B – Lipids and Oxidation

The Separation of Triacylglycerols Using Unpolar and Medium Polar Capillary Columns

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Abstract: Commercial by supplied triacylglycerols and synthesized triacylglycerols were used in this project. Analysed triacylglycerols with the range of carbon number (CN) 24–54 consisted of saturated and unsaturated fatty acids. Two capillary columns were used in the analyses of triacylglycerols, namely non-polar (Optima®-1-TG) and medium polar (Optima®-17-TG) types. In this study the effectiveness of separation (A) of triacylglycerols with different carbon number values, (B) of triacylglycerols with different unsaturation degree, (C) of positional isomers were determined and further possibilities to separate another lipid compounds were analysed. The column Optima®-17-TG separates TAG according to the degree of unsaturation while the column Optima®-1-TG separates saturated and unsaturated TAG. High temperature capillary gas chromatography was used to determine changes in triacylglycerol composition during the transesterification of structured fats.

Keywords: high temperature gas chromatography; triacylglycerol analysis

INTRODUCTION

One way how to determine composition of triacylglycerol's mixtures is the high temperature gas chromatography (HTGC). Highly stable immobilised silicone stationary phases on inert fused-silica columns permit elution of TAG with molecular weights higher than 950 g/mol [1]. One of the main HTGC limits is the thermal degradation of TAG with a high degree of unsaturation. TAG species which have incorporated more than six methylene-interrupted double bonds along the fatty acid acyl chains are basily decomposed. Therefore the elution temperature should as low as possible combined with a high selectivity of the stationary phase to provide an efficient separation of the TAG under low thermal stress [2]. The non-polar methyl silicone phase can be used for the group separation of TAG mixtures according to carbon number (CN), while the distinction between individual TAG according to degree of unsaturation within carbon number groups may be accomplished using more polar silicone phases containing up to 65% of phenyl groups [1].

EXPERIMENTAL

Trisaturated TAG of the type $GS_1S_2S_2$ type were prepared. *Sn*-1-monoacylglycerol (C10–C18) formed the inotial substance. It was prepared at our department. *Sn*-1-monoacylglycerol was acylated with acylchloride in the presence of pyridine [3]. TAG of the $GS_1S_2S_1$ type were prepared, too. Dihydroxyacetone reacted with acylchloride to the *sn*-1,3-diacyloxypropan-2-one. The next step was the reduction of the keto group and the hydroxy group and obtained *sn*-1,3-diacylglycerol was finally acylated [4, 5]. Prepared TAG were purified by crystallisation from isopropylalcohol. The purity of TAG varied from 94% to 99%. *Sn*-1,3-dihexadekanoyl-*sn*-2-(*cis*-oktadec-9-enoyl)glycerol was prepared by T. Kůtek. Other trisaturated (GS_3) and triunsaturated (GU_3) TAGs were bought from Acros and Fluka.

The conditions for the TAGs detection:

– the medium polar column Optima®17 – the stationary phase 50% phenyl 50% methylsiloxan,

Table 1. TAG used in this project

CN	TAG	CN	TAG	CN	TAG	CN	TAG
24	8–8–8[a]	38	14–12–12	42	12–18–12	48	12–18–18
30	10–10–10[a]		18–10–10	44	16–14–14	50	14–18–18
32	12–10–10		10–18–10	44	12–16–16	50	18–16–16
34	14–10–10	40	12–14–14	46	14–16–16		16–18:1–16
	10–12–12		16–12–12		10–18–18	52	16–18–18
36	16–10–10	42	10–16–16		18–14–14	54	18–18–18[b]
	12–12–12[a]		14–14–14[b]		14–18–14		18:1–18:1–18:1[b]
38	10–14–14		18–12–12	48	16–16–16[a]		18:2–18:2–18:2[b]

[a]TAG obtained from the company ACROS; [b]TAG obtained from the company FLUKA

– the non-polar column Optima®1-TG – stationary phase 100% polydimethylsiloxan,
– the dimensions of the columns are following: the length 25 m, the inner diameter 0.32 mm and the thickness of the stationary phase 0.1 μm,
– the inlet temperature 320°C, carrying gas He, make-up gas N_2 and FID detector.

RESULTS AND DISCUSSION

Separation efficiency of TAG with the same carbon number but different degree of unsaturation

The column Optima®1-TG separates only saturated and unsaturated triacylglycerols, it is not possible to separate unsaturated TAG with different number of double bonds on this column. It is possible to separate TAG according to the degree of unsaturation using the column Optima®-17-TG (Figure 1). Further TAGs were analysed namely: *sn*-1,3-dihexadekanoyl-*sn*-2-(cis-oktadec-9-enoyl)glycerol and *sn*-1,2-dihexadekanoyl-*sn*-3-oktadekanoylglycerol but it was not possible to separate this TAGs couple to the base line. The presence of one double bond is not sufficient for the separation of TAG with the same CN.

