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Triacylglycerol analysis of partially hydrogenated fats using high-performance liquid chromatography

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ABSTRACT

This study describes a method that has been developed for the separation and quantification of triacylglycerols in partially hydrogenated fats rich in oleic, elaidic, palmitic and stearic acids. Determination of triacylglycerols is according to carbon number, number of double bonds and geometrical isomers (*i.e. cis* and *trans* configuration). The method described is a two-stage procedure, utilizing RP-HPLC for preliminary fractionation into partition numbers, and following this with subsequent separation into geometrical isomers using RP-HPLC/silver ions. The method has been applied to a number of different model triacylglycerols, and also to partially hydrogenated soya-bean oil and palm oil. The relative standard deviations are 2% for triacylglycerol species on the 15% absolute level, and *ca.* 5% on a 3% absolute level.

INTRODUCTION

The major constituents of natural vegetable fats are triacylglycerols (TGs). Since a TG molecule is built up of three fatty acids, combinations of just a few kinds of fatty acid can give rise to several different TGs. The complexity of TG analysis is therefore obvious.

In a partially hydrogenated fat, the number of fatty acid isomers is dramatically increased by the appearance of geometrical isomers (*i.e. cis* and *trans* configurations) and various positional isomers (*i.e.* different positions of double bonds within the fatty acid chain). Thus the increased number of fatty acid isomers will result in a drastic increase in the number of possible TGs. For example, OOO (three *cis* double bonds) may convert to geometrical isomers OOEl (two *cis*, one *trans*), OElEl (one *cis*, two *trans*) and ElElEl (three *trans* double bonds). Except in the section dealing with positional isomers, in this study all C18:1 *cis* fatty acids have been referred to as oleic acid (O), regardless of the position of the double bond. Similarly, all C18:1 *trans* fatty acids have been referred to as elaidic acid (El), and all C18:2 fatty acids have been referred to as linoleic acid (L).

The importance of being able to determine the geometrical isomers of TGs in partially hydrogenated fats can be ascribed to their effect on the physical behaviour of the fats, such as their polymorphic behaviour and melting properties.

Silver ion chromatography has, since several decades, been utilised for separation of geometrical isomers of lipids, such as fatty acids and TGs [1–7]. This type of separation is possible due to the formation of π-complexes between double bonds and silver ions. *trans*-Olefins form weaker π-complexes with silver ions than do *cis*-olefins; hence *cis–trans* isomers can be separated using silver ion chromatography. Initially silver ions were used in conjunction with TLC, with silver nitrate being incorporated in the silica gel layer. With this approach De Vries and Jurriens [1] have demonstrated resolution of

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just a few geometrical isomers of TGs that include oleic acid (*cis*) and elaidic acid (*trans*). In recent years the technique has been adapted to HPLC. Christie [4] has demonstrated such a separation of some geometrical isomers of TGs into the groups SSEl, SSM, SElM and SMM (S = saturated fatty acid, M = monounsaturated fatty acid) using an ion-exchange column impregnated with silver ions. Hammond and Irwin [5] have demonstrated a similar separation of StElSt and StOSt (St = stearic acid) on a silica column impregnated with silver ions. It is also worth mentioning in conjunction with silver ion chromatography that many workers have shown the separation of symmetric and asymmetric isomers of mixed saturated and unsaturated fatty acids, *e.g.* SOS and SSO [8].

This study deals with separation of the major geometrical isomers of TGs in partially hydrogenated vegetable fats. It includes separation of geometrical isomers, such as all combinations of oleic acid and elaidic acid. The proposed method, which is based on HPLC and includes silver ion chromatography, has been applied to partially hydrogenated soya bean oil and palm oil. To the best of our knowledge, no results of either separation of complete homologous series of geometrical isomers or detailed separation of the complex fats mentioned above have previously been published.

EXPERIMENTAL

Chemicals

Acetone, methanol and 2-propanol, all of analytical-reagent quality and propionitrile of synthesis quality, distilled over Siccapent, were used for the mobile phases. In addition, silver nitrate of analytical-reagent quality was used as a component. For dissolution of PN fractions (PN = partition number), benzene of analytical-reagent quality was used. All these chemicals were produced by Merck (Darmstadt, Germany).

Samples

All model TGs (purity $\geq 99\%$) were purchased from Larodan (Malmö, Sweden). The TGs are listed in Table I. The partially hydrogenated fats were hydrogenated and refined by the company Karlshamns (Karlshamn, Sweden). Both soya-bean oil and palm oil were hydrogenated to a melting point of 41°C and referred to as Soya 41 and Palm 41 respectively. For fatty acid composition, see Table II.

Preparative HPLC separation into partition numbers (PNs)

An LDC pump was used for the PN separation, together with an ERC 7512 refractive index detector (ERMA, Tokyo, Japan). The mobile phase was methanol–acetone (3:2; v/v) at a flow-rate of *ca.* 1 ml/min. The separation was performed on a 5-μm particle size Hibar RP C_{18} column (250 mm × 4 mm I.D.) (Merck) at ambient temperature. About 320 mg fat was dissolved in 1.5 ml acetone in tightly sealed vials. The vials were then tempered at *ca.* 40°C, and 20 μl of the solution were injected into the HPLC system. The PN fractions yielded were collected and evaporated to dryness at 60°C under a stream of nitrogen. Finally, the fractions were dissolved in benzene to give a concentration of *ca.* 20 mg/ml.

