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Journal of Chromatography A, 896 (2000) 239–251

JOURNAL OF CHROMATOGRAPHY A

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Combined liquid and gas chromatographic characterisation of polyglycerol fatty acid esters

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Abstract

In the present study a combined liquid and gas chromatographic technique is described for the analysis of polyglycerol fatty acid esters. Liquid chromatographic fractionation of samples resulted in pure standards of monoesters of di- and triglycerols and diesters of di- and triglycerols. Confirmation of their identity was achieved by LC–MS analysis. Moreover, a chromatographic identification of the mono- and diesters of cyclic diglycerol was proposed. From the isolation of pure esters and their gas chromatographic analysis, it was revealed that co-elution of several compounds occurred. Thus it was shown that prefractionation of the sample using a simplified liquid chromatographic separation, was necessary in order to characterise the esters correctly. In combination with some other chemical analyses, a complete profile of the chemical composition of polyglycerol fatty acid esters can be obtained.

Keywords: Fatty acid esters; Polyglycerols; Glycerols

1. Introduction

Polyglycerol fatty acid esters are important non-ionic surfactants with various applications in food [1], cosmetic, pharmaceutical [2,3] and other industries. Their amphiphilic character enables their use in the stabilisation of food emulsions [4] and various suspensions [5]. They can also be used as a co-emulsifying agent in combination with monoacyl glycerols in the production of fine bakery products [6], in which they exhibit an α-tending effect [1]. Other (possible) applications in food industry relate to texture enhancement of chewing gum [7], to the use as a fat replacer [8] or as a preservative [9].

The functionality of the polyglycerol fatty acid esters are determined by their composition [4,10,11]. Application of theoretical parameters such as the HLB-value to characterise the functional properties of the esters is not sufficient [4]. A more in depth study of these compounds is inevitably in order to link functionality to the molecular structure. This would enable the production of more active mixes by selective chemical synthesis of particular isomers.

Chemically, polyglycerol fatty acid esters are esters of polyglycerol and fatty acid esters. Polyglycerols originate from an alkaline catalysed random polymerisation of glycerol, giving rise to a very complex mixture of compounds [12]. Alternative synthetic pathways may enable a more directed synthesis towards linear polyglycerol isomers [8]. Esterification is achieved using isolated fatty acids or triacyl glycerols in an ad random process, which may be catalysed by alkalics. Again a more directed synthesis would be possible using enzymes [13]. In all cases a very complex mixture of esters is obtained. These vary in polymerisation degree, polyglycerol isomers, degree of esterification, kind of esterified fatty acid and position of esterification.

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0021-9673/00/$ – see front matter
PII: S0021-9673(00)00391-5

Many researchers have tried to analyse polyglycerol fatty acid esters using several chromatographic techniques including paper [2], thin-layer [14], column [15], gas [15,16], high-performance liquid [17,18] and supercritical fluid chromatography [19]. All these techniques were qualitative and full resolution of the different esters present was impossible. Isolation of pure compounds was not reported either.

Recently, De Meulenaer et al. [20] presented a convenient method for the quantitative analysis of the polyglycerol moiety of polyglycerol fatty acid esters. This method is based on a saponification and subsequent gas chromatographic (GC) analysis of the trimethylsilyl derivatised isolated glycerols. In the present research, the problem of the analysis of polyglycerols fatty acid esters is investigated. A combined LC–GC analysis method is presented in order to analyse a mixture of esters up to the diesters of triglycerol.

2. Experimental

2.1. Materials

Silica gel plates (60, F_{254}, 10×5 cm, film thickness 0.25 mm), silica gel 60, dry pyridine (max. 0.01% water) and 2,7-dichlorofluoresceine were obtained from Merck, Germany. Chloroform (analytical-reagent grade), ethyl acetate, isopropanol, ethanol and potassium hydroxide were from Chemlab, Belgium. Acetone, pesticide analysis grade was purchased from Vel, Belgium. Hexamethyldisilazane (HDMS) and trifluoroacetic acid were from Sigma, Belgium. Polyglycerol fatty acid esters were provided by Beldem, Belgium. In Table 1, the main characteristics of the esters are summarised [20].

2.2. Liquid chromatography

For thin-layer chromatography (TLC), a mobile phase of chloroform–acetone (96:4, v/v) was used unless otherwise mentioned. Detection was achieved by spraying the developed plates with a 0.125% (w/v) 2,7-dichlorofluoresceine solution in ethanol.

For open column chromatographic experiments, 50 g silica gel was deactivated with 5% (w/w) water and introduced as a slurry with the mobile phase [chloroform–acetone (96:4, v/v)] in a glass column (40 cm×2.1 cm I.D.). Samples, immobilised on silica gel [2 g, 10% (w/w) sample] were introduced into the column before starting the separation. Elution was performed with chloroform–acetone (96:4) first and subsequently with acetone and methanol.

LC–MS experiments were performed on a quadrupole HP 1100 Series LC–mass selective detection (MSD) set-up (Hewlett-Packard, USA). Samples were dissolved in methanol, 5-μl samples were injected and a flow-rate of 1 ml MeOH min^{-1} was used. Electrospray MS parameters were as follows: positive polarity, gas temperature (N_2) 340°C, nebulising gas pressure: 50 p.s.i., drying gas flow-rate 12 l min^{-1}, voltage at capillary: 4000 V, quadrupole temperature: 100°C, scan range: 150–1000 u, fragmentor (collision-induced dissociation,

Table 1
Polyglycerol esters used and their basic compositional characteristics

Code	Polyglycerol type	Composition (%, w/w)				Esterification degree (%)
		Glycerol	Diglycerol	Triglycerol	Tetraglycerol	
PGE 055	Diglycerol	–[a]	–	–	–	–
PGE 004	Diglycerol	30.18	38.06	23.61	8.37	33
PGE 134	Triglycerol	0.46	8.30	78.56	12.69	32
PGE 145	Triglycerol	0.68	9.39	78.21	11.72	48
PGE 138	Polyglycerol	0.27	17.65	47.64	34.43	38
PGE 147	Polyglycerol	0.21	25.23	47.33	27.23	48

[a] –, No data available.

