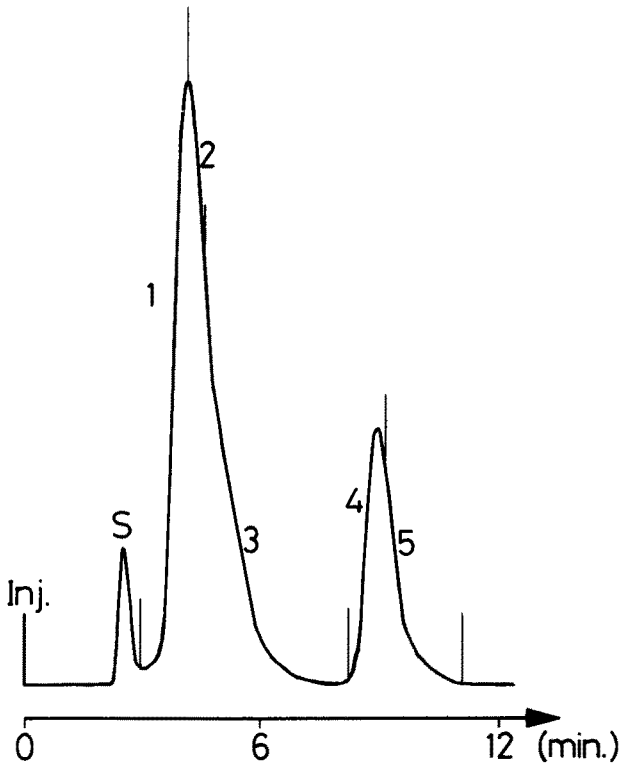

FIG. 2. Preparative HPLC fractionation of a mixture of FAMEs containing about 73% 18:3 ω3. (C18 reverse phase column, 30 cm long and 5.7 cm i.d., acetonitrile at 200 ml/min).

Nitrous acid has been used to isomerize oleic acid (15) and linoleic acid (16). For methyl linolenate, it was necessary to find the best experimental conditions to obtain the highest yield of *mono-trans* trienes and the minimum of *tri-trans* and *di-trans* isomers. Considering the time and cost necessary to prepare methyl linolenate, the tests were effected on the fraction isolated by urea fractionation and containing about 73% of methyl linolenate, 25% methyl linoleate and 1% methyl oleate. Then these tests were verified using a small amount of pure isolated methyl linolenate.

The first test was carried out under the conditions described by Litchfield et al. (15). Two g of methyl esters were mixed at 60 C, with 2 ml of a 2 M aqueous sodium nitrite solution and 1.3 ml of 6 M nitric acid.

