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Regiospecific characterisation of the triacylglycerols in animal fats using high performance liquid chromatography-atmospheric pressure chemical ionisation mass spectrometry

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High performance liquid chromatography-atmospheric pressure chemical ionisation mass spectrometry (HPLC-APCI MS) was applied to the characterisation of triacylglycerols (TAGs) in animal fats. The major TAGs in four fats (beef, chicken, lamb and pork) were identified and positional isomers assigned according to their APCI mass spectra. Beef and lamb fat TAGs were confirmed as containing higher proportions of saturated fatty acids compared with those of chicken and pork. HPLC-APCI MS was also shown to be of value in providing regiospecific information for the fatty acids in individual TAG species. For example, beef and lamb fat were shown to contain both *cis*- and *trans*-isomers of the 18 : 1 fatty acid, whilst chicken and pork contained only the *cis*-isomer. When the position of fatty acid substitution was determined from the APCI spectra, whilst the *cis*-18 : 1 was predominantly found in the 2-position of the TAG, the *trans*-18 : 1 showed a preference for the 1/3-position. Similarly, it was confirmed that although the 2-position of beef, chicken and lamb fat TAGs was dominated by unsaturated fatty acids, in pork fat, a characteristically high proportion of palmitic acid was seen in this position. The TAGs identified compared well with those reported previously. The distributions of 2-position fatty acids seen in lamb and pork fat compared favourably with those obtained by the more traditional method of lipase degradation. Although the distributions for chicken and beef showed some discrepancies, these can be attributed to weaknesses in the quantification procedure or the specificity of the lipase. Overall, the technique of HPLC-APCI MS has been shown to be very powerful for the regiospecific analysis of animal fats.

Introduction

Animal fats comprise complex mixtures of triacylglycerols (TAGs). Characterisation of these mixtures requires determination not only of which fatty acids are present in each triacylglycerol, but also the position at which each fatty acid is esterified to the glycerol backbone, since this has important implications for nutrition.[1] Traditional methods for determining the 2-position fatty acids of TAGs include stereospecific enzymatic hydrolysis[2] and partial hydrolysis of the molecules followed by derivatisation and analysis by chiral chromatography.[3] These methods have the disadvantages of being time-consuming and complex and only provide information on the overall 2-position composition of a mixture rather than information on each molecule.

The most efficient separations of TAGs have been achieved using reversed phase HPLC, with an octadecylsilyl column and acetonitrile- or propionitrile-based mobile phases, allowing separation according to both carbon number and total number of double bonds.[4] Combining HPLC with mass spectrometric detection allows unambiguous identification of compounds, and the relatively new technique of atmospheric pressure chemical ionisation (APCI) has proved to be highly suitable for the analysis of TAG mixtures.[5–7] In addition, it has been shown that APCI spectra of TAGs provide invaluable information on the distribution of fatty acids within individual TAG species, allowing the 2-position fatty acid to be identified.[8] The technique has been successfully applied to the regiospecific analysis of a range of vegetable oils.[9]

The aim of the work presented in this paper was to apply the techniques developed for regiospecific analysis of vegetable oils to the characterisation of a range of animal fats. The four animal fats chosen for analysis, namely beef, lamb, pork and chicken, represent a range of complexities and varying degrees of unsaturation. Whilst the fatty acid compositions and stereospecific distributions of these fats have been widely reported, much less work has been published on the intact TAG composition of the fats.

Materials and methods

Extraction of animal fats

Beef, chicken, lamb and pork samples were obtained from a local supermarket. A portion (*ca.* 2 g) of subcutaneous adipose fat was cut from the meat and ultrasonicated for 15 min with dichloromethane–methanol (2 : 1 v/v; 100 ml). The extract was then dried by passing it through a short column of anhydrous sodium sulfate. The bulk of the solvent was removed by rotary evaporation and the remainder removed under a gentle stream of nitrogen. The extracts were stored in a deep freeze (−20 °C) until required for analysis.

Preparation of fatty acid methyl esters (FAMEs)

The fats were saponified and methylated under conditions similar to those described by Hamilton and Hamilton.[10] NaOH in methanol (2 ml, 0.5 M) was mixed with *ca.* 100 mg of fat in a screw-capped test tube and heated at 70 °C for 45 min. The sample was cooled, then acidified to pH 3 with 1 M HCl. The free fatty acids were extracted with hexane (3 × 5 ml). An aliquot (1 ml) of the combined extracts was taken and blown

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down under a stream of nitrogen. Approximately 100 μl of 14% w/v boron trifluoride–methanol complex (BDH) was added and heated at 70 °C for 45 min. After cooling, water (6 ml) and diethyl ether (3 ml) were added and the FAMEs extracted into the ether. The ether was evaporated under a gentle stream of nitrogen and the methyl esters redissolved in hexane (2 ml) for analysis by GC.