CID) 100 V (1 p.s.i.=6894.76 Pa). Molecular ions with a m e^{-1} of M+23 (due to the addition of sodium ions) are obtained.

2.3. Gas chromatography

Preliminary GC analysis of polyglycerol fatty acid esters was carried out on a Carlo Erba GC 8160 instrument (Interscience, Belgium), equipped with a flame ionisation detector. Chromatographic parameters were: stationary phase: CP Sil 5 CB wall-coated open tubular (WCOT), film thickness 0.25 μm, 25 m×0.25 mm I.D.×0.39 mm O.D. (Chrompack, The Netherlands); mobile phase: He at 0.6 ml min^{-1}, controlled with a DPFC module (Carlo Erba); split 1/100; injector temperature: 340°C; detector temperature: 350°C; injection volume: 1–2 μl; temperature program: 100°C for 1 min – ramp at 10°C min^{-1} to 325°C for 66 min. The flame ionisation detector was operated with hydrogen and oxygen gas at 30 and 300 ml min^{-1}, respectively. Further GC analysis was carried out on a Perkin-Elmer GC 8700 system (Perkin-Elmer, USA), equipped with a flame ionisation detector. Chromatographic parameters were: stationary phase: CP Sil 5 CB WCOT, film thickness 0.25 μm, 5 m×0.25 mm I.D.×0.39 mm O.D. (Chrompack); mobile phase: He at 1 ml min^{-1}; cold on-column injection (1–2 μl), detector temperature: 350°C, temperature program: 100°C for 1 min – ramp at 10°C min^{-1} to 350°C for 30 min. The flame ionisation detector was operated with hydrogen and oxygen gas at 30 and 300 ml min^{-1}, respectively. Chromatographic parameters of the fatty acid analysis were as follows: Carlo Erba GC 5160 (Interscience), stationary phase: CP Sil 88 CB WCOT, film thickness 0.20 μm, 30 m×0.25 mm I.D.×0.39 mm O.D. (Chrompack); mobile phase: He at 1 ml min^{-1}; split–splitless injector, split ratio 1/100 (1–2 μl), injector temperature 200°C, detector temperature: 250°C, temperature program: 120°C for 1 min – ramp at 5°C min^{-1} to 200°C for 20 min. The flame ionisation detector was operated with hydrogen and oxygen gas at 30 and 300 ml min^{-1}, respectively. Correction factors were derived from the injection of a reference oil. Data collection of all GC results was performed using the Gilson Unipoint software (Middleton, USA).

2.4. Sample preparation

For the GC analysis of a polyglycerol fatty acid sample (0.1–10 mg), a dried sample (N_2 gas) was dissolved in 1 ml dry pyridine. To this solution, 1 ml hexamethyldisilazane and 0.1 ml trifluoroacetic acid were added in order to derivatise the esters. The solution was shaken vigorously and subsequently kept for 15 min at room temperature.

Saponification experiments in order to analyse the polyglycerol content of isolated fractions were described previously [20]. Briefly, esters were saponified in an aqueous KOH solution, fatty acids were removed by controlled precipitation and the residue is derivatised to silyl ethers for GC analysis.

Fatty acid analysis was performed by transesterification of the esters in a 2 *M* methanolic KOH solution and subsequent extraction of the fatty acid methyl esters in hexane.

3. Results

3.1. Development of a column chromatographic prefractionation

In the preliminary experiments, esters were analysed using TLC on silica gel. Basically two solvent mixtures were evaluated: chloroform–acetone (96:4, v/v), and chloroform–acetone–methanol (94:4:2, v/v/v). The first mixture allowed separation into three distinct groups of constituents (R_F values 0.02, 0.1–0.25 and 0.67–0.92) according to their polarity. The second solvent mixture allowed a more detailed separation, especially for the more complex triglycerol and tetraglycerol fatty acid esters for which spots were observed spread out over the total TLC plate (results not shown).

The separation scheme as outlined in Fig. 1 was subsequently followed throughout the analysis of all samples. Thus three fractions, eluted with chloroform–acetone (96:4, v/v) were obtained. More polar compounds were eluted with acetone, while methanol elution allowed a final complete stripping of the column. All samples were analysed on their composition and purity using GC.

Column chromatographic fractionation was carried out for samples PGE 055, 004, 134 and 145. In all

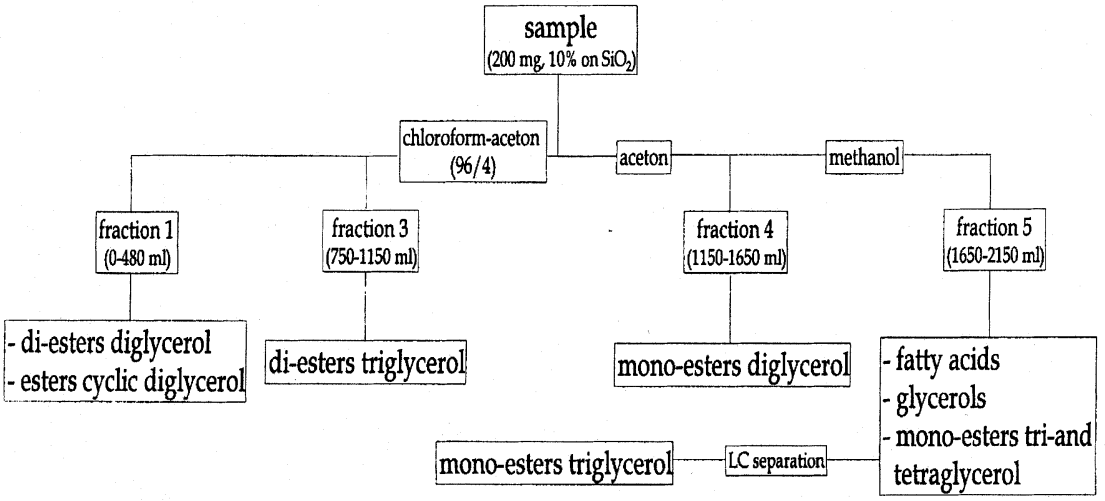

Fig. 1. Fractionation scheme followed for the isolation of chromatographically pure compounds.

cases similar results were obtained. The fractionation was not accomplished for samples PGE 138 and 147 as comparable results were to be expected.