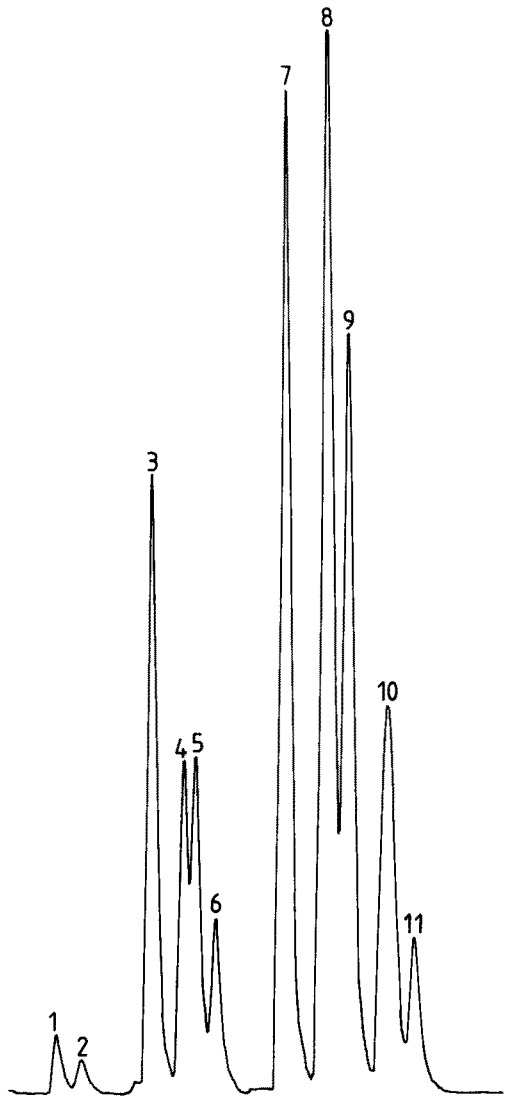

FIG. 3. GLC analysis on a Silar 10 C glass column of a mixture of isomeric 18:1, 18:2 and 18:3 FAME (isomerization at 60 C, during 30 min, using 1 ml 2M aqueous sodium nitrite and 0.65 ml 6M nitric acid/g of FAME). (1) 18:1 *t*; (2) 18:1 *c*; (3) 18:2 *tt*; (4) 18:2 *ct*; (5) 18:2 *tc*; (6) 18:2 *cc*; (7) 18:3 *ttt*; (8) 18:3 *ctt* + 18:3 *tct*; (9) 18:3 *ttc* + 18:3 *cct*; (10) 18:3 *tcc* + 18:3 *ctc*; (11) 18:3 *ccc*.

Sampling was effected every five min at the beginning, then every 15 min, to follow the isomerization reaction. Figure 3 shows the fatty acid composition of the mixture after 30 min of reaction. Many isomeric fatty acids can be detected. The chemical nature of these components was studied after silver-nitrate thin layer chromatography (TLC) and gas liquid chromatographic analysis (GLC) of each TLC band, using an internal standard (17:0). Each component was identified by comparison with the TLC data of Rakoff and Emken (9) and the GLC data published recently (2,9). The evolution of the isomerization reaction is shown in Figure 4. The three *mono-trans-di-cis* trienes, present in similar quantities, were pooled, as well as the three *di-trans-mono-cis* isomers, in order to visualize the important changes as the reaction proceeded. A rapid decrease in linolenic acid was observed. After 10 min of reaction, the 18:3 ω3 represented less than 10% of the total 18:3 isomers. Then, its level diminished slowly to reach 4% after 45 min. Consequently, all seven geometrical isomers of linolenic acid were detected after 5 min. of reaction time. The levels of *di-trans-mono-cis* increased up to 15 min and then became stable (46% of total trienes). The *tri-trans* level increased rapidly at the beginning and then slowly, whereas the *mono-trans-di-cis* decreased regularly. Moreover, as the mixture became more and more viscous and yellow-orange, the yield in fatty acids decreased. These yellow-orange materials, insoluble in hexane but soluble in chloroform, acetone or THF, may be polymeric and/or nitro compounds. From these results, it is evident that the conditions used in this first test are too drastic and that moderate conditions should be used.