Ag–HPLC separation into geometrical isomers

The chromatographic system used comprised an Optilab 5931 liquid chromatograph (Tecator, Höganäs, Sweden), with the refractive index detector equipped with a 10-mm measuring cell. The separation was performed on a 5-μm particle size Hibar RP C_{18} LiChrosorb column (250 mm × 4 mm I.D.) from Merck. The mobile phase was methanol–2-propanol (3:1; v/v), with $AgNO_3$ in a concentration of 0.085 *M*, at a flow-rate of *ca.* 1 ml/min. The $AgNO_3$ was dissolved in methanol by stirring, before the 2-propanol was added, to reach dissolution at room temperature. The temperature of the total chromatographic system was kept at 20°C. The major PN fractions, at a concentration of 20 mg/ml benzene, were injected in volumes of 5–10 μl. A computer-based system, CHROMATIC (KEBO Computer Applications, Stockholm, Sweden) was used for integration purposes.

TABLE I

CHARACTERISTICS OF TRIACYLGLYCEROL STANDARDS

Structure refers to the sum of the carbon atoms in the fatty acid moieties in relation to the sum of the number of double bonds. The abbreviations c and t refer to the double bonds in *cis* and *trans* configuration, respectively, in the linoleic acid moieties. In the interests of simplification, all $C_{18:2}$ fatty acid moieties have been designated as linoleic acid (L), regardless of *cis* or *trans* configuration.

Triglyceride	Abbreviation	Structure	PN
Dioleolinolein (c,c)	OOLc,c	$C_{54:4}$	46
Dioleolinolein (t,t)	OOLt,t	$C_{54:4}$	46
Oleoelaidolinolein (c,c)	OElLc,c	$C_{54:4}$	46
Dielaidolinolein (c,c)	ElElLc,c	$C_{54:4}$	46
Palmitooleolinolein (c,c)	POLc,c	$C_{52:3}$	46
Palmitooleolinolein (t,t)	POLt,t	$C_{52:3}$	46
Palmitoelaidolinolein (c,c)	PElLc,c	$C_{52:3}$	46
Dipalmitolinolein (c,c)	PPLc,c	$C_{50:2}$	46
Dipalmitolinolein (t,t)	PPLt,t	$C_{50:2}$	46
Triolein	OOO	$C_{54:3}$	48
Dioleoelaidin	OOEl	$C_{54:3}$	48
Oleodielaidin	OElEl	$C_{54:3}$	48
Trielaidin	ElElEl	$C_{54:3}$	48
Palmitodiolein	POO	$C_{52:2}$	48
Dipalmitoolein	PPO	$C_{50:1}$	48
Dipalmitoelaidin	PPEl	$C_{50:1}$	48
Tripalmitin	PPP	$C_{48:0}$	48
Stearodiolein	StOO	$C_{54:2}$	50
Stearooleoelaidin	StOEl	$C_{54:2}$	50
Stearodielaidin	StElEl	$C_{54:2}$	50
Palmitostearoolein	PStO	$C_{52:1}$	50
Distearoolein	StStO	$C_{54:1}$	52
Distearoelaidin	StStEl	$C_{54:1}$	52

RESULTS AND DISCUSSION

Preparative fractionation into triacylglycerol groups

The aim of the preparative fractionation was to divide the TGs into well-defined groups, each containing a moderate number of TGs. Three procedures were considered for this purpose: separation according to PN, separation according to number of double bonds (NDB) and separation according to carbon number (CN). However, since CN analysis is performed by GC this procedure is not suitable for fractionation. Separation according to NDB can be performed by separation of brominated TGs. However, the bromination reaction produces irreversibly chemically altered compounds that become identical for both *cis* and *trans* configurations, and this procedure was therefore also excluded. The third procedure, separation according to PN [9] accordingly proved to be the best solution. It follows the simple relation that $PN = CN - 2 \cdot NDB$. By way of example, PN separation of Soya 41 is illustrated in Fig. 1A. Since the PN48 fraction from Soya 41 includes the geometrical isomers of at least nine different TGs, the PN peak is broadened. Among others, it includes the four geometrical isomers OOO, OOEl, OElEl and ElElEl, which are partly separated as pure standards (Fig. 1D). For purposes of comparison, Fig. 1C shows the resolution of OOO and ElElEl only. The triunsaturated OOO and the saturated PPP (Fig. 1B) are nearly separated at baseline but, since Soya 41 includes several

TABLE II

FATTY ACID COMPOSITION OF PALM 41 AND SOYA 41

Structure refers to number of carbon atoms in relation to the number of double bonds. The abbreviations c and t refer to the double bonds in *cis* and *trans* configuration, respectively. Fatty acid composition has been determined by GC on a polar fused-silica column.