3.2. GC of polyglycerol fatty acid esters

In preliminary experiments, silylated esters were analysed on a 30 m apolar column, using a split–splitless injector. Results indicated that injection temperatures should be as high as 340°C in order to allow sufficient sample evaporation. Even under those experimental conditions however, analysis was restricted up to the monoesters of triglycerol, indicating that discriminating evaporation and possible column adsorption took place during these experiments. Therefore, cold on-column injection was combined with a separation on a short column (5 m) with the same stationary phase. Typical chromatograms for samples PGE 004, 145 and 138 are shown in Fig. 2.

The chromatogram of fraction 1 obtained from PGE 055 is given in Fig. 3a, indicating two distinct peak groups at, respectively, 14.5–16.5 and 22–27 min. For PGE 004, the first eluting group was not intense. Polyglycerol analysis revealed the presence of diglycerol as principle component together with an earlier eluting compound (glycerol eluted before this compound). Again in sample PGE 004, this unidentified peak was present to a much lower extent compared to sample PGE 055 (not shown). Similar results were obtained for the esters PGE 134 and 145 (not shown).

Fraction 2 revealed the presence of mono-palmitine and mono-stearine glycerol (12.5–14.5 min), as confirmed by the injection of pure standards. These monoacyl glycerols were only present in the diglycerol esters PGE 004 and 055.

The chromatogram obtained from the third fraction of sample 004 and 055 only revealed the residual presence of monoacyl glycerols. For the triglycerol derived esters however, the peaks eluting between 26 and 34.5 min (Fig. 2b) were only observed. As can be seen from Fig. 2b, groups of triplets can be differentiated. Polyglycerol analysis revealed the presence of triglycerol and its branched isomers (not shown).

Further elution with chloroform–acetone (96:4, v/v) could not elute more components from the column. Therefore the polarity of the mobile phase was drastically increased. Thus by eluting with acetone, a fourth fraction was obtained. For the diglycerol fatty acid esters, the only peaks observed were those eluting in Fig. 2a between 16 and 17.5 min. Analysis of the polyglycerol composition indicated that diglycerol was solely present. For the triglycerol esters similar peaks were observed next to

those present in fraction 3. Polyglycerol analysis proved that both di- and triglycerols were present in this fraction.

Total stripping of the column was achieved by elution with methanol. For all samples, the different polyglycerol molecules which were present in the original sample (Fig. 2) could be observed again, next to the presence of palmitic and stearic acid. For the diglycerol esters especially the compounds present in fraction 4 were detected. For the triglycerol esters, dominating presence of peaks eluting at 19–21 min could be revealed (Fig. 4). As this fifth fraction seemed to contain a major compound, which was not isolated yet, a supplementary column chromatographic separation was achieved for this fraction after precipitation of the fatty acids by cooling [5.25 g silica, 5% inactivated, 1.2 cm I.D., ethyl acetate–isopropanol–water (5:2:1, v/v/v)]. The dominating peaks present in fraction 5 of sample PGE 134 and 145 (Fig. 4, 19–21 min), were purely present in the volume fraction 10–35 ml (chromatogram not shown). Again polyglycerol analysis revealed the presence of triglycerol and its branched isomers (not shown).

3.3. Detailed chromatographic analysis of fraction 1 for diglycerol esters

As in the first fraction for diglycerol esters PGE 004 and especially PGE 055, two groups of peaks were observed (Fig. 3a, 14.5–16 and 22–27 min) and the polyglycerol analysis indicated that apart from the diglycerol also another alcohol, different from glycerol, was present, some efforts were made in order to elucidate the identity of these peaks. Therefore a TLC separation using hexane–ethyl acetate (50:50, v/v)) was performed resulting in a clear separation of two groups (R_F 0.24 and 0.62). GC analysis of the most polar spot (R_F 0.24) showed similar results as the chromatogram shown in Fig. 3a