Lipase degradation

The determination of the composition of fatty acids in the 2-position was carried out using a method based on the British Standards Institution method BS 684, section 2.39:1986. A test portion of the fat (*ca*. 0.5 g) was dissolved in hexane and passed through an alumina column in order to remove non-triacylglycerol components. A portion of this purified fat (0.1 g) was weighed into a centrifuge tube and placed in a water bath at 65 °C for not more than 40 s. Once liquid, they were immediately transferred to a water bath at 40 °C. Buffer solution [2-amino-2-(hydroxymethyl)propane-1,3-diol, 1 M (Sigma), adjusted to pH 8 with aqueous 6 M HCl, 2 ml], aqueous sodium cholate solution (1 g l^{-1}, 0.5 ml) and aqueous calcium chloride solution (220 g l^{-1}, 0.2 ml) were added. Pig pancreatic lipase (20 mg, Sigma) was added and the mixture shaken for the exact length of time determined for each lipid, or for 0 to 20 min when assessing the activity of the pancreatic lipase. The tube was then removed from the water bath and vortex mixed for a further 2 min. Hydrochloric acid (6 M, 1 ml) and diethyl ether (1 ml) were added and the tube shaken vigorously. After centrifugation (1200*g*, 5 min) the organic phase was transferred to a vial and the extraction repeated. When assessing the activity of the lipase, a portion of the extract was taken, blown down under nitrogen gas and derivatised with *N*,*O*-bis(trimethylsilyl) trifluoroacetamide (BSTFA, 20 μl (Sigma), 80 °C, 1 h). and assessed by high temperature gas chromatography (HT-GC) as described below.

The 2-monoacylglycerols (MAGs) were separated from the other components of the organic phase using thin layer chromatography (TLC). The mixture was applied to a silica TLC plate (LK6F, 250 μm layer thickness, 60 Å particle size, 20 × 20 cm, Whatman) and developed using a mixture of hexane:diethyl ether:formic acid (70:30:1 v/v/v). The plate was visualized in an iodine tank and the band corresponding to the MAGs (R_f 0.06) scraped off. Fatty acid methyl esters of the MAGs were prepared directly from the silica and analysed as described below.

HPLC-APCI MS analysis of animal fats

HPLC-MS analyses were performed on a Waters 600MS quaternary solvent delivery system, coupled to a Finnigan MAT TSQ700 fitted with an APCI source. This was operated with a vaporizer temperature of 450 °C, capillary temperature of 280 °C and corona current of 5 μA. High purity nitrogen was used for the sheath and auxiliary gases, at 60 psi and 20 ml min^{-1} respectively. Spectra were obtained over the range *m*/*z* 200 to 1000, with a scan time of 2 s. A Supelcosil LC-18 column (octadecylsilyl bonded phase, 25 cm × 10 mm id, 5 μm particle size, 100 Å pore size) was used, with propionitrile as the mobile phase at a flow rate of 0.8 ml min^{-1}. All samples were dissolved in propionitrile to a concentration of 5% (v/v) for injection (20 μl) onto the HPLC column. The mass spectral data were expressed as base peak chromatograms, in which the intensity of the base peak of each scan was plotted against time. Since APCI spectra exhibit little fragmentation, base peak chromatograms help to remove background noise.

Gas chromatography

Gas chromatography analyses were carried out on a HP 5890A GC using on-column injection (1 μl) and the following chromatographic conditions.

Fatty acid methyl esters. Fatty acid methyl ester (FAME) analyses were carried out using a 25 m × 0.32 mm id fused silica capillary column coated with BPX70 stationary phase (immobilized 70% cyanopropyl equivalent modified siloxane, 0.12 μm film thickness, SGE). The oven temperature program was 50 °C (2 min) to 150 °C at 15 °C min^{-1} then to 240 °C (20 min) at 4 °C min^{-1}. Helium was used as the carrier gas at a column head pressure of 15 psi.

Lipase reaction mixtures. High temperature GC analysis of the derivatised mixture of fatty acids, mono-, di- and triacylglycerols obtained when assessing the activity of the lipase was carried out using a DB1-HT fused silica capillary column (100% polydimethylsiloxane, 15 m × 0.32 mm id, 0.1 μm film thickness, J&W Scientific). The carrier gas was hydrogen and the oven temperature program was 50 °C (2 min) to 350 °C (10 min) at 10 °C min^{-1}.

Gas chromatography-mass spectrometry (GC-MS)

Identification of FAMEs and components present in the lipase hydrolysis mixtures was carried out using GC-MS. These analyses were performed on a Carlo Erba Mega Series gas chromatograph coupled to a Finnigan MAT 4500 mass spectrometer which was operated in electron ionisation mode (70 eV). Scans were performed from *m*/*z* 50 to 850 at a frequency of two scans per second. Data were collected using an INCOS data system and processed using the Interactive Chemical Information Software (ICIS) package. The chromatographic conditions employed were identical to those described above.