Fatty acid	Abbreviation	Structure	Palm 41 (%)	Soya 41 (%)
Lauric acid	La	$C_{12:0}$	0.3	0.1
Myristic acid	M	$C_{14:0}$	1.2	0.1
Palmitic acid	P	$C_{16:0}$	42.3	10.7
Palmitoleic acid	Po	$C_{16:1}$	0.3	0.1
Margaric acid	Mg	$C_{17:0}$	0.0	0.1
Stearic acid	St	$C_{18:0}$	7.0	18.9
Oleic acid + elaidic acid	O + El	$C_{18:1c} + C_{18:1t}$	45.1	65.4
Linoleic acid	L	$C_{18:2}$	3.4	3.4
Arachidonic acid	A	$C_{20:0}$	0.4	0.4
Gadoleic acid	G	$C_{20:1}$	0.2	0.3
Behenic acid	B	$C_{22:0}$	0.0	0.4
Unknowns	–	–	0.0	0.3
Total	–	–	100	100

TGs with retention times between those of OOO and PPP, the PN48 peak looks like the peak of a single component. This is also due to the relative proportions of the different TGs within the peak. If the relative proportions are altered, the peak may become asymmetrically shaped, as for the PN50 peak of Palm 41 (Fig. 8). As the retention times of the different TGs within a PN differ, it is important to collect the whole peak at fractionation. One total fractionation, with an elution time of *ca.* 1.5 h, will provide enough material for the subsequent separation procedure.

Subsequent separation into geometrical triacylglycerol isomers

Optimization of analytical system. The subsequent separation procedure was based on argentation HPLC, in order to separate geometrical isomers.

When work on developing the method was first started, some initial experiments were carried out with silver ions bonded to the stationary phase [10]. However, this procedure also separated the positional isomers to a high degree, and the resulting chromatograms were too complex. The procedure made both identification and quantification difficult, due to the broadening of the peaks. Another procedure making use of silver ions in the mobile phase was therefore tested. This gave good separation between geometrical isomers, but separation of positional isomers was less pronounced. Also the separation of symmetric and asymmetric TG isomers (*e.g.* POP and PPO) were studied using silver ions in the mobile phase. No resolution occurred which means that our intended separation into geometrical isomers was not disturbed. Another advantage of utilizing silver ions in the mobile phase was that a RP C_{18} column could be successfully used for the separation.

Several points had to be taken into consideration in choosing the mobile phase. First, the mobile phase had to dissolve the silver nitrate properly while at the same time being sufficiently non-polar for the elution of saturated TGs. Secondly, the mobile phase had to be inert to the silver ions, so that no reaction would take place. Thirdly, the refractive index of the mobile phase had to be different from that of the TGs, since TGs were to be detected using a refractive index detector. For these reasons mobile phases such as propionitrile, acetonitrile, methanol and 2-propanol were tested. The mobile phase that

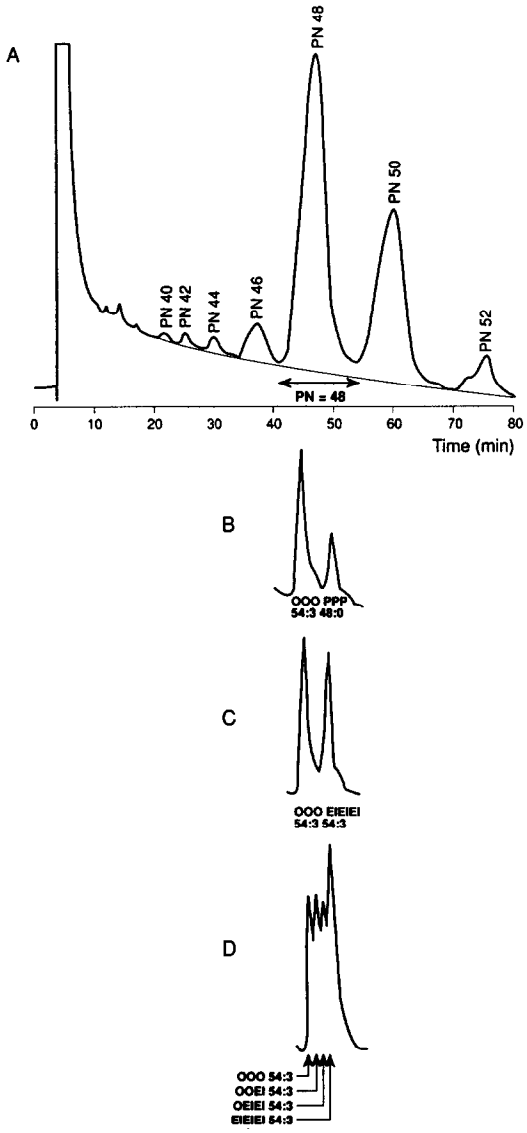

Fig. 1. PN separation of Soya 41 and illustration of the separation of different types of TG isomers included in the PN48 peak. Conditions: column Hibar RP C_{18} (250 mm × 4 mm I.D.), mobile phase methanol–acetone (3:2; v/v). Ambient temperature, flow-rate 1 ml/min. For abbreviations see Table I; for A, B, C and D see text.