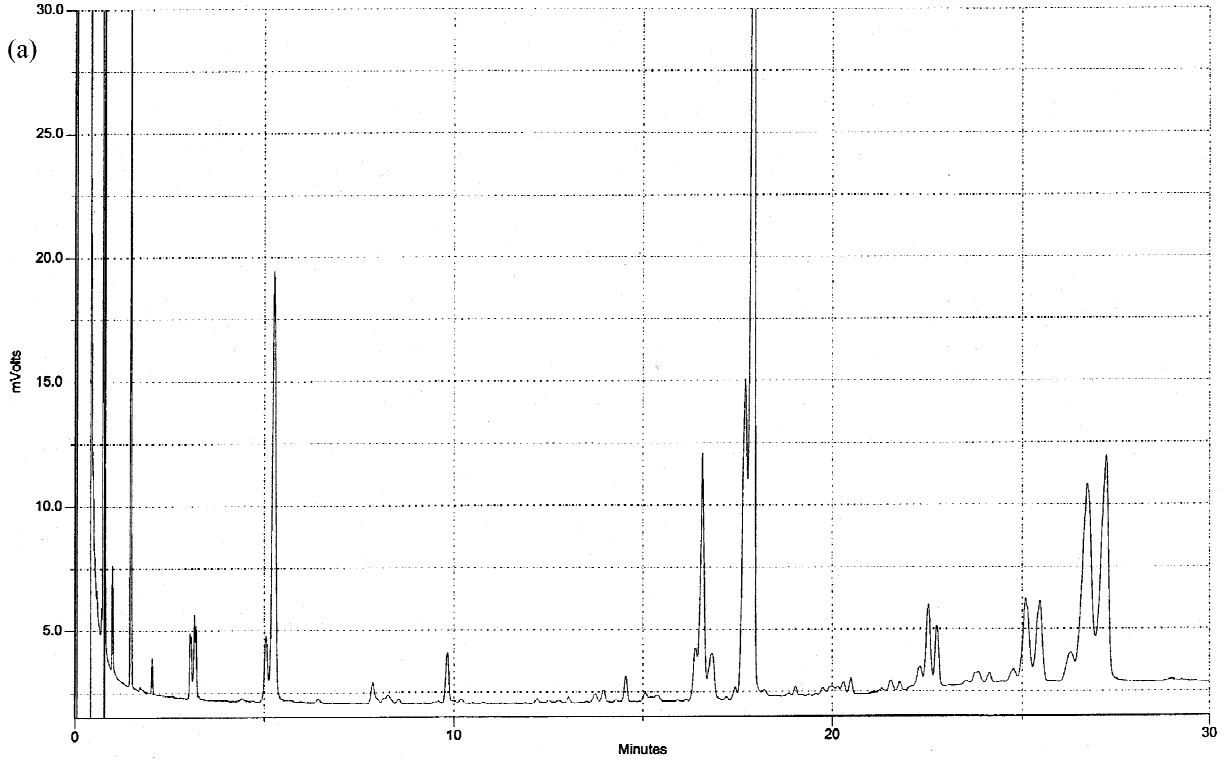

Fig. 2. GC of polyglycerol fatty acid esters PGE 004 (a), PGE 145 (b) and PGE 138 (c).

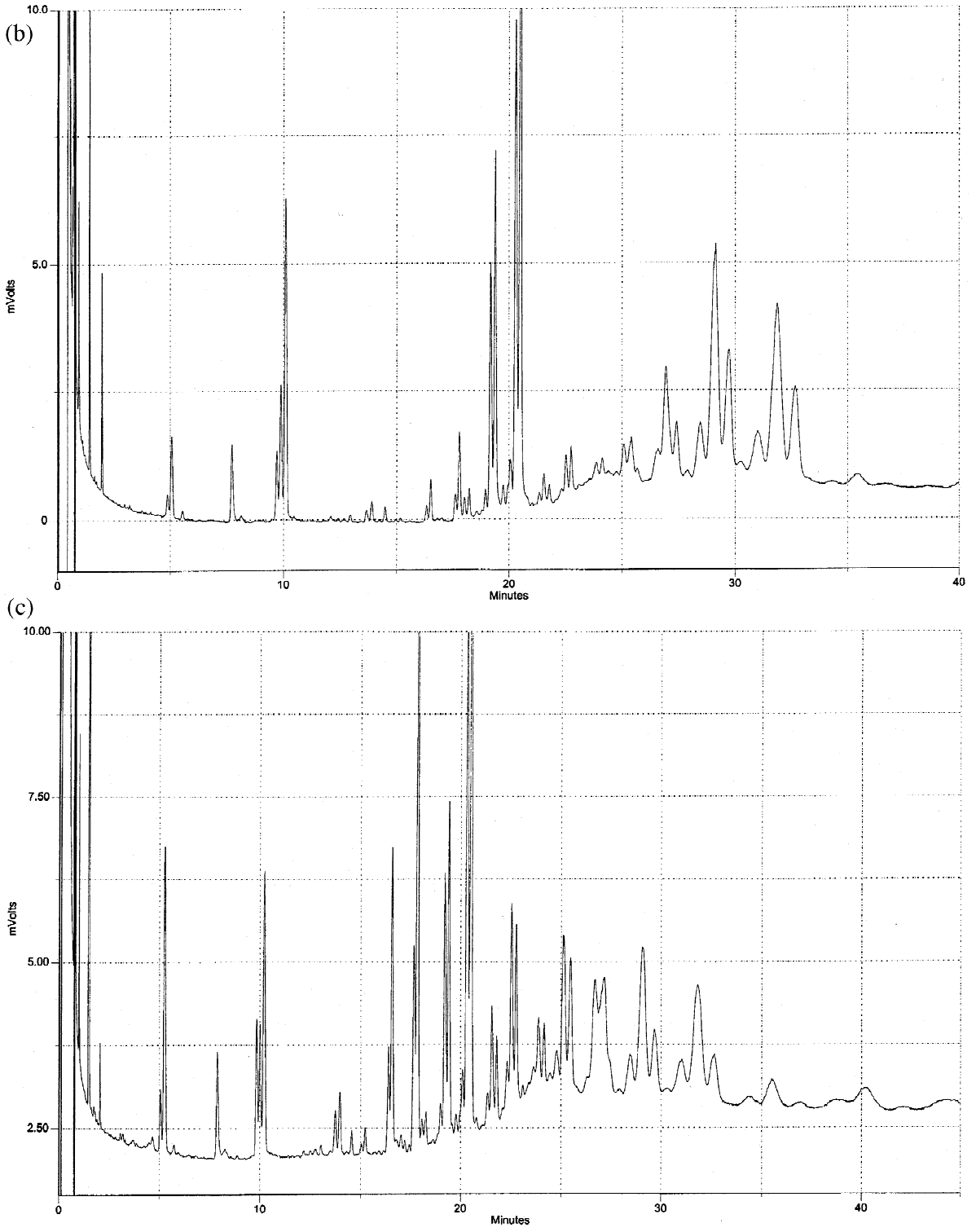

Fig. 2. (*continued*).