Quantification of TAG components

TAG components present in HPLC-APCI MS chromatograms were quantified according to the method described by Byrdwell and Neff.[7]

Results and discussion

Interpretation of APCI mass spectra of TAGs

The four animal fats, namely beef, chicken, lamb and pork, were analysed by HPLC-APCI MS. The component TAGs were identified according to their mass spectra and positional isomers assigned according to the relative abundances of the DAG ions, as described previously,[9] whereby the least abundant of the $[M-RCO_2]^+$ ions resulting from loss of a fatty acyl moiety (diglyceride ions, $[DG]^+$) corresponds to the loss of the fatty acid in the 2-position. Similarly, the regiospecific configuration of AAB and ABA type TAG species can be determined by the ratio of the resulting $[AA]^+$ and $[AB]^+$ ions. For an AAB type TAG, this ratio is around 1, whereas an ABA type molecule exhibits a spectrum with a significantly lower $[AA]^+:[AB]^+$ ratio.

The TAG species identified in each of the fats are listed in Table 1, where the nomenclature reflects the position of fatty acid substitution. Hence, OOO represents triolein, LOL represents 1,3-dilinoleoyl-2-oleoyl glycerol and POS represents 1(3)-palmitoyl-2-oleoyl-3(1)-stearoyl glycerol. No distinction

Table 1 Qualitative acylglycerol composition of a range of animal fats

	Beef	Chicken	Lamb	Pork
Diacylglycerols				
MyO[a]	*[b]	—[c]	*	—
LL	—	*	—	*
LO	*	*	—	*
PL	*	*	—	*
OO	*	*	—	*
PO	*	*	*	*
OS	*	—	*	—
Triacylglycerols				
LnLnL	—	3[d]	—	3
LLnL	—	3	—	3
LnLnO	—	2	—	3
PoLLn	—	2	—	—
PoPoLn	—	3	—	—
LnLnP	—	2	—	—
LLL	—	*	—	*
LLnO	—	3	—	3
LPoL	—	2	—	—
PLLn	2	2	—	3
PLnPo	—	2	—	—
PoLPo	—	—	—	3
MyLPo	—	3	—	—
LLO	—	3	LOL 2	3
PoLO	—	3	—	1
LLP	3	3	—	2
PLnO	3	2	2	POLn 2
PMxO	3	—	—	—
PoMyO	2	—	—	—
MyPoP	2	—	—	—
PLnP	—	3	—	—
OLO	3	3	LOO 2	3
OOPo	3	3	—	—
LLS	LSL 3	—	2	2
OPoO	—	—	—	2
OMyO	—	—	3	—
OPL	OLP 3	PLO + POL	POL + LPO	3
PPoO	2	3	3	3
PSLn	—	—	—	2
PLP	—	3	—	3
MyOP	PMyO 3	2	3	—
PPoP	—	2	—	—
OOO	*	*	*	*
SLO	—	3	—	3
OPO	3	OPO + OOP	OOP 3	3
OPO[e]	3	—	3	—
SPL	—	—	—	3
POP	3	3	3	3
PPO[e]	3	—	3	—
PPoS	2	—	—	3
PPP	*	*	—	—
PMoS	2	—	—	—
PMaO	1	—	2	—
PMyS	3	3	2	—
OSO	2	3	OOS 3	3
OOS[e]	2	3	OSO 3	—
POS	3	POS + PSO	3	3
PSO[e]	3	—	SPO 3	—
PPS	PPS + PSP	3	3	3
MaOS	3	3	—	—
PMaS	2	—	3	—
POA	2	—	—	3
MoSS	2	—	2	—
SOS	3	SSO + SOS	3	3
SSO[e]	3	—	3	—
SPS	PSS + SPS	PSS + SPS	2	3
MaSS	2	—	1	—
SPA	1	—	—	3
SSS	*	—	*	*

[a] Abbreviations for fatty acids: My, myristic (tetradecanoic) acid (14:0); Mx, myristoleic (9-tetradecenoic) acid (14:1); P, palmitic (hexadecanoic) acid (16:0); Po, palmitoleic (9-hexadecenoic) acid (16:1); Ma, margaric (heptadecanoic) acid (17:0); S, stearic (octadecanoic) acid (18:0); O, oleic (9-octadecenoic) acid (18:1); L, linoleic (9,12-octadecadienoic) acid (18:2); Ln, linolenic (9,12,15-octadecatrienoic) acid (18:3); A, arachidic (eicosanoic) acid (20:0). [b] *No positional isomers possible or not distinguished. [c] Not found. [d] 1,2,3 relative confidence of positional isomer assignment—3 highest, 1 lowest. Unless indicated, positional configuration is as shown in column 1. [e] *trans*-18:1.

is made between the *sn*-1 and *sn*-3 positions. In addition to the TAG components, some diacylglycerols (DAGs) were identified in the fats. Since certain co-eluting TAGs can give rise to common diacylglycerol ions (*e.g*. POP and PPoS both give rise to *m*/*z* 577) it can be difficult to assign a positional isomer to the two components with confidence. Consequently, each of the TAG species listed in Table 1 is accompanied by a confidence rating out of three for the positional isomer assignment, where 3 is the highest and 1 the lowest. The relative percentages of each component are listed in Table 2. The overall distributions of fatty acids in the 2-position of each fat were calculated from these data and are given in Table 3.