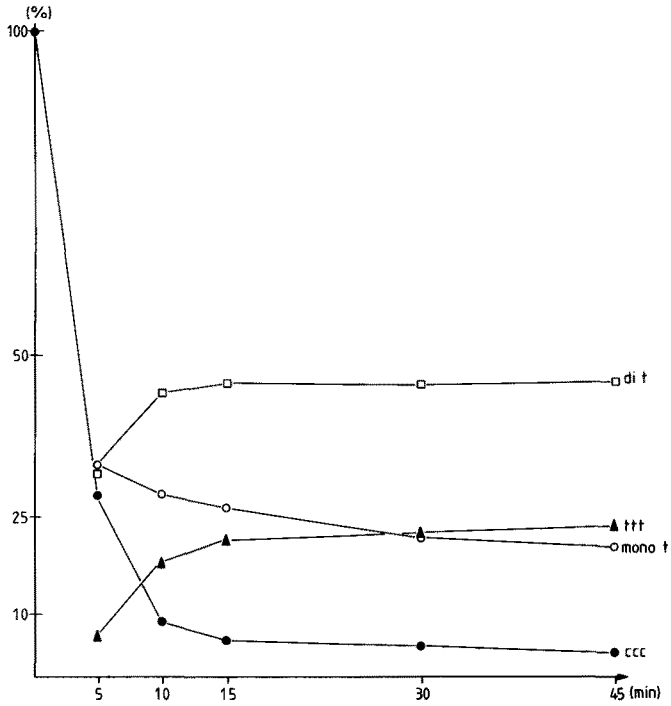

FIG. 4. Influence of the length of the nitrous acid isomerization of a mixture of 18:3 ω3 (73%), 18.2 ω6 (25%) and 18:1 ω9 (1%) on the formation of 18:3 geometrical isomers (results expressed as % of total trienes. Isomerization at 60 C, using 1 ml 2 M aqueous sodium nitrite and 0.65 ml 6M nitric acid/g of FAME).

Several other conditions of isomerization were then tested (Table 3), with changes in temperature, time and nitrous acid concentration. Nitrous acid concentration is very important because if the concentration is divided by two (tests 2 and 3), the *tri-trans* content decreases from 10.3% to 0.5 and the *di-trans-mono-cis* from 37.4 to 9.0. Further reduction of the concentration in nitrous acid by half (test 4) resulted in a moderate decrease in the levels in *tri-trans* and *di-trans-mono-cis*. In this case, 60% of *tri-cis* 18:3 remained unchanged. The effect of temperature was studied in tests 3, 5 and 6. The results show that the reduction from 50 C to 40 C resulted in a decrease in the level of *tri-trans* and of *di-trans-mono-cis*, but that the reduction from 40 C to 30 C did not modify the composition of the final mixture appreciably. Since our aim was to obtain the maximum amount of *mono-trans-di-cis* and the minimum amounts of *di-trans-mono-cis* and of *tri-trans*, it seems that the optimum conditions would be a 40 C temperature and a level of nitrous acid as low as possible. However, under these conditions, the yield of *mono-trans-di-cis* is low. Comparison of tests 3 and 7 showed that an increase in reaction time from 10 to 20 min. resulted in an important increase in *di-trans-mono-cis* and even in *tri-trans*, without a noticeable

TABLE 3

Influence of Various Reaction Parameters During the Isomerization of Methyl Linolenate by Nitrous Acid

	Conditions			Results (% of total 18:3)			
	Temperature (°C)	Nitrite 2M (ml/g fatty acid)	Time (min)	*ttt*	Σ*di-trans*	Σ*mono-trans*	*ccc*
Test 2	40	0.4	10	10.3	37.4	36.9	15.4
Test 3	40	0.2	10	0.5	9.0	36.7	53.8
Test 4	40	0.1	10	0.3	6.7	33.4	59.6
Test 5	50	0.2	10	1.4	13.4	39.3	45.6
Test 6	30	0.2	10	0.6	9.6	37.3	52.3
Test 7	40	0.2	20	2.1	19.2	41.7	37.1

improvement in *mono-trans-di-cis*. The conditions of test 4 were therefore selected: 10 min. at 40 C with 0.1 ml of 2M sodium nitrite and 62.5 μl of 6M nitric acid/g of FAMEs. Isomerization of the fraction containing at least 98% of methyl linolenate (instead of 73%) carried out under the same experimental conditions gave good results. Minor amounts of *tri-trans* (about 1%) were obtained.

The isomerized methyl esters were then purified in order to eliminate any secondary reaction products which could have been formed. The method of Litchfield et al. (15) was not used because it apparently removes only about one-half of the contaminating nitrogen (15). The method which was used (silica column chromatography) is similar to that usually used for determining the amount of polar components in heated fats (17,18), recently developed for methyl esters (19). No nitrogen contamination could be shown in the resulting FAMEs after micro-Kjeldahl analysis. The composition of the fraction obtained finally is reported in Table 4 (first method).