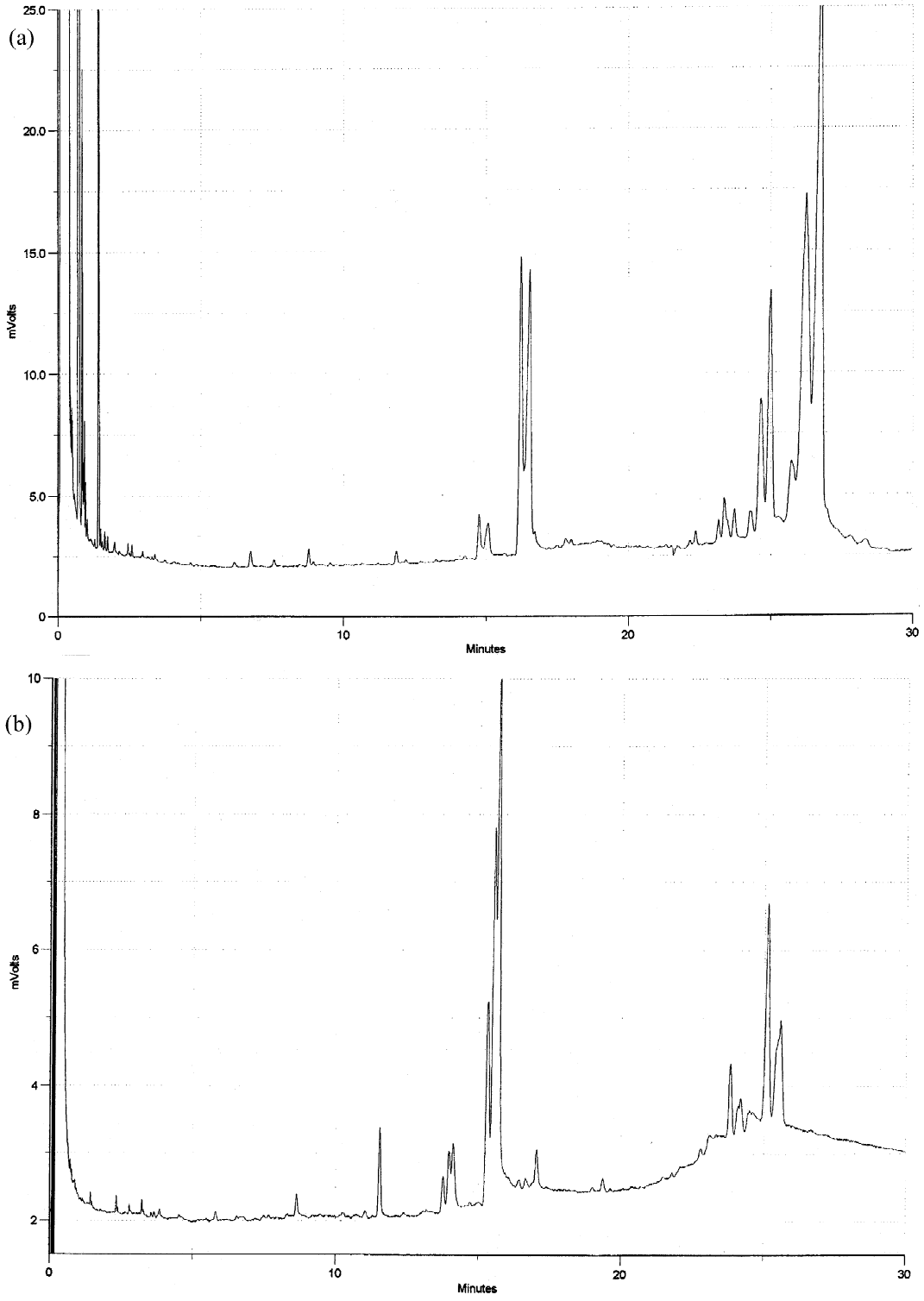

Fig. 3. GC of fraction 1 isolated from sample 055 (a) after trimethylsilyl derivatisation and (b) without trimethylsilyl derivatisation.

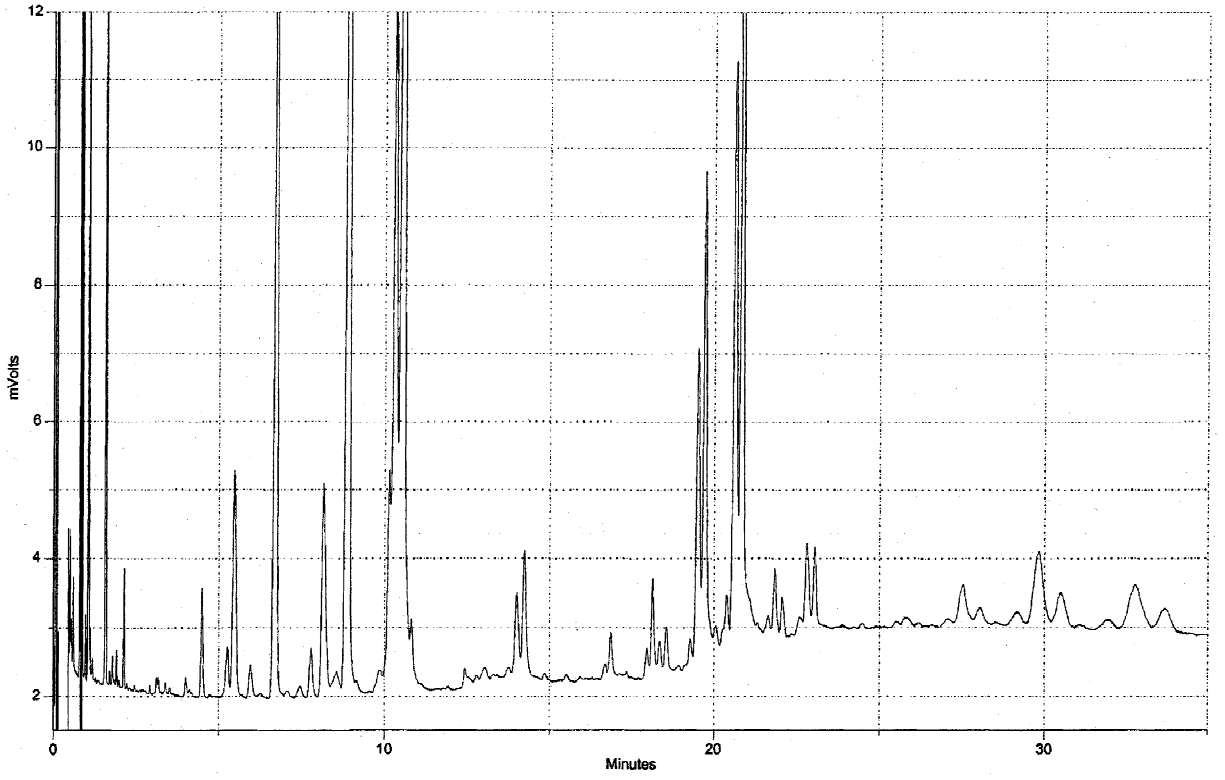

Fig. 4. GC of fraction 5 isolated from sample PGE 145.