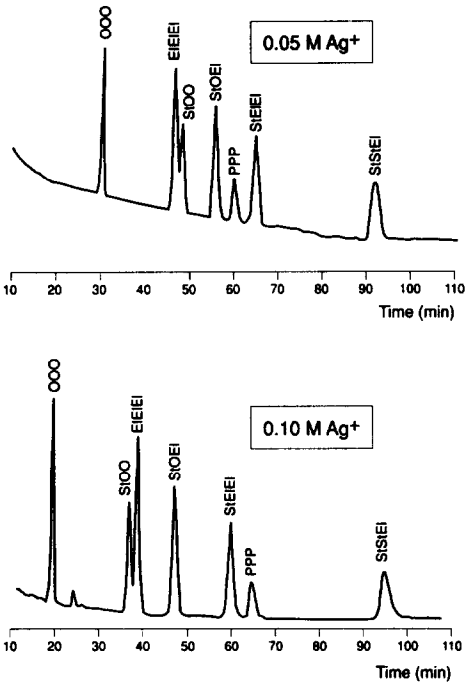

Fig. 2. Influence of silver nitrate concentration on retention times of different TGs, including geometrical isomers. Conditions: Column Hibar RP C_{18} LiChrosorb (250 mm × 4 mm I.D.), mobile phase methanol–2-propanol (3:1; v/v), system temperature 20°C, flow-rate 1 ml/min. For abbreviations see Tables I and II.

gave the best separation results was methanol–2-propanol (3:1; v/v) with dissolved silver nitrate.

The silver ions affect in two ways. They act as complexing agents with double bonds (π-complexes), and they also increase the polarity of the mobile phase. From Fig. 2 it can be seen that a saturated TG (PPP) has a longer retention time when the silver concentration is increased. The silver ions cannot form π-complexes with a saturated compound and this longer retention time must be attributable to the increased polarity of the mobile phase. Unsaturated TGs are of course also affected by the polarity of the mobile phase. The formation of π-complexes is also illustrated in Fig. 2, from which it can be seen that PPP-StEIEI and EIEIEI-StOO are eluted in inverted order when the silver concentration is increased. When the elution order of PPP and StEIEI is inverted in this way, the *trans* double bonds must have formed a complex with silver ions as otherwise, with only a change in the polarity of the mobile phase taking place, no inversion would have resulted. The inversion in the order in which EIEIEI and StOO are eluted demonstrates that there is a difference in the

strength of silver complexes with *cis versus trans* double bonds. StOO with only *two cis* double bonds is so strongly affected by the increase in the silver concentration that it elutes before ElElEl, in which there are *three trans* double bonds. Since, due to the increased polarity of the mobile phase, the stearic acid in StOO contributes to an increase in retention time, it is clear that the silver complexes with *cis* double bonds are stronger than those with *trans* double bonds. This is probably due to a sterical hindrance from the *trans* double bonds.

Silver concentrations of 0.01–0.10 *M* $AgNO_3$ were tested on mixtures with standards and on partially hydrogenated fats mainly based on monounsaturated and saturated C_{16} and C_{18} fatty acids. Higher concentrations than 0.10 *M* are not practicable because of the limited solubility of $AgNO_3$. The effect of increased silver concentration on the PN48 of a partially hydrogenated soya-bean oil is shown in Fig. 3. Here a low concentration of 0.02 *M* $AgNO_3$ is compared with a high concentration of 0.10 *M*. As can be seen, the high silver concentration gives much better separation of geometrical isomers. A silver concentration of 0.085 *M* was finally chosen, based on total overall evaluation of separation of all major PN fractions and also bearing in mind the risk of precipitation occurring with higher concentrations.

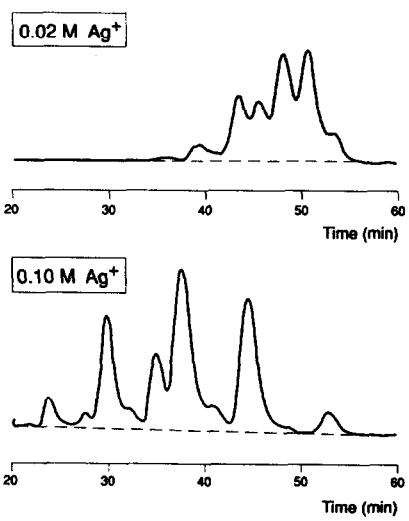

Fig. 3. Influence of silver nitrate concentration on separation of the PN48 fraction of a partially hydrogenated soya-bean oil. Conditions as in Fig. 2.

The use of silver in the mobile phase made special precautions necessary to avoid its reduction, which might have damaged the detector cell. The mobile phase had to be kept in dark bottles to protect it from light. Furthermore, the light source emitting radiation through the refractive index detector had to be in the OFF position whenever samples were not being analyzed. In the event of precipitation occurring, the silver nitrate might have damaged the pump. To avoid damage to either the detector cell or the pump, low flow had to be on even when no analysis was being performed. During longer periods with no analysis, the mobile phase with silver nitrate had to be changed to the mobile phase without. By taking these precautions we have used an HPLC system for several years without encountering any serious problems.

The influence of temperature on separation was tested briefly. Temperatures ranging from 18 to 30°C for the mobile phase and the column were tested. It was noticed that lower temperatures in this range gave better resolution, and a temperature of 20°C was finally chosen. Temperatures lower than 18°C were not considered advantageous, due to the risk of precipitation occurring.

