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QUANTITATIVE DETERMINATION OF TRIACYLGLYCEROLS SEPARATED ON CAPILLARY COLUMNS ACCORDING TO ACYL CARBON NUMBER AND LEVEL OF UNSATURATION

PAAVO KALO*
Department of General Chemistry, University of Helsinki, SF-00710 Helsinki (Finland)
and
KAI VAARA and MATTI ANTILA
Department of Dairy Science, University of Helsinki, SF-00710 Helsinki (Finland)
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SUMMARY

The quantitative determination of triacylglycerols separated according to acyl carbon numbers and level of unsaturation was studied on two 10-m immobilised SE-54 columns. The fat samples were silylated before on-column injection. The 0.2 mm I.D. column showed higher resolving power and the 0.32 mm I.D. column better repeatability. The application to real quantitations was investigated with analyses of butter-fat solid and liquid fractions and of untreated and interesterified 70–30 mixtures of butter-fat solid fraction and hydrogenated rapeseed oil. Changes in the triacylglycerol composition induced by interesterification and fractionation are so pronounced that analysis on capillary columns can be recommended, in spite of only fair repeatability.

INTRODUCTION

The possibility of altering the physical properties of fats through fractionation, hydrogenation and interesterification has aroused considerable interest in recent years. Changes in physical properties have been followed by measurement of melting and crystallisation curves and measurement of solid fat content by NMR method or dilatometry, and the changes in triacylglycerol composition have been followed by mass spectrometry, enzymatic deacylation, stereospecific analysis, argentation thin-layer chromatography (TLC) and gas chromatography (GC)[1]. In the low resolution separations on packed and short capillary columns, triacylglycerols are separated into groups of triacylglycerols with the same number of carbon atoms. Quantitative determinations on packed columns are commonly made[2], though problems due to irreversible absorption and polymerisation have been reported[3]. Takagi and Itabashi[4] have quantitated triacylglycerols separated according to degree of unsaturation on Silar 10°C packed column. Mordret *et al.*[5]. D'Alonzo *et al.*[6,7] and Monseigny *et al.*[8] have made separations on short capillary columns according to acyl carbon

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numbers. Schomburg *et al.*[9], Grob *et al.*[10], Geeraert *et al.*[11,12], Traitler and Prevot[13], Lercker[14] and Lercker *et al.*[15] and Amer *et al.*[16] have used 8- to 20-m columns for high resolution separations of triacylglycerols. Monseigny *et al.*[8], D'Alonzo *et al.*[6,7] and Geeraert *et al.*[11] and Amer *et al.*[16] have reported on quantitative determinations on capillary columns for low resolution separations and Kalo *et al.*[17] for separations according to the level of unsaturation. Grob[18] has suggested that losses of higher triacylglycerols in capillary columns are mainly the result of degradation to free fatty acid, and to prevent such degradation reactions D'Alonzo *et al.*[6,7] introduced prior silylation of the free fatty acids and mono- and diacylglycerols present in the fat. Grob *et al.*[10], Traitler and Prevot[13], Geeraert *et al.*[11] and Geeraert and Sandra[12] noted the advantages of on-column injection technique in the separations on capillary columns. In the present work, the method of prior silylation[6,7] is combined with the use of on-column injection technique for the quantitative determination of triacylglycerols separated on SE-54 silica capillary columns according to the level of unsaturation.

EXPERIMENTAL

A Carlo Erba 5300 gas chromatograph with an on-column injector and a flame ionisation detector was used in conjunction with a Hewlett-Packard 3390A integrator. The chromatograms were run with 0.32 mm I.D.(I) and 0.20 mm I.D.(II) 10-m immobilised SE-54 columns with 0.10 μm film thickness. The inlet pressure of hydrogen carrier gas was 0.22 kg/cm^2 with column I and 0.7 kg/cm^2 with column II at the beginning of the run, and was raised to 0.9 kg/cm^2 32.5 min after injection.