Lamb fat

The base peak mass chromatogram of lamb fat (Fig. 1a) is extremely complex and reflects the high proportion of saturated fatty acids as determined by GC (Table 4). Stearic and palmitic acid containing components dominate the profile and several odd-chain fatty acid containing TAGs can be seen, including PMaS and MaSS. Many of the TAGs identified, including OOP, POP, OOS and SOS, are present as two distinct chromatographic peaks, due to the two different geometric isomers of the monounsaturated C_{18} fatty acid known to be present in lamb fat. The *cis*- and *trans*-isomers were assigned by comparing the relative proportions of the two peaks and by comparing their relative retention times with those of the single peak observed in vegetable oil and pork fat chromatograms, where only the *cis*-isomer is present. It is interesting to note that whilst the *cis*-18:1 is found in the *sn*-2 position of the TAG, the *trans*-18:1 tends to be found in the *sn*-1/3 position. This is entirely consistent with stereospecific analyses on sheep adipose tissue[11] and is possibly because the *trans* double bond gives a straighter chain than the *cis*, and thus acts more like a saturated fatty acid.

The complexity of the chromatogram means that certain components, particularly those which elute at shorter retention times, have not been identified. Mass chromatograms of potential $[DG]^+$ ions relating to TAGs containing odd-chain fatty acids, suggest that several may be present, however the complexity of the spectra means unambiguous identification is difficult without enhanced chromatographic resolution.

Beef fat

The fatty acid composition of beef adipose tissue is similar to that of sheep, although it has a slightly lower melting point due to a lower proportion of stearic acid (Table 4). The TAG profile of beef fat (Fig. 1b) reflects the fatty acid composition, showing a high proportion of saturated fats. As with lamb fat, geometric isomers of 18:1 give rise to two peaks for certain components, which is consistent with previous reports.[12] In addition, some saturated TAGs, such as SPS/SSP, are present as coeluting positional isomers. Whilst the left hand side of the peak shows a $[SS]^+$:$[SP]^+$ ratio of less than 1, indicating SPS (Fig. 2a), the right hand side of the peak shows a ratio much closer to one, suggesting SSP (Fig. 2b).

The most abundant fatty acid in the 2-position is 18:1, with stearic and palmitic acids present in lower abundance (Table 3). This is in agreement with the results of stereospecific analysis.[13,14] However, the percentage of 17:1 in the 2-position is far higher than that observed in previous studies, because the response factor calculated for 17:1 was anomalously high. The high response factor is due to a discrepancy between the percentage of 17:1 noted in the HPLC-APCI MS data (0.26%) and that seen in the GC fatty acid composition (1.4%).

Perrin and Prévot[15] identified fifteen TAG species in beef tallow, the majority of which were noted in this study along with several additional components. The relative proportions of the

TAGs present in the two studies compare reasonably well, although there are discrepancies for certain components, such as OOP, OPO and OSS. This is possibly due to the increased sensitivity and specificity afforded by the mass spectrometer compared with the laser light scattering detector used by Perrin and Prévot, allowing the unambiguous identification of more components. Differences in the diets of the animals from which fats derive may also affect the TAG compositions.