(not shown). If the total fraction is analysed by GC without prior silyl derivatisation, a more complex chromatogram is obtained (Fig. 3b).

If fraction 1 of sample 004 is column chromatographed (2.125 g 5% inactivated silica, 1.2 cm I.D.) with chloroform–acetone (96:4, v/v) in fractions of 2 ml, a separation can be obtained between the compounds eluting at 22–24 min and 24.5–27 min (Fig. 5). The first, most apolar fraction, seemed to be enriched with the compounds eluting at 22–24 min. GC of the apolar fraction in the above described TLC experiment showed similar results. The more polar compounds, eluting in the GC experiment at 24.5–27 min, were comparable to the major compounds present in the total fraction 1 of samples 004 and 055 (Fig. 3a).

3.4. LC–MS spectral data of isolated fractions

Mass spectral data were collected of fractions which seem to contain esters of one particular polyglycerol. These are summarised in Table 2. In interpreting these data it should be kept in mind that due to the addition of sodium ions to the compounds, the molecular ions observed have an m/z increased by 23 u.

4. Discussion

4.1. Column chromatographic fractionation and total GC analysis

As a restricted group separation was thought to be more appropriate for the goal of this research, the acetone–chloroform (96:4) mixture was selected instead of the mix containing methanol. As TLC experiments revealed the presence of very polar compounds, however (R_F 0.02), elution of these compounds in the column chromatographic separation was achieved by using acetone and subsequently methanol. The fact that indeed all com-

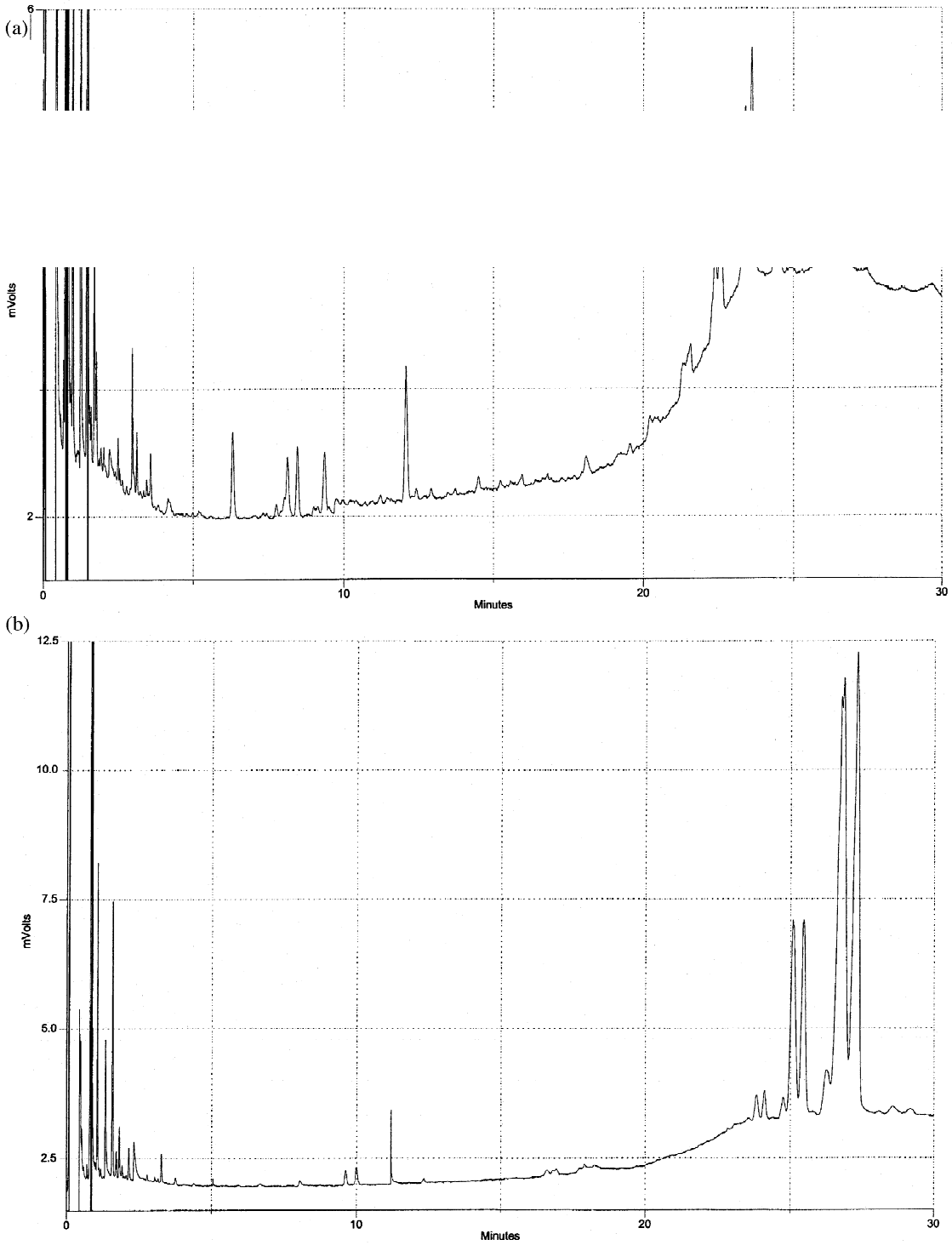

Fig. 5. GC of two fractions isolated from a column chromatographic separation of fraction 1 (sample PGE 004): 6–8 ml (a) and 18–20 ml (b).