To derivatise the free fatty acids and mono- and diacylglycerols the fat sample was treated with BSTFA (Merck) according to D'Alonzo *et al.*[6,7] as follows: 50 μl of BSTFA were added to a 2.5-mg or 0.25-mg sample of fat in a screw-cap vial,

TABLE I

FATTY ACID COMPOSITIONS (MOL %) OF THE TRIACYLGLYCEROL FRACTIONS ISOLATED BY ARGENTATION TLC

Fatty acid	*Fraction of saturated TAG of butter-fat*	*Fraction of monoene TAG of butter-fat*	*Fraction of monoene TAG of butter-fat rapeseed oil*	*Fraction of diene TAG of butter-fat rapeseed oil*
C 8:0	4.6	3.9	–	–
C 10:0	1.5	2.0	–	–
C 12:0	2.2	3.1	–	–
C 14:0	14.1	11.6	8.3	15.4
C 15:0	–	–	1.0	–
C 16:0	46.3	30.9	32.1	11.8
C 17:0	–	–	1.6	7.9
C 18:0	27.6	15.6	16.4	5.7
C 18:1	1.4	30.6	34.9	39.9
C 18:2	2.3	2.4	3.5	6.2
C 20:1	–	–	2.1	13.2

TABLE II

EMPIRICAL CORRECTION FACTORS OF TRIACYLGLYCEROLS

Component	*Correction factor on column I*	*Correction factor on column II*
C 30	1.03	1.23
C 36	1.00	1.00
C 42	0.98	0.94
C 48	0.95	0.94
C 54	1.09	1.21

which was shaken vigorously for a few seconds and heated at 100°C for 15 min. In the analyses on column I fat samples of *ca.* 2 μg, and in those on column II samples of *ca.* 0.15 μg, were injected at a column temperature of 160°C. After an isothermal period of 1 min, the column temperature was quickly raised to 245°C and then at 2°C/min to 300°C and at 3°C/min to 345°C.

A Nu Check Prep (Elysian, MN, U.S.A.) calibration mixture containing equal amounts of tricaprin, trilaurin, trimyristin, tripalmitin and tristearin was used for determination of empirical correction factors. The correction factors of other peaks were calculated from the nearest calibration peak. Chromatograms were calculated by normalisation.

For identification of triacylglycerols separated by level of unsaturation, interesterified butter-fat and a 60–40 butter-fat–rapeseed oil mixture were purified from free fatty acids and mono- and diacylglycerols on Silica Gel G plates (Merck) with a 0.25-mm layer thickness. Plates were eluted with hexane–acetone (100:8)[19]. Triacylglycerols were separated according to level of unsaturation on Silica Gel G (Merck) plates, impregnated with 25% of silver nitrate in a 0.25-mm layer thickness. The eluent was benzene–chloroform–methanol (70:30:0.25)[20]. The fatty acid compositions of the fractions isolated by argentation TLC were determined by the sodium methoxide method[21] on a 25-m Silar 10C glass capillary column with 0.25-μm film thickness and 0.32 mm I.D. The fatty acid compositions of the purified fractions are in accordance with trisaturated, monoene and diene triacylglycerols (Table I).

RESULTS AND DISCUSSION

The empirical correction factors of medium-molecular-weight saturated triacylglycerols show lower values than those of triacylglycerols with 30 and 54 acyl carbon numbers (Table II). This is in accordance with the correction factors determined by Watt and Dils[22] and Lichtfield *et al.*[23] on packed columns, but the deviation is clearly more pronounced than reported by Kuksis[24] for packed columns and Monseigny *et al.*[8] for capillary columns. The higher value of the empirical correction factor for C_{54} triacylglycerols than for medium-molecular-weight triacylglycerols most probably indicates partial degradation of high-molecular-weight triacylglycerols. The prior silylation of fat probably did not totally prevent the degradation of higher triacylglycerols in the analytical conditions used. However, in the analysis in the presence of silylating reagent the degradation products are silylated and elute

TABLE III

VARIATION COEFFICIENTS (%) OF SATURATED, MONOENE AND DIENE TRIACYLGLYCEROLS

On column I determined with interesterified butter-fat, $n = 7$; on column II determined with butter-fat solid fraction S_{24}, $n = 6$.