Isolation of geometrical isomers of linolenic acid from a heated linseed oil. Heated linseed oil is a good starting material to isolate 18:3 geometrical isomers which are formed during the heat treatment of the triglycerides. However, it is necessary to eliminate the other compounds formed during heating such as polymers, polar compounds and cyclic fatty acid monomers. The method used has been described elsewhere (10) and is summarized in Figure 1 (part of method 2). The FAMEs of a linseed oil, heated for 12 hr under nitrogen, were purified on a silicic acid column; the non-polar fraction was submitted to two urea inclusions (ratio of urea/FAMEs = 3); the non-urea adduct fraction contained the cyclic monomers and the adduct fraction the *cis and trans* aliphatic fatty acids (isomers). This urea adduct fraction was then submitted to a urea inclusion with a urea/fatty acids ratio of 1.5 and preparative HPLC (Fig. 1, method 2).

The amount of trienes in the fraction isolated from a heated linseed oil (Table 5) was smaller than that in the fresh linseed oil (28.8% compared to 54.6), due to the polymerization and cyclization which occur during the heat treatment. The urea inclusion of this frac-

TABLE 4

Fatty Acid Composition (%) of the Two Fractions Obtained Using Methods Described in Figure 1

Fatty acid	Method 1	Method 2
18:1 *t*	—	0.1
18:1 *c*	—	0.4
18:2 *ct*	< 0.2	0.1
18:2 *tc*	< 0.2	0.1
18:2 *cc*	0.6	0.5
18:3 *ttt*	1.2	1.1
18:3 *di-trans-mono-cis*	12.0	45.6
18:3 *mono-trans-di-cis*	35.4	42.2
18:3 *ccc*	50.5	4.4
Unknowns		4.2[a]

[a]Traces of cyclic fatty acids and other components not yet identified.

TABLE 5

Fatty Acid Composition of the Fractions Obtained After Urea Inclusion of a Mixture of Methyl Esters Obtained From a Heated Linseed Oil

	Purified FAME from heated linseed oil[a]	Fractions obtained after urea inclusion (ratio urea/fatty acids, 1.5) Nonurea adduct fraction	Urea adduct fraction	Inclusion yield (%)[b]
Weight of each fraction (g)	200	114	83.7	
FAME (%)				
Σ *saturates*	16.1	1.0	38.3	96.6
Σ *monoenes*	30.1	25.6	37.3	51.7
18.2 ct	4.6	4.5	4.2	40.9
18:2 *tc*	4.6	5.8	2.4	23.5
18:2 *cc*	11.2	17.9	2.2	8.1
18:2 *tt* conj.	0.6	—	1.2	100
Σ *dienes*	21.0	28.2	10.0	21.0
18:3 *tct*	13.8	17.8	7.8	24.3
18:3 *cct*	5.8	9.8	1.4	9.5
18:3 *ctc* 18:3 *tcc*	7.9	11.6	2.8	15.1
18:3 *ccc*	1.3	2.3	0.1	7.6
Σ *trienes*	28.8	41.5	12.1	17.6

[a]Fraction obtained after column chromatography of the total methyl esters of a heated linseed oil (275 C, 12 hr, under nitrogen), and after urea inclusion (ratio urea/fatty acid methyl esters 3).

[b]Ratio (FAME in urea adduct fraction)/(FAME in starting mixture).

tion (200 g) with 300 g of urea gave 114 g of filtrate and 83.7 g of urea adduct fraction. The analyses of these two fractions (Table 5) indicated that the non-urea adduct fraction is enriched in trienes (41.5 compared to 28.8). Almost all the saturates and half of the monoenes are included (respectively 96.6 and 51.7%). However, the dienes are included in about the same proportions as the trienes (Table 5, last column). Among these trienes, the geometrical isomers of linolenic acid are much more included than linolenic acid. It would then be very difficult, using one more urea inclusion step, to increase the level of 18:3 geometrical isomers in the non-urea adduct fraction, without further increasing the amount of linolenic acid. We therefore decided to use this nonurea adduct fraction, containing only 1% of saturates and 41.5% of trienes, for the HPLC fractionation.