Table 2
Mass spectral data of isolated liquid chromatographic fractions

M+23/z	Relative intensity	Identification
Fraction 1 – PGE 004		
721.5	100	$C_{18:0}$–$C_{18:0}$ diesters diglycerol
693.5	38	$C_{16:0}$–$C_{18:0}$ diesters diglycerol
681.5	18	Unknown
455.3	12	$C_{18:0}$ monoesters diglycerol
777.6	10	$C_{20:0}$–$C_{20:0}$ diesters diglycerol
Fraction 3 – PGE 134		
823.6	100	$C_{18:0}$–$C_{20:0}$ diesters triglycerol
851.6	87	$C_{20:0}$–$C_{20:0}$ diesters triglycerol
795.5	38	$C_{18:0}$–$C_{18:0}$ diesters triglycerol
767.5	7	$C_{16:0}$–$C_{18:0}$ diesters triglycerol
Fraction 4 – PGE 004		
455.3	100	$C_{18:0}$ monoesters diglycerol
427.2	14	$C_{16:0}$ monoesters diglycerol
795.6	6	$C_{18:0}$–$C_{18:0}$ diesters triglycerol
Fraction isolated from chromatographic separation of fraction 5, sample PGE 134 (10–35 ml)		
529.3	100	$C_{18:0}$ monoesters triglycerol
501.3	56	$C_{16:0}$ monoesters triglycerol
603.4	8	$C_{18:0}$ monoesters tetraglycerol
557.3	7	$C_{20:0}$ monoesters triglycerol
473.3	7	$C_{14:0}$ monoesters triglycerol

pounds were eluted, was derived qualitatively from superposition of the chromatograms of the different fractions obtained and comparison with those of the original samples.

It is obvious that an increasing polymerisation degree renders the chromatograms more complex. Differences in esterification degree were however not clearly reflected in a more complex chromatogram, although peaks could be observed with longer retention times for sample PGE 138 [Fig. 2c, for sample PGE 147 these peaks had higher intensity (not shown)].

Another interesting observation is the presence of the free polyglycerols present in the chromatograms (retention times: diglycerol 5 min; triglycerol 10 min, tetraglycerol 13 min), which is most probably due to a partial hydrolysis of the samples upon their storage period. From this observation, a first idea of the polyglycerol content of an esters studied can be obtained.

4.2. Identification of isolated fractions

From the obtained results, the various isolated fractions could be identified. Fraction 1 seemed to contain esters derived from diglycerol and another unidentified alcohol. Considering the major peaks in this fraction (Fig. 3a, 22–27 min), it was concluded that these were diesters of diglycerol. This is based on their low polarity, on the complexity of the peaks (similar to diacyl glycerols) and their lower concentration in the original sample. Final certitude was obtained from LC–MS experiments. As stearic acid is the most abundant (60%) in all esters studied, the triplet with the highest intensity, eluting at 26.5–27 min, correspond to the $C_{18:0}$–$C_{18:0}$ diesters. The triplet eluting before is most probably due to the $C_{16:0}$–$C_{18:0}$ diesters, while the $C_{16:0}$–$C_{16:0}$ diester triplet is only present in small quantities. The fact that triplets for each ester were observed is due to the presence of place isomers and not due to the presence of various linear diglycerol isomers as a previous study revealed the presence of one dominating isomer [20] (Table 1). As no diesters of diglycerol were observed in the second fraction, total elution in the first fraction was achieved.

From the third fraction of triglycerol esters PGE 134 and 145 another group of pure esters could be

isolated (eluting from 26 to 34.5 min, Fig. 2b). As polyglycerol analysis indicated that only triglycerol was present and because the compounds were more polar compared to the diesters of diglycerol, it was supposed that diesters of triglycerol were isolated. This hypothesis was confirmed by the LC–MS data. These indicated that especially esters of $C_{20:0}$ were present, which is quite surprising since $C_{20:0}$ was only present in minor quantities (2%, w/w). No explanation could be found for this observation. As GC and LC–MS data are not completely consistent, no clear identification of the different peaks with regard to their fatty acid composition was possible.

It can be observed from the total chromatograms of the tri- and polyglycerol esters studied (Fig. 2b and c), that the diesters of triglycerol are the last eluting components which are clearly resolved. For the polyglycerol esterified samples, peaks eluting at higher retention times could be observed, but these were broad and low in intensity. Most probably these are originating from diesters of tetraglycerol. They are not due to, for example, triesters of diglycerol, since these should then also be present in the first fraction collected because of their low polarity, which was not the case. Consequently, the proposed GC technique is restricted up to the analysis of diesters of triglycerol.

Further elution with acetone, enabled isolation of pure monoesters of diglycerol esters from samples PGE 004 and 055 in a fourth fraction. This is confirmed by their high polarity, their abundance in the original samples and LC–MS data. The two major couple of peaks in the gas chromatogram are, respectively due to the stearic ester (most intense, Fig. 2a, 17.5 min) and palmitic ester of diglycerol, which is in correspondence with their elution behaviour and fatty acid composition.

For the triglycerol esters, monoesters of diglycerol and diesters of triglycerol eluted together, indicating that chloroform–acetone (96:4) was not able to completely elute the diesters of triglycerol. Apart from free glycerols and fatty acids, monoesters of triglycerol and tetraglycerol were also expected to be present in samples PGE 134 and 145, since these were not observed in the former isolated fractions. From the chromatogram of the fifth fraction and the elution behaviour of the monoesters of diglycerol, it was expected that the most dominating peaks around 20 min (Fig. 4) were due to the monoesters of triglycerol. After these, monoesters of tetraglycerol most probably elute. A supplementary column chromatographic separation allowed the isolation of the peaks eluting between 18.4 and 21 min, which indeed seemed to consist of triglycerol esters (polyglycerol analysis, LC–MS data). In correspondence with the fatty acid analysis, the most intense peak is due to the $C_{18:0}$ isomer, while the earlier eluting peak is due to the $C_{16:0}$ ester.