The number of columns, *i.e.* the number of theoretical plates was arrived at from the separation point of view. With two columns, peak broadening and analysis time increased but no appreciable improvement in separation took place. One column was therefore chosen. The number of theoretical plates for the TGs present was 36 000 to 42 000.

Separation capacity. The resolution of unsaturated and saturated TGs with the same PN is strongly increased in the subsequent separation, using silver in the mobile phase, compared to in the initial fractionation. For example the separation of OOO and PPP increased dramatically (see Fig. 4 and Fig. 1B). Consequently, an extended space between the first and the last eluted component, within a PN, becomes avail-

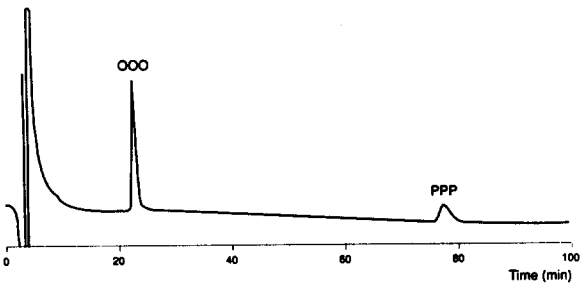

Fig. 4. Separation of two TGs, OOO and PPP, with a substantial difference in saturation but with the same PN. Conditions as in Fig. 2, but with silver concentration of 0.085 *M*. For abbreviations see Table I. PN = CN − 2 · NDB; here: $PN_{OOO} = 54 - 2 \cdot 3 = 48$ and $PN_{PPP} = 48 - 2 \cdot 0 = 48$.

able for the geometrical isomers of that particular PN. Such separations of geometrical isomers of homologous series within PN46, PN48 and PN50 are illustrated in Fig. 5A–C. PN, CN and NDB remain constant within a series and only the geometrical configurations vary. A transition from oleic acid with a *cis* configuration to elaidic acid with a *trans* configuration contributes to a major change in retention time, due to the effect of the silver ions in the mobile phase. As a result, resolution between isomers which differ in their geometrical configuration by just one double bond is excellent. From Fig. 5A–C it can also be seen that it would not have been possible to analyze the different PNs in a single analysis, as this would have resulted in overlapping peaks. Also for TGs containing palmitic acid (P), the resolution between isomers which differ in their geometrical configuration by just one double bond is excellent (Fig. 6A). Fig. 6B illustrates the separation of two isomers that differ only in the geometrical configuration of linoleic acid. In the first peak, both the double bonds in the linoleic acid are in *cis* configuration. In the second, both are in *trans* configuration.

Fig. 7A illustrates the way in which a change from one oleic acid moiety in OOLc,c to one elaidic acid moiety (OElLc,c), affects retention time to the same extent as a corresponding change from linoleic acid with both double bonds in *cis* configuration in OOLc,c to linoleic acid with both double bonds in *trans* configuration (OOLt,t). A similar change is illustrated in Fig.

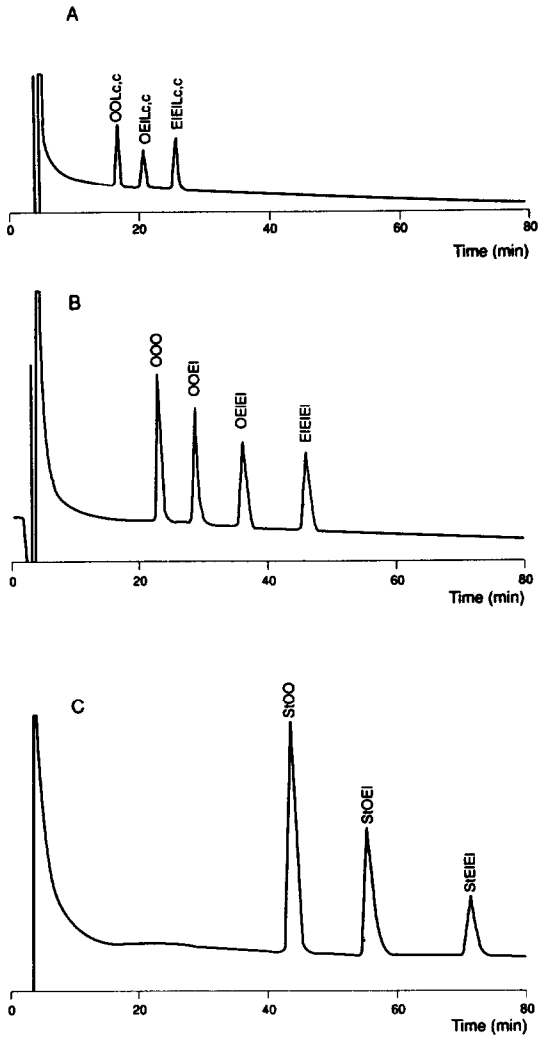

Fig. 5. Separation of homologous series of geometrical TG isomers, with C_{18} fatty acid moieties, for PN46 (A), PN48 (B) and PN50 (C). Within each series the difference between adjacent peaks can be seen to be the geometrical configuration of just one double bond. Conditions as in Fig. 4. For abbreviations see Table I.