Acyl carbon number	*Column I*		*Column II*		
	Saturated TAG	*Monoene TAG*	*Saturated TAG*	*Monoene TAG*	*Diene TAG*
26	13	–	18	–	
28	14	–	21	–	
30	3.8	8.1	27	33	
32	3.1	5.8	12	4.2	5.8
34	7.6	13	8.7	5.7	25
36	5.5	4.0	9.2	17	
38	1.8	4.2	11	11	17
40	5.7	5.0	4.8	6.1	
42	13	9.6	11	19	
44	1.7	6.9	5.0	21	
46	3.6	5.7	3.6	7.7	
48	5.0	3.1	5.4	26	
50	3.9	2.0	9.1	7.9	21
52	7.6	4.1	7.9	14	11
54	–	2.6	9.1	9.4	15

from the column, enabling continuous analysis over a period of several months without intermediate washing of the column.

The repeatability of quantitations was studied by repeating the quantitation of triacylglycerols of interesterified butter-fat (column I) seven times and of butter-fat solid fraction (column II) six times and calculating the coefficients of variation for each peak (Table III). The coefficients of variations on 0.32 mm I.D. column (I) are an order of magnitude higher than the best values reported for low resolution separations[8,11]. On 0.2 mm I.D. column (II) the coefficients of variation of saturated triacylglycerols are a little higher and those of monoene triacylglycerols clearly higher than on column I.

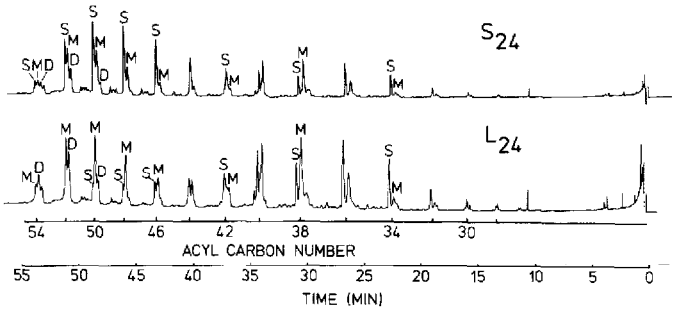

Fig. 1. Chromatogram of butter-fat solid fraction S_{24} and liquid fraction L_{24} run on a 10-m SE-54 column with 0.2 mm I.D. (II). For conditions see text.

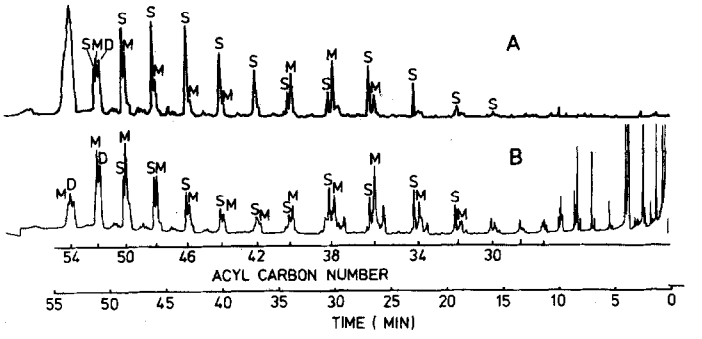

Fig. 2. Chromatograms of 70–30 butter-fat solid fraction S_{24}–hydrogenated rapeseed oil mixtures run on a 10-m SE-54 column with 0.2 mm I.D. (II). For conditions see text. (A) Untreated mixture; (B) interesterified lipase as catalyst.

