DETERMINATION OF THE TRISATURATED GLYCERIDE CONTENT OF FATS BY COLUMN CHROMATOGRAPHY OF THE MERCURIC ACETATE ADDUCTS ON FLORISIL*

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INTRODUCTION

The trisaturated glyceride (GS_3) content of natural fats can be determined by the traditional permanganate oxidation techniques described by HILDITCH[1]. It has been shown by ESHELMAN AND HAMMOND[2] that these methods do not yield reliable results with milk fat. In addition, the procedure is involved and time consuming. In a research project designed to study the trisaturated glyceride content of milk fat (to be published elsewhere) a method has been developed which proved suitable for the rapid determination of the GS_3 content of fats. It is based on the quantitative addition of mercuric acetate to the double bonds of all unsaturated glycerides (GU) followed by separation of the GS_3 and GU by column chromatography on Florisil. The GU can be recovered from the adducts with simultaneous re-establishment of the original structure by elution with an acid-containing solvent.

EXPERIMENTAL

Margarine fat was obtained from commercial samples of margarine by melting, washing with warm water to remove non lipid material, drying under reduced pressure and filtration. The other fats were obtained from commercial sources and used without further treatment.

The trisaturated glyceride content was determined by weighing 700–800 mg of fat into a 50 ml Erlenmeyer flask with ground joint, and adding 3 g of mercuric acetate, 12 ml of methanol and a few boiling chips. The mixture was refluxed for 30 min and the contents of the flask transferred to a 500 ml separatory funnel fitted with a Teflon stopcock which contained 200 ml of water. The flask was rinsed several times with a total volume of 75 ml of chloroform, which was added to the contents of the separatory funnel. The organic solvent layer was removed and the aqueous layer washed 4 times with 50 ml of chloroform. The combined chloroform extracts were evaporated. The chromatographic separation was carried out on a column of Florisil (activated magnesium silicate). The Florisil was deactivated with 14 % by wt. of distilled water. The columns were Pyrex tubes, about 400 × 20 mm in size each fitted with a fritted glass disk and a Teflon stopcock. The elution solvent was a

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mixture of hexane–diethyl ether, 8:2. The columns were charged with 30 g of deactivated Florisil slurried in elution solvent.

The reacted fat, after evaporation of the solvent, was taken up in 10 ml of the elution solvent and introduced into the chromatographic column. The GS_3 were eluted with 500 ml of the hexane–ether mixture and weighed after evaporation of the solvent.

The unsaturated glycerides could be obtained and regenerated from the adducts adsorbed on the Florisil by a second elution with 180 ml of a solvent mixture consisting of 95 % ethanol, chloroform and hydrochloric acid, 9:8:1. The eluent was transferred to a 500 ml separatory funnel, 200 ml of water and 200 ml of petroleum ether added and the organic solvent layer washed several times with water. The organic layer was then neutralized, washed again and evaporated.

The fatty acid compositions of the GS_3 and GU were determined by GLC using a method similar to the one described by DEMAN[3]. The results are expressed in weight % as methyl esters.

RESULTS AND DISCUSSION

The reliability of the outlined procedure is indicated by the fatty acid analysis of the GS_3. In no case was there more than a trace (less than 0.1–0.2 %) of unsaturated fatty acid in the GS_3. Fig. 1 shows the gas chromatograms of a sample of shortening (sample No. 2), and of the GS_3 obtained from it. Recovery trials of added trilaurin and trimyristin to vegetable oils indicated recoveries within ± 0.6 %, when GS_3 was added at levels of 20–40 %. Repeatability was checked by performing 10 replications on a sample of milk fat, which resulted in values ranging from 35.2 to 36.6 % with a standard deviation of the mean of 0.15 %.

The GS_3 contents of two samples of lard (Table I) were 10.3 and 11.1 %. The fatty acid compositions of sample 1, its GS_3 and GU are given in Table II. The values for GS_3 content correspond with figures reported by COLEMAN[4], who found 9.2 and 12.9 mole % in two samples. VANDER WAL[5] found only 0.6 mole % and YOUNGS[6] 8.0 mole %. The GS_3 contains palmitic and stearic acid as major components in approximately equal amounts, and small amounts of lauric and myristic acid. The

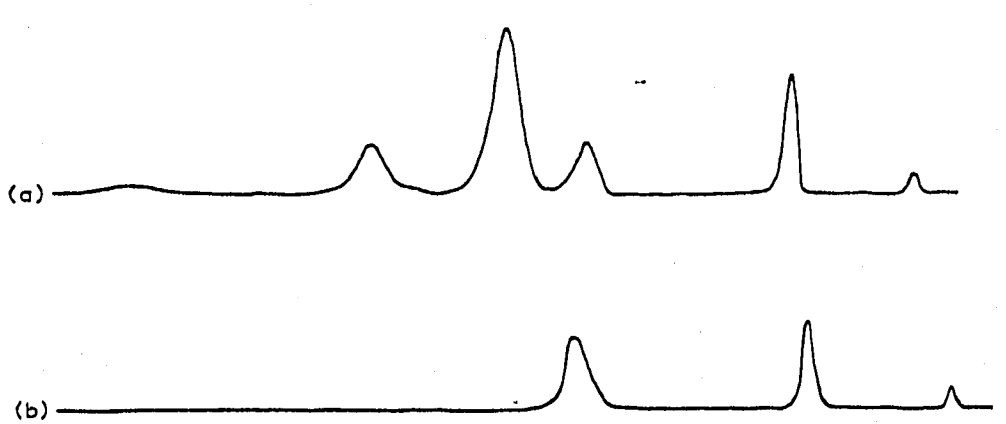

Fig. 1. Gas chromatograms of the fatty acid methyl esters of a sample of shortening (No. 2, Table I) and of the trisaturated glycerides obtained from it by the mercuric acetate method. Peaks from right to left: (a) 14:0, 16:0, 18:0, 18:1, 18:2, and 18:3; (b) 14:0, 16:0, and 18:0.