7B. From this it would seem that a change in the configuration of the only double bond in a monounsaturated fatty acid in a given TG corresponds to a change in the geometrical configuration of both double bonds in a diunsaturated fatty acid in the same TG.

A strong separation capacity of the system for other types of TG isomers, such as the positional

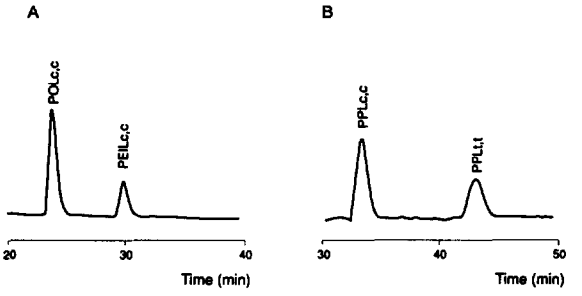

Fig. 6. Separation of homologous series of geometrical TG isomers containing palmitic acid, for PN46. Conditions as in Fig. 2, but with silver concentration of 0.085 *M*. For abbreviations see Table I; for A and B see text.

and symmetric/asymmetric (*e.g.* POP and PPO), was undesirable because this could cause overlapped and broadened peaks as well as identification problems. To check the degree of separation of positional isomers in the silver nitrate system used, two extremes were tested. These extremes, OOO (oleic acids with double bonds in position 9) and PePePe (petroselinic acids with double bonds in position 6) were clearly separated. Separation occurred in spite of the fact that attempts were made to suppress it with a view to obtaining better defined chromatograms. However, it seems unlikely that hydrogenation results in any considerable amounts of such extremes. The broadening of peaks for a partially hydrogenated fat, compared to those for model substances, can be explained by a slight variation in positional isomers (Figs. 8 and 9). The separation of symmetric/asymmetric isomers has also been studied. The pairs POP/PPO and StOSt/StStO showed no resolution.

Applications

With a view to illustrating some applications two fats were chosen, both hydrogenated to melting points of 41°C but with different fatty acid compositions (Table II).

Fig. 8 illustrates the separation and identification of Palm 41, which included a high percentage of palmitic acid (42.3%). With nearly 70% of total TGs, the peak of PN48 dominates. The sizes of the remaining PNs can be seen in Table III. The peaks that elute immediately after the solvent peak are also worthy of note. These are the diglycerides included in the fat.

The PN46, PN48 and PN50 fractions were subsequently separated into geometrical isomers (Fig. 8). The largest fractions, PN48 and PN50,

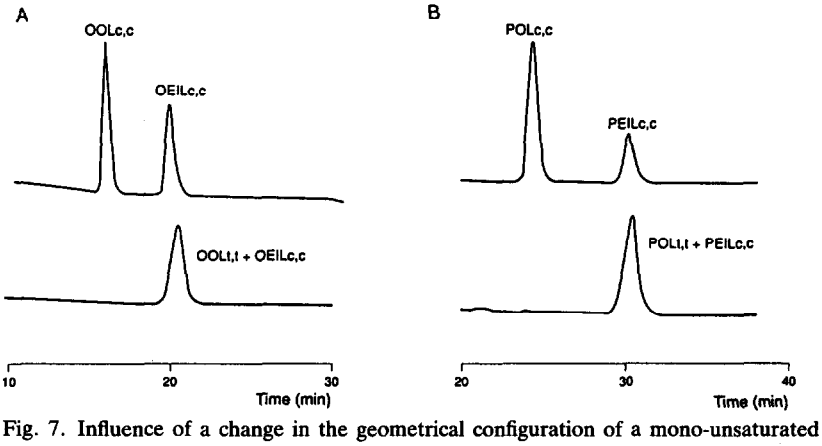

Fig. 7. Influence of a change in the geometrical configuration of a mono-unsaturated fatty acid moiety within a TG compared with that of a di-unsaturated fatty acid moiety. Conditions as in Fig. 2, but with silver concentration of 0.085 *M*. For abbreviations see Table I; for A and B see text.

