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[Received November 1, 1963—Accepted March 3, 1964]

Determination of the Glyceride Structure of Fats: Distribution of Individual Saturated and Unsaturated Acids[1]

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Abstract

A method has been devised which gives the distribution of saturated and unsaturated fatty acids. It involves fractionation of the triglycerides into groups on the basis of total unsaturation by employing chromatography on a silicic acid-silver nitrate column. The glyceride composition of each fraction is then determined by gas-liquid chromatography (GLC) of the oxidized glycerides. Using this method, the glyceride composition of lard and cocoa butter was determined to give quantitative amt of 24 and 18 glycerides, respectively. Duplicate analyses agreed to within ±0.5%. The fatty acid composition calculated from the glyceride composition agreed to within ±1.5% with that of the original fat. This approach provides a new basis for the evaluation of the glyceride tyes in natural fats and for the first time permits the quantitative determination of all the chemically different glycerides of myristic, palmitic, stearic, oleic, linoleic and linolenic acids in a fat.

Introduction

IN A RECENT publication from this laboratory (16), we have reported a method for the determination of glyceride composition of natural fats which involves oxidation of the fat by permanganate-periodate and subsequent GLC of the oxidized esterified glycerides. Although this method gives the distribution of the individual saturated fatty acids in the glycerol moiety, the unsaturated acids are estimated together as azelaoglycerides. In order to obtain the distribution of the unsaturated acids as well, it is necessary to first quantitatively separate the fat into groups differing in unsaturation. de Vries (3) has recently described such a method. Using a column of silicic acid impregnated with silver nitrate, and varying proportions of benzene in petroleum ether as eluting solvent, he obtained clear cut separation of tristearin, oleodipalmitin, stearodiolein and triolein. In this paper we describe a method of glyceride analysis based on fractionation of a fat into groups differing in the degree of unsaturation followed by GLC analysis of oxidized, esterified glyceride fractions thus obtained. The method has been applied to the study of glyceride composition of lard and cocoa butter.

[1] Presented at AOCS Meeting in Minneapolis, 1963. Issued at NRC 7947.

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Experimental

Materials

Benzene was purified as outlined by Vogel (14), by shaking it with coned H_2SO_4, washing, drying and distilling using a fractionating column. The firt and the last 50 ml were rejected and the middle fraction was collected.

Mallinckrodt silicic acid, 100 mesh, analytical grade was used.

Samples of synthetic triglycerides were obtained from Canada Packers, Toronto, and purified by silicic acid chromatography (6).

Lard and cocoa butter were commercial samples and had iodine values of 66 and 39 respectively.

Methods

Column Chromatography. A mixture of tristearin, oleodistearin, palmitodiolein and triolein was separated on a silver nitrate impregnated silicic acid column as described by de Vries (3). Subsequently, lard was fractionated into 5 fractions as follows: 20 g silver nitrate impregnated silicic acid (3) and 10 g celite were mixed together in a mortar and pestle and packed on to a column (18 mm diam) with 30%, by volume, of benzene in Skellysolve F (a hydrocarbon fraction with a br of 35–58C) to give a column length of 28 cm. The column was covered with black paper during operation. Lard (146 mg) in 3 ml 30% benzene in Skelly F was added at the top of the column. Elution was commenced with 40% benzene in Skelly F. The rate of elution was 0.5 ml/min and fractions of 20 ml were collected. A change in the eluting solvent was made only after a peak started coming down. Thus 55, 80 and 100% benzene solutions were used to elute fractions 2,3 and 4, respectively. Fraction 5 was eluted with ether. Solvent systems for effecting separations were arrived at on the basis of preliminary runs. Cocoa butter was fractionated into 4 fractions using the same general procedure. Fractions belonging to each peak were pooled together. Thin layer chromatography (TLC) of these fractions by the method of Padley (1) gave single spots. The wt of each fraction was then determined.

GLC Analysis. A portion of each fraction was in-

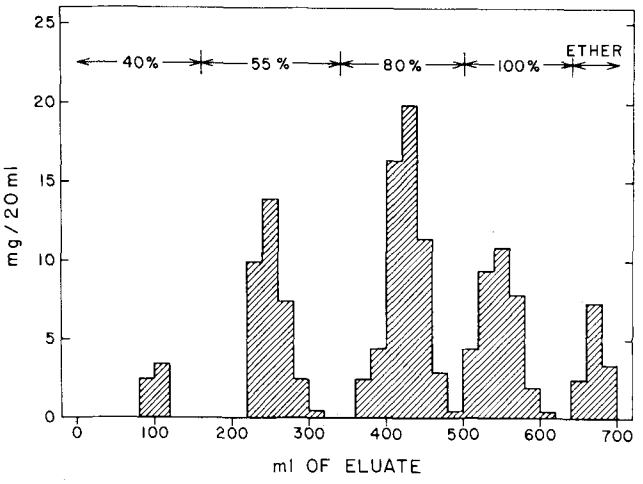

FIG. 1. Fractionation of lard.

teresterified with methanolic HCl and the fatty acid composition of the esters was determined by GLC using a column containing phthalic ethylene glycol on chromosorb W.