Positional isomers separation

It is not possible to separate positional isomers of trisaturated TAGs using neither non-polar Optima®1-TG column nor medium polar Optima®17-TG capillary column. The TAG summarised in Table 2 were used for positional isomer separation.

Table 2. TAG used for the positional isomers separation

	CN 38	CN 40	CN 46
Positional	18:0–10:0–10:0	18:0–12:0–12:0	18:0–14:0–14:0
Isomer	10:0–18:0–10:0	12:0–18:0–12:0	14:0–18:0–14:0

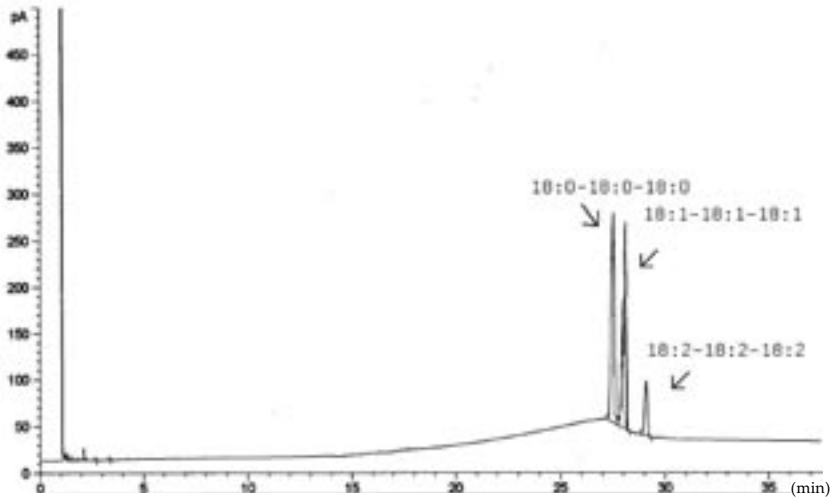

Figure 1. Chromatogram of the analysis of the mixture 18:0–18:0–18:0; 18:1–18:1–18:1 and 18:2–18:2–18:2 on the column Optima®17-TG

Table 3. The elution order of other lipids

Order	Lipid	Order	Lipid	Order	Lipid	Order	Lipid
1	MAG – 10	6	MAG – 16	11	cholesterol	15	sitosterol
2	MAG – 11	7	MAG – 18	12	DAG – 24	16	DAG – 28
3	MAG – 12	8	DAG – 20	13	campesterol	17	DAG – 30
4	MAG – 13	9	squalene	14	stigmasterol	18	DAG – 32
5	MAG – 14	10	α–cholestan				

Column efficiency for the separation of trisaturated TAG with the same CN but different fatty acid composition

The efficiency for the separation trisaturated TAG with the same CN but different fatty acids composition was studied. It was found out that it is not possible to separate these TAG with CN 48–54. Partial separation was reached for TAG with CN 36, 38 and 46 when both types of the columns were used.

Separation of the other lipids

It is possible to separate other lipids such as monoacylglycerols, diacylglycerols, free sterols and hydrocarbon (e.g. squalene) on non-polar and medium polar capillary columns – Table 3.

Analysis of natural fats using non-polar and medium polar capillary columns

The natural fats were analysed using both column types. The palm oil was chosen and it is possible to see separative efficiency in Figure 2 (medium polar capillary column Optima®-17-TG) and in Figure 3 (nonpolar capillary column Optima®-1-TG). It is obvious that medium polar column gives better separation of the TAGs. This column separates the TAG mixtures according to the degree of unsaturation what is not possible on non-polar column.