4.3. Chromatographic identification of cyclic diglycerol esters

From the in depth chromatographic analysis of the first isolated fraction of samples PGE 004 and 055 some interesting observations could be made enabling the identification of the unknown peaks present in Fig. 2a (14.5–16 min). It was supposed that these peaks were due to the presence of cyclic diglycerol esters, as confirmed by the presence of an earlier eluting alcohol then the linear diglycerol, corresponding with our previous observations with regard to the elution behaviour of diglycerol isomers [20]. It is proposed that the compounds eluting around 15 min are monoesters of cyclic diglycerol because their polarities are similar to those of the diesters of diglycerol and because the compounds seemed to contain a free hydroxyl group, which could be silylated (Fig. 2b). Again, the fatty acid composition of the total ester is reflected in the presence of a smaller and intense couple of peaks. Probably the existence of various cyclic diglycerol isomers [20] explains the presence of double peaks for each ester. From the unsuccessful preliminary attempts to isolate these esters, a second most interesting observation was made during a column chromatographic experiment on fraction 1 of sample PGE 004, as indicated in Fig. 5. Although it was impossible to separate the monoesters of cyclic from the diesters of linear diglycerol, some compounds could be detected which were more apolar compared to the diesters of linear diglycerol (Fig. 5a). These compounds had a comparable retention behaviour in GC as the diesters of diglycerol, but were more apolar. Thus, it can be supposed that these were the diesters of cyclic diglycerol. Confirmation of these suppositions by mass spectral data was impossible because of the low concentrations of these compounds.

4.4. Proposed analytical scheme for the analysis of polyglycerol fatty acid esters

GC analysis of isolated pure compounds and total samples, revealed that some esters co-eluted: diesters of diglycerol and diesters of triglycerol, diacyl glycerols and monoesters of di- and triglycerols. This co-eluting behaviour would even be more emphasised if other fatty acids would be used in the manufacture of the polyglycerol fatty acid esters (e.g., $C_{14:0}$ and $C_{20:0}$). Thus it can be concluded that a single chromatographic analysis of a polyglycerol sample is not sufficient in order to get correct compositional information. Thus a prefractionation scheme as outlined in Fig. 6 can be proposed. In a first fraction, eluted with chloroform–acetone (96:4), apolar compounds up to diesters of diglycerol can be isolated (e.g., diacyl glycerols, triacyl glycerols). Afterwards, acetone elution isolates diesters of triglycerol (partially) and monoesters of diglycerol (completely). Finally a methanol elution allows isolation of other monoesters and the remaining diesters of triglycerol. These isolated fractions can subsequently be analysed by GC without any risk of interfering peaks. In combination with the results presented in a previous study [20] and some additional chemical analyses, a total analytical scheme for the characterisation of polyglycerol fatty acid esters is presented.

5. Conclusions

The present study combined liquid and gas chromatography in order to isolate and characterise pure esters of polyglycerols. Identification was confirmed by mass spectral analysis. A simplified fractionation scheme was proposed in order to allow a reliable LC–GC combined analysis of polyglycerol fatty acid esters up to the diesters of triglycerol. It is proposed to fractionate the sample in three different fractions, which are all analysed by GC without risk of interfering peaks. Since pure standards could be isolated of all diglycerol and triglycerol mono- and diesters, a quantitative analysis should be possible as well.

Acknowledgements

The authors wish to thank Mr. M. Meulemans and P. Goossens (Beldem) for providing the different samples and for the interesting discussions throughout this research. Professor P. Sandra (Organic

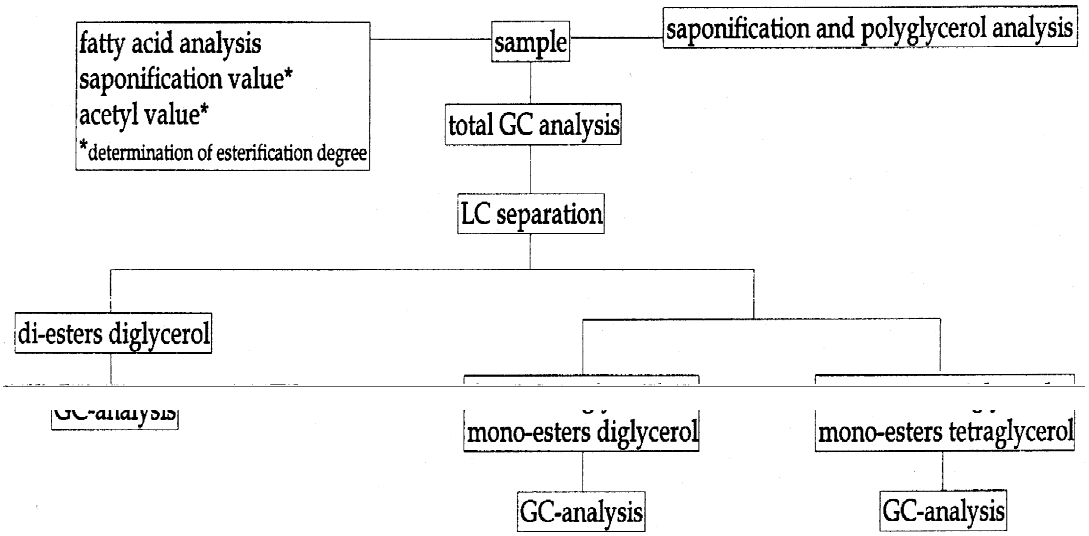

Fig. 6. Proposed analytical scheme for the analysis of polyglycerol fatty acid esters.

Chemistry Department, Ghent University) is acknowledged for providing the mass spectra of the isolated compounds. Furthermore Mr. L. De Smijtere is thanked for his technical support during the experimental work.

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