Table 2 Percent compositions of TAG components identified in animal fats

	Beef	Chicken	Lamb	Pork
MyO[a]	< 0.05	—[b]	—	—
LL	—	< 0.05	—	0.28
LO	< 0.05	< 0.05	—	< 0.05
PL	< 0.05	< 0.05	—	< 0.05
OO	< 0.05	< 0.05	< 0.05	< 0.05
PO	< 0.06	< 0.05	< 0.05	< 0.05
OS	< 0.07	—	< 0.05	—
LnLnL	—	0.09	—	0.06
LLnL	—	0.38	—	0.34
LnLnO	—	0.09	—	0.11
PoLLn	—	0.31	—	—
PoPoLn	—	0.16	—	—
LnLnP	—	0.10	—	—
LLL	—	1.05	—	1.44
LLnO	—	1.33	—	1.27
LPoL	—	0.90	—	—
PLLn	0.35	0.80	—	0.80
PLnPo	—	0.31	—	—
PoLPo	—	0.41	—	0.13
MyLPo	—	4.28	—	—
LLO	—	2.94	0.08	4.41
PoLO	—	—	—	1.07
LLP	0.08	3.22	—	3.89
PLnO	1.89	1.44	2.88	1.42
PMxO	1.37	—	—	—
PoMyO	0.80	—	—	—
MyPoP	1.26	—	—	—
PLnP	—	0.35	—	—
OLO	0.15	7.05	0.26	6.68
OOPo	0.71	4.43	—	—
LLS	0.26	—	0.21	1.64
OPoO	—	—	—	1.34
MyOO	—	—	1.56	—
OPL	0.79	7.05	0.84	8.20
PPoO	3.57	4.92	2.83	1.78
PSLn	—	—	—	0.80
PLP	—	3.31	—	1.81
MyOP	3.16	1.31	3.63	—
PPoP	—	0.82	—	—
OOO	1.13	7.77	1.54	6.85
SLO	—	6.06	—	4.74
OPO	4.13	9.14	4.56	14.77
OPO[c]	1.70	—	1.10	—
SPL	—	—	—	8.14
POP	4.30	6.97	3.04	3.32
PPO[c]	—	—	1.45	—
PPoS	—	—	—	0.61
PPP	—	0.99	—	—
PMoS	7.50	—	—	—
PMaO	2.25	—	2.64	—
PMyS	4.62	0.28	5.82	—
OSO	4.80	7.47	6.79	4.45
OOS[c]	2.24	0.79	1.98	—
POS	12.34	7.97	8.05	13.56
PSO[c]	2.64	—	4.64	—
PPS	5.27	1.61	5.05	0.85
MaOS	3.21	1.24	—	—
PMaS	3.76	—	5.04	—
POA	—	—	—	1.12
MoSS	—	—	3.42	—
SOS	10.18	1.86	10.05	1.68
SSO[c]	1.55	—	5.03	—
SPS	7.81	0.65	8.13	1.82
MaSS	2.13	—	3.10	—
SPA	0.54	—	—	0.32
SSS	3.44	—	6.24	0.22

[a] Abbreviations are given in Table 1. [b] — = not detected. [c] *trans*-18:1.

Pork fat

The composition of pork adipose tissue is distinctly different from that of either beef or sheep, since it contains a higher proportion of unsaturated fatty acids. In addition, only the *cis*-isomer of 18:1 is present, which gives rise to an HPLC profile which is simpler than that observed for lamb or beef fat (Fig. 3a). The relatively high proportion of linoleic (L) and linolenic (Ln) acids present is reflected by the TAGs at shorter retention times, such as LnLnL and LnLL. The TAG composition compares reasonably well with that reported by Perrin and Prévot[15] using laser light scattering detection. Twelve additional TAGs were identified, most of which appeared at relatively short retention times. The 2-position of the pork fat is

Table 3 Distribution of fatty acids in the 2-position, determined by quantification of the TAG species present in the HPLC-APCI MS profiles

	Percentage of total			
Fatty acid	Beef	Chicken	Lamb	Pork
14:0[a]	8.6	—[b]	7.4	—
14:1	1.4	—	—	—
15:0	—	—	—	—
16:0	11.6	9.4	20.8	54.8
16:1	4.8	1.9	2.8	2.0
17:0	6.0	—	7.7	—
17:1	7.5	—	—	—
18:0	21.4	14.3	19.8	7.2
18:1	35.8	40.5	38.4	15.3
18:2	1.0	29.8	0.2	19.1
18:3	1.9	4.1	2.9	1.7

[a] Carbon number:number of double bonds. [b] — = Not detected.

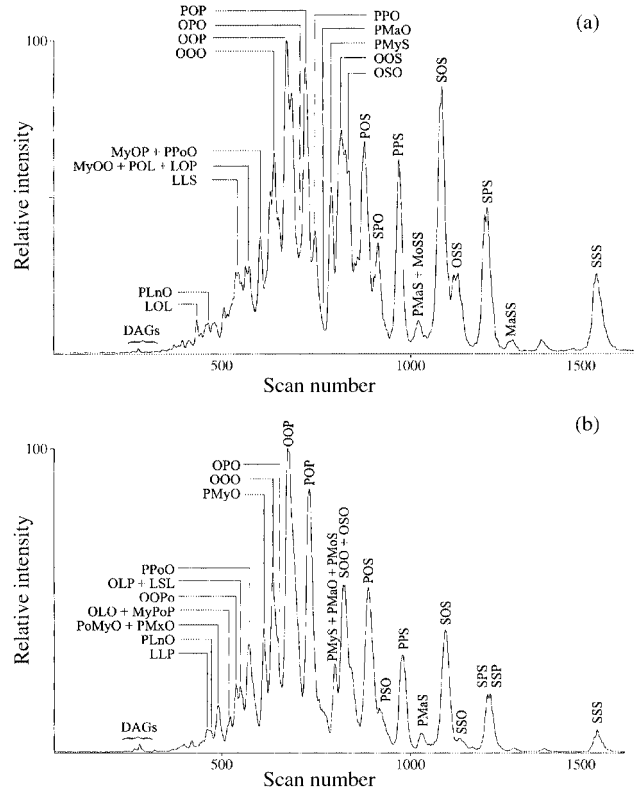

Fig. 1 The HPLC-APCI MS base peak mass chromatograms of (a) lamb fat and (b) beef fat.