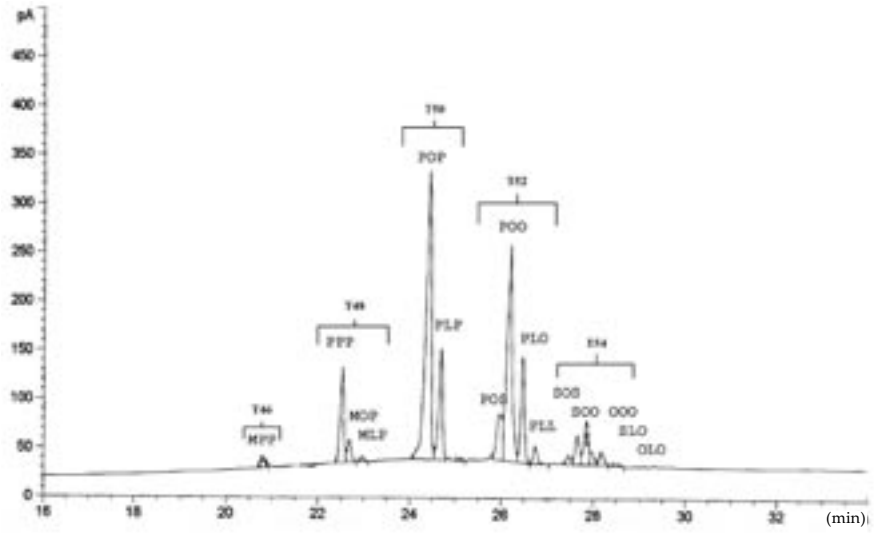

Figure 2. Chromatogram of palm oil analysis on the column Optima®-17-TG

Table 4. The analysis of cocoa butter by using medium polar capillary column Optima®-17-TG

TAG	Determined (%)	Published (%)	TAG	Determined (%)	Published (%)
POP	18	17.8–22.6	SOS	25.9	22.8–31.1
PLP	1.8	0.7–1.2	SOO	2.6	2.9–6.7
POS	42.9	37.1–40.6	SLS + OOO	1.5	1.7–3.2
POO	2.0	1.2–3.9	SLO	0.1	–
PLS	3.0	2.8–3.7	SOA	1.0	1.0
PLO	0.3	–			

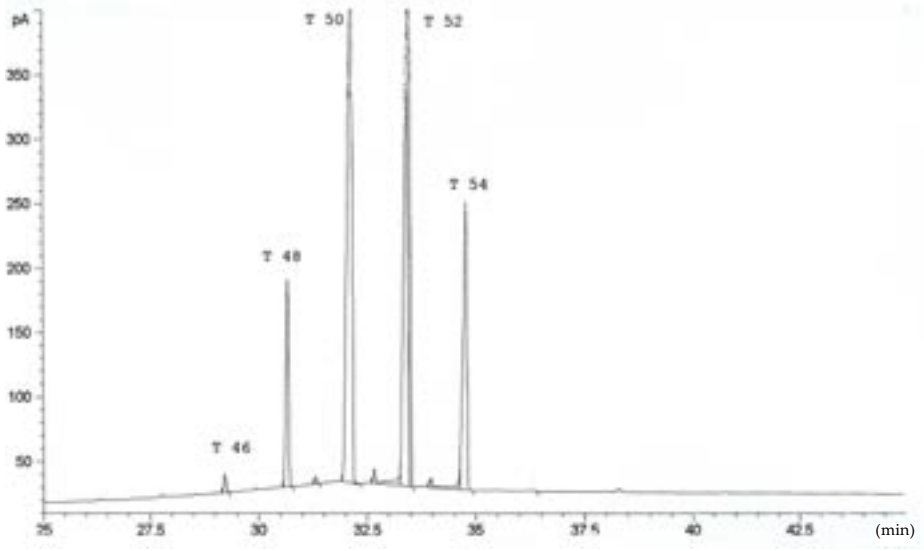

Figure 3. Chromatogram of palm oil analysis on the column Optima®-1-TG

Cocoa butter analysis

The results of cocoa butter analysis were compared to the published data [6]. The analysis was carried out using medium polar capillary column Optima®-17-TG.

CONCLUSIONS

It is possible to separate TAG with the same carbon number but different degree of unsaturation using medium capillary column Optima®-17-TG. The column Optima®-1-TG separates saturated and unsaturated TAG, it is not possible to separate unsaturated TAG according to the degree of unsaturation. It is not possible to separate neither positional isomers nor TAG with the same CN (range 48–54) but different fatty acid composition when columns Optima®-17-TG and Optima®-1-TG are used. Partial separation was reached during the analysis of TAG with CN 36, 38 and 46. Column Optima®-17-TG offers better separative efficiency for analysis of natural fats. There is a higher number of peaks when compared to Optima®-1-TG. The reason for this effect is that Optima®-17-TG allows TAG separation according to the degree of unsaturation. It is possible to separate monoacylglycerols, diacylglycerols and free sterols on this columns.

References

[1] Hinshaw J.V., Seferovic W. (1986): J. High Resolut. Chromatogr., **9**: 731.

[2] Aichholz R., Lorbeer E. (1998): J. High Resolut. Chromatogr., **21**: 363.

[3] Chandran D.V., Bhatanagar R.K. (1968): J. Am. Oil Chem. Soc., **45**: 581.

[4] Barry P.J., Crig B.M. (1955): Can. J. Chem., **33**: 716.

[5] Bentley P.H., McCrae W. (1970): J. Org. Chem., **35**: 2082.

[6] Ulberth F., Buchgraber M. (2003): Eur. J. Lipid Sci. Technol., **105**: 32.