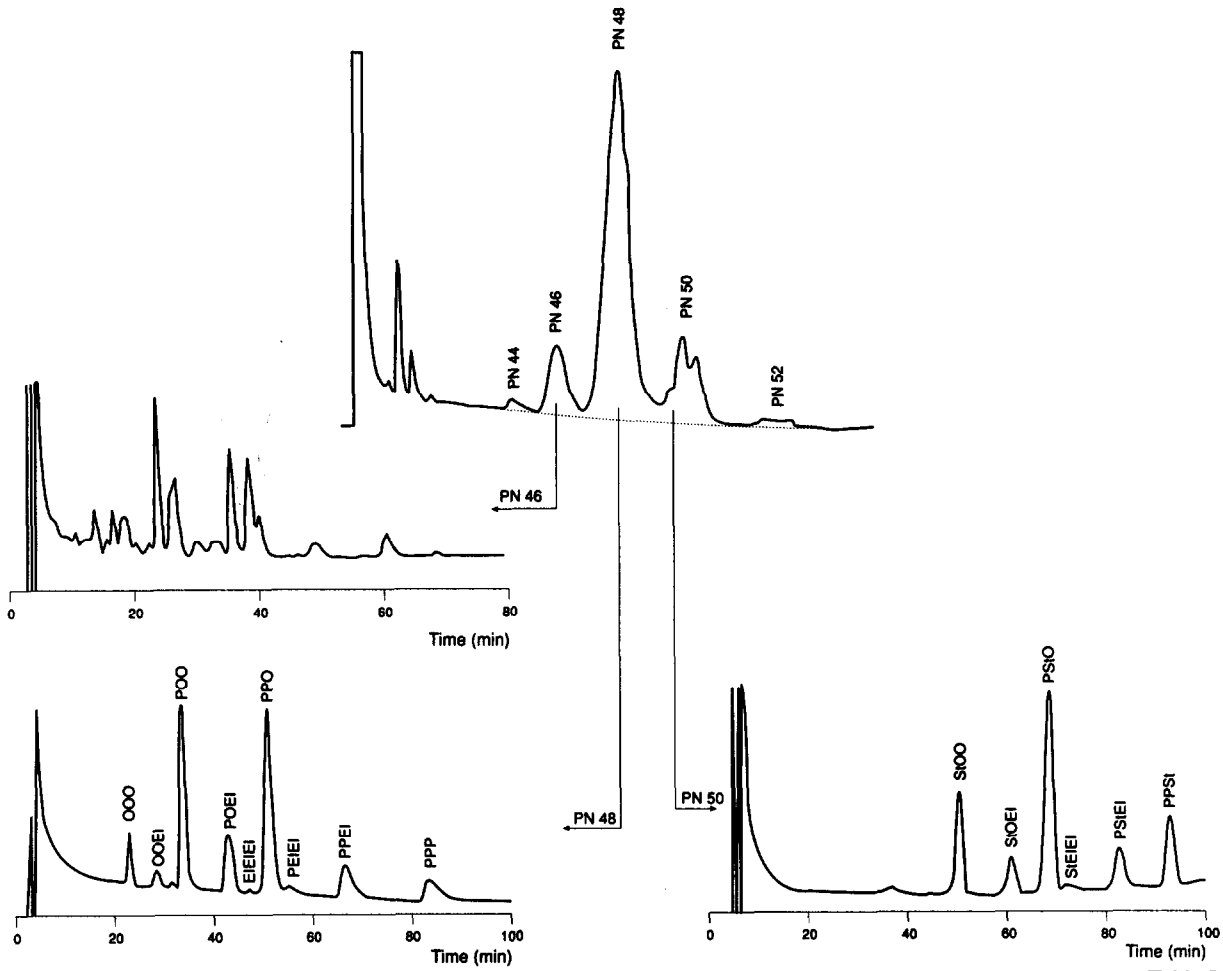

Fig. 8. Application of the proposed method on Palm 41. For conditions see under Experimental. For abbreviations see Table I.

were identified with the help of standard solutions. The PN46 peaks have not been indicated, due to incomplete identification as a result of the wide variety of TGs included. One of the reasons for this variety is that the TGs in this fraction include a large percentage of linoleic acid, which as a fatty acid can appear in four different geometrical isomers. However, since most of the TGs belong to PN48 and PN50, many of them have been identified anyway. The TGs have been separated into geometrical isomers nearly at baseline (Fig. 8).

Fig. 9 illustrates separation and identification in the case of Soya 41. This fat is especially rich in oleic, elaidic and stearic acid. Consequently, PN distribution becomes displaced to higher PNs than in the case of Palm 41 (Table III). The PN52 fraction was therefore analyzed and identified as geometrical isomers, and has been included in Table IV.

In the case of Soya 41 too, the diacylglycerols can be seen to have eluted after the solvent peak. If the chromatograms for Soya 41 and Palm 41 are compared it can be seen, as might