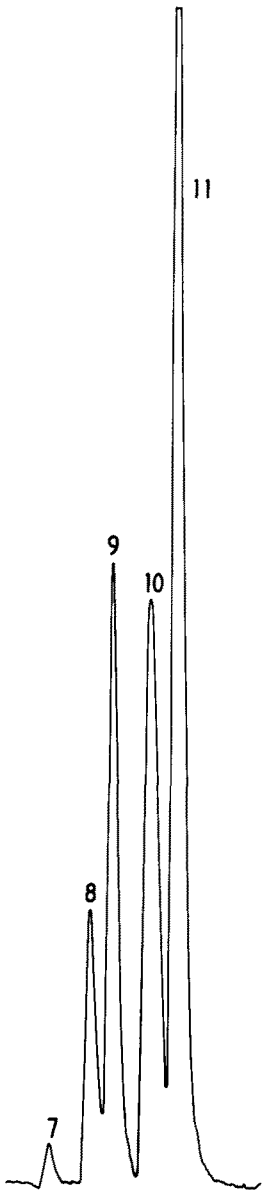

FIG. 5. Preparative HPLC fractionation of a mixture of FAMEs isolated from a heated linseed oil (275 C, 12 hr, under nitrogen, same HPLC conditions as in Fig. 2).

Two recycles were necessary (Fig. 5). The first part of the peak (fraction 1) contained pure trienes and was collected. Fraction 2 was recycled and fraction 3 discarded. The first part of the recycled peak (fraction 4) was recycled again and fraction 5 discarded. Fraction 6 was pure in trienes and fraction 7 discarded. With 11.5 g injected at once, 3.5 g of trienes were obtained. The composition of this fraction (Table 4, method 2) was quite different if compared to the mixture obtained using method 1. The amount of all *cis* 18:3 ω3 was much smaller in the fraction isolated using method 2. It is important to note that 18:3 *ttt* is present in minor quantities in the fraction isolated from heated linseed oil. This component was not detected in the starting fraction, probably due to the overlap with traces of cyclic fatty acid monomers. The *tri-trans* 18:3 also was not determined in a rapeseed oil heated at 240 C for 10 hr (2). It is possible that this component was present in too small a quantity to be detected that the different heating conditions (275 C, 12 hr under nitrogen versus 240 C, 10 hr with air) can lead to different fatty acid compositions.

An advantage of the second method is the possibility of stopping the preparation when cyclic monomers are removed (Fig. 1). The linolenic acid geometrical isomers are then present in a mixture containing straight-chain aliphatic fatty acids suitable for nutritional purposes. Another advantage of this method is that different heating conditions can be used to obtain different fatty acid compositions. For example, 200 C is a better temperature to obtain *mono-trans-di-cis* compounds with very little *di-trans-mono-cis* content. A higher temperature (240 C, for example) yields higher proportions of geometrical isomers with regard to linolenic acid. However, in this case, the *di-trans-mono-cis* isomers are present in important quantities and become the major isomers if the heating time is sufficient (20). The 18:3 geometrical isomers mixtures could be further fractionated in order to obtain pure *mono-trans, di-trans* and *tri-trans* fractions by silver resin chromatography on a macroreticular column, according to the procedure of Lanser and Emken (21).

REFERENCES

1. Ackman, R.G., S.N. Hooper and D.L. Hooper, *J. Am. Oil Chem. Soc. 51*:42 (1974).
2. Grandgirard, A., J.L. Sebedio and J. Fleury, *Ibid. 61*:1563 (1984).
3. Sebedio, J.L., A. Grandgirard, Ch. Septier and J. Prevost, *Rev. Fr. Corps Gras 34*:15 (1987).
4. Piconneaux, A., A. Grandgirard and J.L. Sebedio, *C.R. Acad. Sci. Paris 300*:353 (1985).
5. Piconneaux, A., A. Grandgirard, J.L. Sebedio and J.L. Le Quere, *Lipids*, submitted for publication.
6. Artman, N.R., *Adv. Lipid Res. 7*:245 (1969).
7. Perkins, E.G., *Rev. Fr. Corps Gras 23*:257 (1976).
8. Ottaviani, P., J. Graille, P. Perfetti and M. Naudet, *Chem. Phys. Lipids 24*:57 (1979).
9. Rakoff, H., and E.A. Emken, *Chem. Phys. Lipids 31*:215 (1982).
10. Sebedio, J.L., and J. Prevost and A. Grandgirard, *J. Am. Oil Chem. Soc. 64*:1026 (1987).
11. Ros, A., *J. Gas Chromatogr., 3*:252 (1965).
12. Privett, O.S., *Riv. Ital. Sost. Grasse 46*:433 (1969).
13. Potteau, B., *Ann. Nutr. Alim. 30*:89 (1976).
14. Bascetta, E., F.D. Gunstone and C.M. Scrimgeour, *Lipids 19*:801 (1984).