dominated by palmitic acid, with oleic and linoleic acids also present (Table 4); this is characteristic of pork fat and consistent with previous reports.[16]

Chicken fat

Chicken fat is shown to have a considerably higher proportion of unsaturated fatty acids than sheep or beef lipids and a lower proportion of stearic acid than beef, sheep or pork fat (Table 4). The TAG composition (Fig. 3b) reflects the unsaturated nature of the fatty acid composition, with the most abundant components being POO, POL, LOO, OOO and PPO (Table 2). No geometric isomerism is observed, but some TAGs are present as two positional isomers. All but one of the components identified by Viau and Gandemer[17] are noted in similar relative proportions, as well as nineteen TAGs previously unreported in chicken. Unsaturated fatty acids predominate in the 2-position (Table 3), with most diacid TAGs present in the symmetrical ABA form, which is in agreement with previous reports.[18]

Table 4 Fatty acid composition of reference fats determined by GC analysis

	Percentage composition			
Fatty acid	Beef	Lamb	Chicken	Pork
14:0[a]	3.7	4.1	0.7	1.2
14:1	0.8	—[b]	—	—
15:0	1.3	1.0	—	—
16:0	25.0	19.8	22.3	21.1
16:1	3.5	1.6	5.5	1.7
17:0	3.1	2.5	0.3	0.5
17:1	1.4	0.5	0.2	0.5
18:0	31.4	32.1	10.0	14.1
18:1	25.1	31.4	41.2	37.3
18:2	1.0	2.1	16.9	18.7
18:3	1.4	1.2	2.0	1.6
19:0	1.2	0.9	—	—
19:1	0.1	1.0	—	—
20:0	0.7	0.8	1.0	0.5
20:1	0.1	0.7	—	2.8

[a] Carbon number:number of double bonds. [b] — = Not detected.

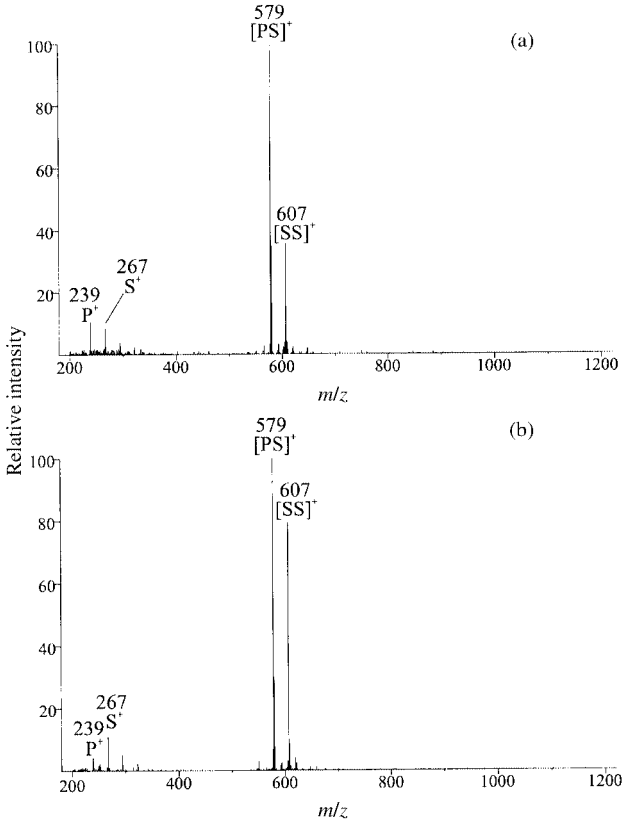

Fig. 2 APCI mass spectra of co-eluting positional isomers seen in the HPLC profile of beef fat. The spectrum of the left hand side of the peak (a) corresponded to 1,3-distearoyl-2-palmitoyl glycerol (SPS) whilst the spectrum of the right-hand side of the peak (b) corresponded to 1(3),2-distearoyl-3(1)-palmitoyl glycerol (SSP).