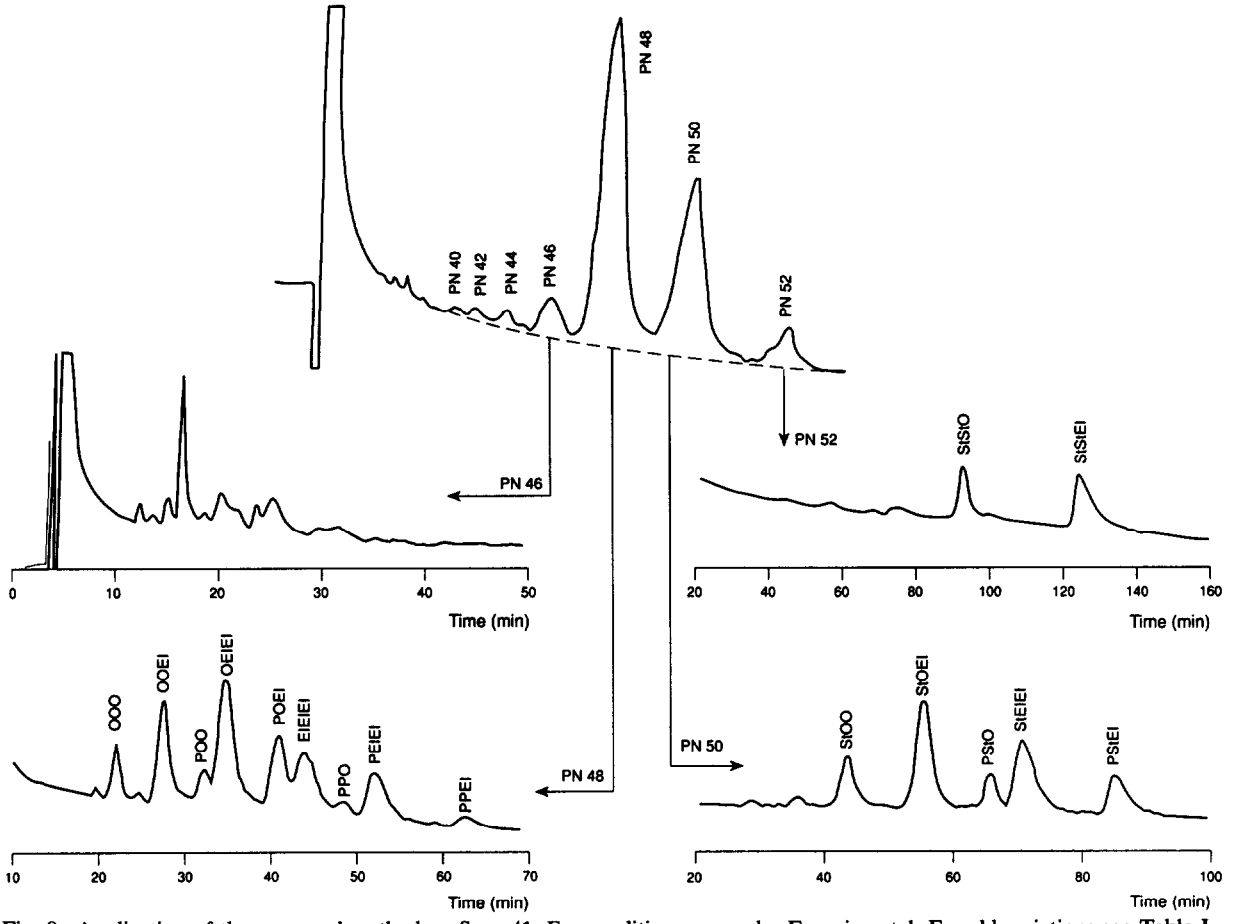

Fig. 9. Application of the proposed method on Soya 41. For conditions see under Experimental. For abbreviations see Table I.

TABLE III

PARTITION NUMBER DISTRIBUTION OF PALM 41 AND SOYA 41

PN	Palm 41 (%, w/w)[a]	Soya 41 (%, w/w)[a]
40	0.0	0.6
42	0.2	0.9
44	1.2	1.4
46	9.9	5.5
48	68.3	54.5
50	18.0	32.8
52	2.5	4.5
Total	100	100

[a] % (w/w) of total amount of TGs.

be expected, that Soya 41 contains a much smaller percentage of diacylglycerols.

Quantification of TGs for the two fats is as shown in Tables III and IV. The amounts have been expressed in % (w/w), which is approximately equal to area% when TGs do not differ much in CN and NDB.

Reproducibility

The relative standard deviation of the total analysis of TG amounts of 15 and 3% has been calculated as 2 and 5%, respectively. The calculations of the standard deviations are based on that the total analysis can be designated as a multiplicative expression [11].

TABLE IV

TRIACYLGLYCEROL COMPOSITION OF PALM 41 AND SOYA 41, INCLUDING GEOMETRICAL ISOMERS

For abbreviations see Table I.

Triglyceride			Palm 41 (%, w/w)[a]	Soya 41 (%,w/w)[a]
Abbreviation	Structure	PN		
OOO	$C_{54:3}$(c,c,c)	48	3.6	2.7
OOEl	$C_{54:3}$(c,c,t)	48	1.7	8.1
POO	$C_{52:2}$(c,c)	48	17.8	2.5
OElEl	$C_{54:3}$(c,t,t)	48	0.1	14.9
POEl	$C_{52:2}$(c,t)	48	7.9	7.3
ElElEl	$C_{54:3}$(t,t,t)	48	0.4	8.7
PPO	$C_{50:1}$(c)	48	24.3	1.5
PElEl	$C_{52:2}$(t,t)	48	1.4	6.3
PPEl	$C_{50:1}$(t)	48	5.9	1.1
PPP	$C_{48:0}$	48	4.8	0.0
Unidentified peaks	–	48	0.4	1.4
StOO	$C_{54:2}$(c,c)	50	3.1	4.0
StOEl	$C_{54:2}$(c,t)	50	1.6	10.8
PStO	$C_{52:1}$(c)	50	8.1	3.0
StElEl	$C_{54:2}$(t,t)	50	0.4	9.1
PStEl	$C_{52:1}$(t)	50	2.1	4.9
PPSt	$C_{50:0}$	50	2.8	0.0
Unidentified peaks	–	50	0.0	1.0
StStO	$C_{54:1}$(c)	52	–	1.4
StStEl	$C_{54:1}$(t)	52	–	2.5
Unidentified peaks	–	52	–	0.6

[a] % (w/w) of total triacylglycerols.

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