15. Litchfield, C., R.D. Harlow and R. Reiser, *J. Am. Oil Chem. Soc. 42*:73 (1965).
16. Harlow, R.D., C. Litchfield and R. Reiser, *Ibid. 40*:505 (1963).
17. Guhr, G., and J. Waibel, *Fette, Seifen, Anstrichm 80*:106 (1978).
18. Standard Methods for the Analysis of Oils, Fats and Derivatives, 6th Edn., 1st suppl., part 4, *Pure Appl. Chem. 54*:233 (1982).
19. Perrin, J.L., P. Perfetti, C. Dimitriades and M. Naudet, *Rev. Fr. Corps Gras 32*:151 (1985).
20. Grandgirard, A., and F. Julliard, *Rev. Fr. Corps Gras 34*:213 (1987).
21. Lanser, A.C., and E.A. Emken, *J. Chrom. 256*:460 (1983).

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ERRATA

An error occurred in a figure legend which appeared in the July issue of the *Journal of the American Oil Chemists' Society*. The legend to Figure 3 in "A Methodology Study to Evaluate Quality of Soybeans Stored at Different Moisture Levels" should have read, "Phosphorus removed by degumming oils from soybeans stored at different moisture levels."

The paper was written by E. N. Frankel, A. M. Nash and J. M. Snyder. It appeared in *J. Am. Oil Chem. Soc. 64*:987-992 (1987).

The following material is reprinted, in its correct form, from a paper which appeared in the July issue of the *Journal of the American Oil Chemists' Society*. The paper, "Formation of Short Chain Volatile Organic Acids in the Automated AOM Method," was by J. M. deMan, Fan Tie and L. deMan. It appeared on pages 993 through 996.

The formation of formic acid in autoxidizing oils has been elucidated by Loury (6). He postulated that formic acid is formed by peroxidation of aldehydes. A resonance equilibrium is established between two limiting forms of an aldehyde molecule:

$$R{-}CH_2{-}\overset{+}{C}H{-}\overset{-}{O} \rightleftharpoons R{-}CH_2{-}CHO \rightleftharpoons R{-}\overset{-}{C}H{-}\overset{+}{C}HOH$$

or, more exactly, between two limiting forms of the carbonyl-free radical that initiates the autoxidation chain:

$$R{-}CH_2{-}\dot{C}O \rightleftharpoons R{-}\dot{C}H{-}CHO$$

The first hybrid yields a peracid-free radical by autoxidation, and chain transfer gives the peracid:

$$R{-}CH_2{-}\dot{C}O + O_2 \rightarrow R{-}CH_2{-}\overset{\cdot}{\underset{O}{C}}{-}O$$

The second hybrid is able to tie up oxygen at the α carbon to yield the α-hydroperoxy aldehyde by a similar mechanism:

$$R{-}\dot{C}H{-}CHO + O_2 \rightarrow R{-}\overset{\cdot}{\underset{O}{C}}H{-}CHO$$

The latter results in formic acid and a new aldehyde by breakdown of the —O—O and —C—C bonds:

$$R{-}\overset{H}{\underset{O}{\overset{|}{\underset{|}{C}}}}{-}CHO \rightarrow HCOOH + R{-}CHO$$