Determination of 2-position fatty acids by lipase degradation method

In order to validate the HPLC-APCI MS method for determining the 2-position fatty acids in TAGs, the data were compared with those obtained using a conventional lipase degradation method in which the 1- and 3- position fatty acids are cleaved and the fatty acids in the 2-position determined by GC analysis. The lipase shows differing specificities for different fatty acids and in addition, a secondary function of the enzyme enables conversion of 2-monoacylglycerols to 1-monoacylglycerols. Consequently, since the four animal fats exhibited distinctly different fatty acid profiles, it was necessary to determine the optimum hydrolysis time for each sample, in order to achieve the maximum amount of TAG hydrolysis with the minimum degree of transesterification. To assess these factors, each of the animal fats was treated with lipase for varying lengths of time. The resulting lipase digests were then analysed by HT-GC and the components identified using GC-MS.

The HT-GC profiles for chicken fat after 5, 10, 15 and 20 mins of lipase treatment are shown in Fig. 4. The sequential hydrolysis of TAGs is clearly indicated by the decreasing relative abundance of TAGs, the initial increase and subsequent decrease in the amount of DAGs and the increase in the

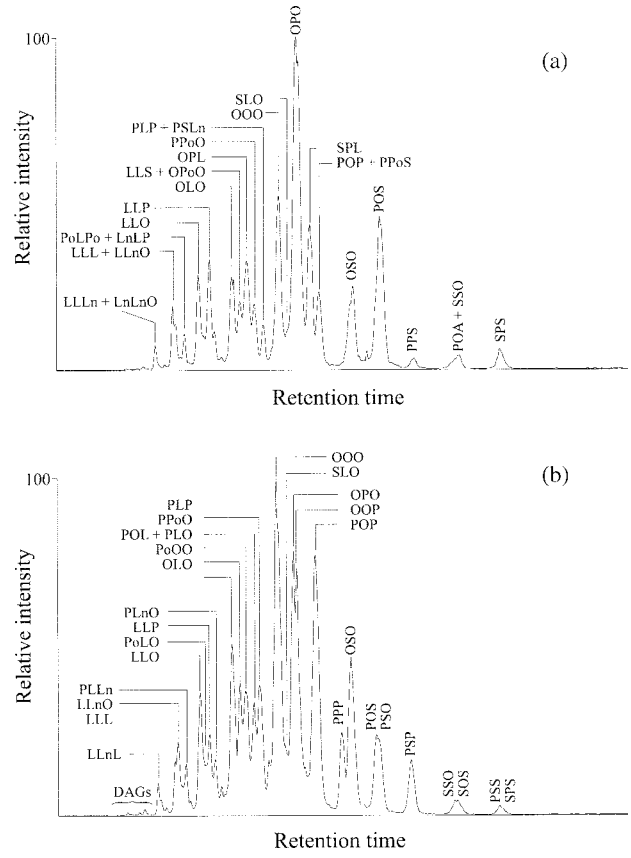

Fig. 3 The HPLC-APCI MS base peak mass chromatograms of (a) pork fat and (b) chicken fat.

quantities of MAG components. However, by 15 min, certain MAG components, such as 2-monoolein (2-O) have decreased in abundance and are replaced by higher levels of the corresponding 1-MAG, suggesting that some transesterification has occurred. Consequently, an optimum hydrolysis time of 12 min was chosen for chicken fat. The optimum times for the beef, lamb and pork fats were 7, 15 and 10 min, respectively. The variation in these times is probably due to the selectivity of the lipase. Lamb fat appears to contain a higher proportion of fatty acids which are cleaved more slowly by the lipase, whilst beef fat appears to contain a higher proportion of those fatty acids which are rapidly hydrolysed. Alternatively other, non-TAG components of the fat may interfere with the lipase to differing degrees.

Comparison of HPLC-APCI MS with lipase degradation

Once the optimum hydrolysis time for each fat had been established, the 2-MAGs resulting from enzymatic hydrolysis of the TAGs could be isolated by TLC, then derivatised to yield fatty acid methyl esters, which were subsequently analysed by GC. The results of the lipase degradation method are compared with those obtained from HPLC-APCI MS in Fig. 5. The two sets of data compared favourably for both lamb and pork fat, however for beef and chicken fats, there was some discrepancy. Specifically, stearic acid was present at a higher abundance in the HPLC-APCI MS method whilst 18:1 and palmitic acids were generally underrepresented compared with the lipase degradation. This may be due to the specificity of the lipase giving rise to differential rates of hydrolysis for different acids. Alternatively, the presence of unidentified TAGs in the early part of the chromatogram and the anomalously high response factor obtained for 17:1 in beef fat, may cause the overall distribution of TAGs to be distorted somewhat. The advantage of the HPLC-APCI MS method over the lipase degradation is that the mass spectrometric method gives the regiospecific configuration for individual TAG species within the mixture, even where two positional isomers co-elute. In contrast, the lipase degradation can only give an overall distribution.