TABLE I

TRISATURATED GLYCERIDE (GS_3) CONTENT OF SOME FATS AS DETERMINED BY THE MERCURIC ACETATE ADDUCT PROCEDURE

Product	% GS_3
Lard 1	11.1
Lard 2	10.3
Shortening 1	17.1
Shortening 2	11.5
Coconut oil	81.9
Margarine fat 1	4.1
Margarine fat 2	9.0
Margarine fat 3	6.3
Margarine fat 4	5.4
Margarine fat 5	9.3

TABLE II

FATTY ACID COMPOSITION IN WEIGHT % AS METHYL ESTERS OF LARD SAMPLE I AND THE TRISATURATED (GS_3) AND UNSATURATED GLYCERIDES (GU) OBTAINED FROM IT BY THE MERCURIC ACETATE ADDUCT PROCEDURE

Fatty acid	*Whole fat*	*GS_3*	*GU*
12:0	0.1	0.9	0
14:0	1.2	3.6	1.3
16:0	25.1	49.7	23.7
16:1	1.8	0	2.3
18:0	12.1	45.6	10.7
18:1	45.7	trace	49.1
18:2	12.6	0	10.8
18:3	1.5	0	2.0

TABLE III

FATTY ACID COMPOSITION IN WEIGHT % AS METHYL ESTERS OF SHORTENING SAMPLE I AND THE TRISATURATED (GS_3) AND UNSATURATED GLYCERIDES (GU) OBTAINED FROM IT BY THE MERCURIC ACETATE ADDUCT PROCEDURE

Fatty acid	*Whole fat*	*GS_3*	*GU*
12:0	0.1	0.9	0
14:0	6.5	7.8	5.0
16:0	26.3	49.4	20.9
16:1	8.3	1.0	10.5
18:0	13.8	40.8	9.8
18:1	29.7	trace	34.3
18:2	11.2	0	14.1
18:3	4.2	0	5.4

lauric acid was found to be associated with the GS_3. MAGIDMAN *et al.*[7] have reported 0.07 % lauric acid in lard. The stearic acid content of the GS_3 was close to four times higher than in the whole fat, whereas the palmitic acid content was only about twice as high. This indicates that, in lard, palmitic acid seems to combine more readily with unsaturated acids into glycerides than does stearic acid.

The shortening samples analyzed contained 17.1 and 11.5 % GS_3. There seems to be no published information on the content and composition of the GS_3 of shortenings. Table III gives the fatty acid composition of shortening sample No. 1 and its GS_3 and GU. As in the lard there was a relatively greater accumulation of stearic acid than palmitic acid in the GS_3.

Coconut oil had a GS_3 content of 81.9 %. ESHELMAN *et al.*[8] reported 72.2 and 76.2 % in two samples analyzed by the mercaptoacetic acid method. COLLIN AND HILDITCH[9] found 84–86 mole %. The fatty acid composition of coconut oil, its GS_3 and GU is listed in Table IV. It is interesting to note that palmitic acid is present in a higher percentage in the GU than in the GS_3.

TABLE IV

FATTY ACID COMPOSITION IN WEIGHT % AS METHYL ESTERS OF COCONUT OIL AND THE TRISATURATED (GS_3) AND UNSATURATED GLYCERIDES (GU) OBTAINED FROM IT BY THE MERCURIC ACETATE ADDUCT PROCEDURE

Fatty acid	*Whole fat*	*GS_3*	*GU*
6:0	0.8	0.9	0.5
8:0	7.6	8.6	4.6
10:0	6.5	7.1	2.9
12:0	47.3	53.8	23.1
14:0	17.3	19.3	9.5
16:0	8.2	7.2	11.2
16:1	0.1	0	0.5
18:0	2.7	3.0	2.6
18:1	7.2	trace	35.5
18:2	2.3	trace	9.6
18:3	trace	0	trace

Five samples of margarine fat analyzed had GS_3 contents ranging from 4.1 to 9.3 %. The fatty composition of sample No. 1 and its GS_3 and GU is given in Table V.

The examples given above indicate that this method can be applied to determine the GS_3 content of a variety of fats, it is considerably easier to perform and less time consuming than other methods.

TABLE V

FATTY ACID COMPOSITION IN WEIGHT % AS METHYL ESTERS OF MARGARINE FAT SAMPLE I AND THE TRISATURATED (GS_3) AND UNSATURATED GLYCERIDES (GU) OBTAINED FROM IT BY THE MERCURIC ACETATE ADDUCT PROCEDURE

Fatty acid	*Whole fat*	*GS_3*	*GU*
14:0	1.2	16.8	0.4
16:0	12.3	61.0	10.4
18:0	5.9	22.2	5.4
18:1	49.0	trace	51.0
18:2	31.6	0	32.8

SUMMARY

A new method for the determination of the trisaturated glyceride (GS_3) content of fats is described. The method is based on the formation of mercuric acetate adducts of the unsaturated glycerides (GU) of the fat and the subsequent column chromatographic separation of the GS_3 and the mercuric acetate adducts on deactivated Florisil. The GU can be regenerated and eluted with an acid-containing solvent. The fatty acid compositions of the fats, GS_3 and GU of lards, shortenings, coconut oil, and margarine fats were determined.

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