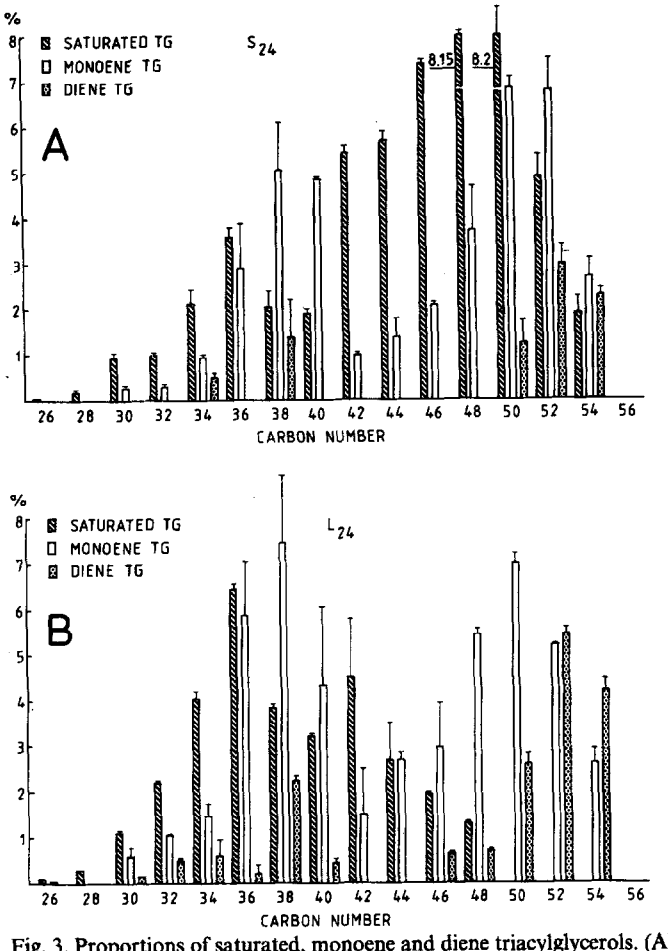

Fig. 3. Proportions of saturated, monoene and diene triacylglycerols. (A) Butter-fat solid fraction S_{24}; (B) butter-fat liquid fraction L_{24}. The mean and the higher value of a double determination.

The 0.32 mm I.D. column (I)[17] separated saturated and monounsaturated triacylglycerols of butter fat and allowed their quantitation. But the diene and triene triacylglycerols did not separate properly from each other and could be quantitated only as a group[17]. The 0.2 mm I.D. column (II) separated in addition the diene and triene triacylglycerols, allowing quantitation of diene triacylglycerols (Figs. 1 and 2). In the analysis of butter-fat fractions the saturated and monoene and diene triacylglycerols could be quantitated in the acyl carbon number range 26–54. Fig. 3 shows the triacylglycerol contents of butter-fat solid fraction S_{24} and liquid fraction L_{24} measured in double determinations on column II. The proportions of trisaturated triacylglycerols with 44–52 acyl carbon numbers were high in the solid fraction. The trisaturated triacylglycerols could not be detected in the liquid fraction in the acyl carbon number range 50–54 and their concentrations were low within acyl carbon numbers 44–48. The concentrations of saturated and monounsaturated triacylglycerols with acyl carbon numbers 34–38 were higher in the liquid fraction.

Chromatograms of an untreated 70–30 mixture of butter-fat solid fraction S_{24} and hydrogenated rapeseed oil, and of the same mixture interesterified with *Candida cylindracae* lipase as catalyst, are shown in Fig. 2[25]. Interesterification decreased the proportion of saturated triacylglycerols especially in the acyl carbon number range 42–52, and increased the proportion of monoene triacylglycerols especially in the acyl carbon number range 48–52. A separate analysis with another temperature program was necessasry for determination of the proportions of triacylglycerols with 54 acyl carbon atoms.

These examples show the changes in the triacylglycerol composition induced by fractionation or interesterification to be so marked that the GC quantitative determination of triacylglycerols separated according to the level of unsaturation can give valuable information, in spite of the only fair repeatability occasioned by the low capacity of the column.

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