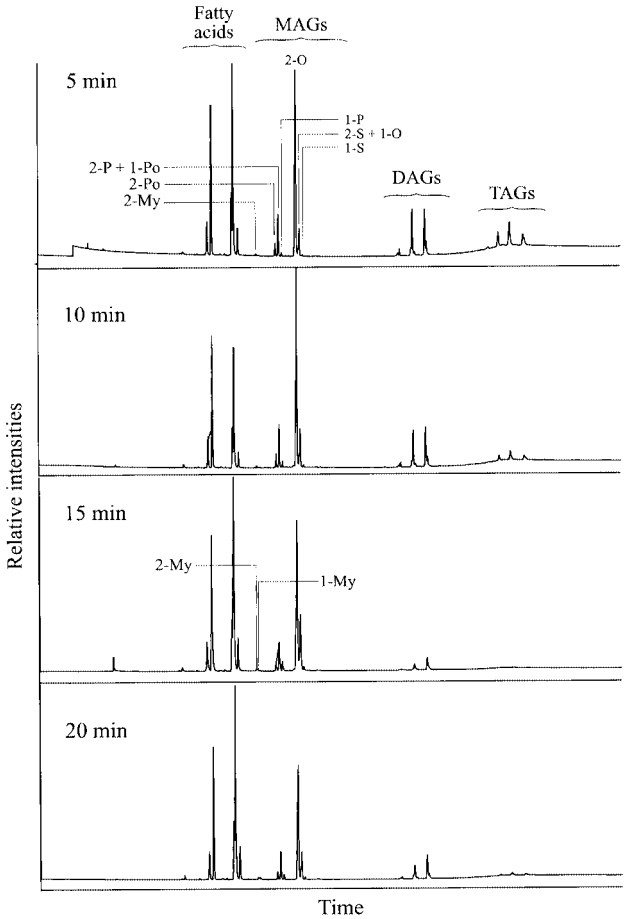

Fig. 4 HT-GC profiles of lipase digest of chicken fat at 5, 10, 15 and 20 min.

Conclusions

Analysis of four animal fats by HPLC-APCI MS allowed the major TAGs to be identified, as well as the position of fatty acid substitution within those TAGs to be determined. Lamb and beef fat were shown to be highly saturated, containing odd-chain fatty acids, as well as more than one isomer of the 18:1 fatty acid. Whilst the *cis*-18:1 was predominantly found in the 2-position of the TAG, the *trans*-18:1 showed a preference for the 1/3-position. Pork and chicken fat contained relatively high proportions of unsaturated fatty acids and a single isomer of 18:1. Whilst the 2-position distribution of chicken fat was dominated by oleic and linoleic acids, in pork the 2-position was characteristically rich in palmitic acid. Quantification of the peaks present in the HPLC-APCI MS profiles of the four fats allowed the distribution of 2-position fatty acids to be calculated. The distributions obtained for lamb and pork fat compared well with those obtained from a lipase degradation of

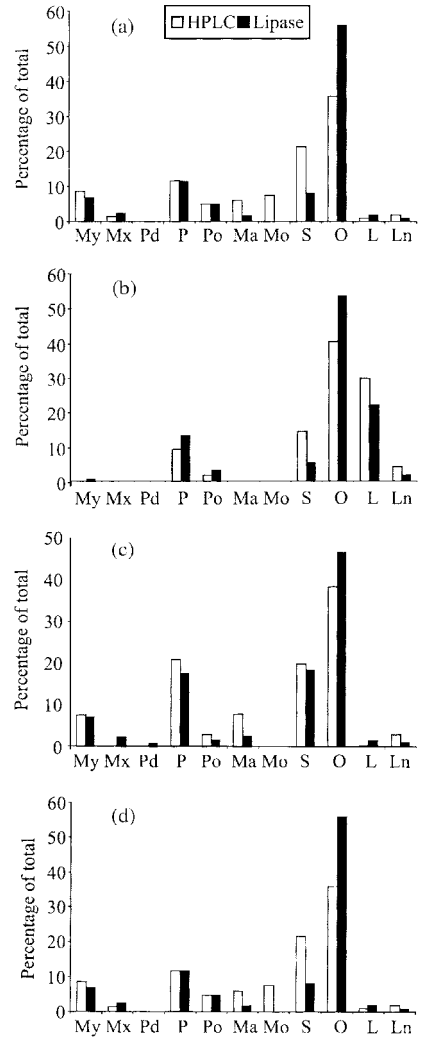

Fig. 5 Comparison of the 2-position distribution of (a) beef, (b) chicken, (c) lamb and (d) pork fats, determined by HLPC-APCI MS and lipase degradation.

the fats, whilst those obtained for chicken and beef fat had some discrepancies between the two approaches, probably due to problems with the quantification procedure and the specificity of the lipase for particular fatty acids and potential interference from other fat components. APCI-HPLC MS was shown to be a powerful technique for the regiospecific characterisation of TAGs in animal fats.

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