Glyceride composition of a further portion of each of these fractions was determined by the method of Youngs and Subbaram (16). Details of the method of oxidation and GLC analysis are described in that paper.

Pancreatic Lipase Hydrolysis. In order to calculate triglyceride composition according to Vander Wal (13), pancreatic lipase hydrolysis of lard and cocoa butter was carried out by the procedure described by Youngs (15).

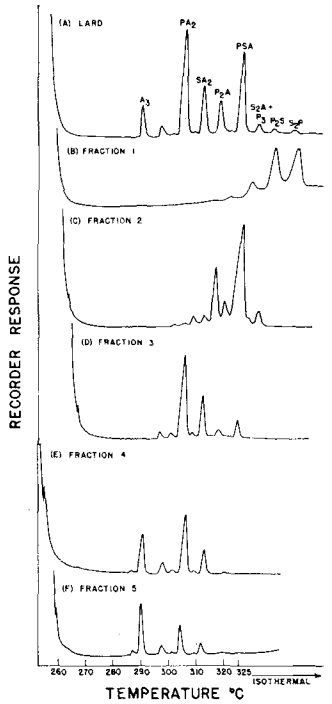

FIG. 2. GLC charts of oxidized methylated glycerides of lard.

Column—4 ft x 3/16 in. in S.S.
Packing—2% SE30 on Anakrom A.B.S.
Helium flow rate—100 ml/min
Hydrogen flow rate—30 ml/min
Air flow rate—400 ml/min
Temp program—260–340C at 3°/min
Inj. port temp—385C
Block temp—355C
Attenuation—800
Chart speed—2 min/in

TABLE I
Separation of Lard and Cocoa Butter on Silicic Acid-Silver Nitrate Column

Fraction No.	Number of double bonds	Eluting solvent	Lard wt %		Cocoa butter wt %
			Run 1	Run 2	
1	0	40[a]	4.7	4.0	1.4
2	1	55[a]	23.3	24.4	73.8
3	2	80[a]	37.7	38.2	18.1
4	3	100[a]	24.0	24.4	6.7
5	4	Ether	10.3	9.0	

[a] % Benzene in Skelly F.

Results and Discussion

A mixture of synthetic triglycerides, tristearin, oleodistearin, palmitodiolein and triolein was separated on the silicic acid-silver nitrate column. Clear cut fractions were obtained and the recovery was 98–102%. This determined the pattern of elution of the triglycerides under the present experimental conditions.

Before attempting the fractionation of lard and cocoa butter by column chromatography, a preliminary TLC separation was carried out by the method of Padley (1) on silver nitrate coated silica gel plates. From this an idea of the components present was obtained. The fractionation of natural fats was then carried out using column chromatography. Lard was thus separated (Fig. 1) into five fractions with 0,1,2,3 and 4 double bonds by eluting respectively, with 40,55,80 and 100% benzene in Skelly F, and finally with ether. Table I gives the wt per cent of fractions as obtained by column chromatography of duplicate runs for lard and a single run for cocoa butter. Duplicate analysis of lard agreed with 1%. Assignment of the degree of unsaturated for each fraction was substantiated by the determination of the fatty acid composition.

These fractions were oxidized, esterified and analysed gas chromatographically by the method of Youngs and Subbaram (16). Figure 2 shows the GLC separations of lard. The separation of oxidized, esterified lard is shown in 2A, while 2B–2F give the glyceride composition of the fractions separated from lard on the silicic acid-silver nitrate column. Figure 2B shows the separation of fraction 1, which should consist of fully saturated glycerides (no double bond)

TABLE II
Glycerides in Lard

Fraction from silicic acid silver nitrate column	Glyceride composition of fraction[a]	Final composition
	Mole %	Mole %
1	PS_2 46.8	2.2
	P_2S 38.2	1.8
	P_3 10.6	0.5
	MP_2 4.4	0.2
2	S_2O 6.0	1.4
	PSO 67.0	15.6
	P_2O 23.2	5.4
	MPO 3.4	0.8
	M_2O 0.4	0.1
3	PSL 5.9	2.2
	P_2L 3.2	1.2
	SO_2 23.0	8.7
	PO_2 64.5	24.3
	MO_2 3.4	1.3
4	PSLi 1.2	0.3
	PPLi 0.4	0.1
	SLO 16.8	4.0
	PLO 46.6	11.2
	MLO 2.4	0.6
	O_3 32.6	7.8
5	SL_2 7.8	0.8
	PL_2 23.4	2.4
	ML_2 3.9	0.4
	O_2L 64.9	6.7

[a] M—myristic, P—palmitic, S—stearic, O—oleic, L—linoleic, Li—linolenic.

TABLE III

Comparison of Glyceride Composition of Lard[a]

		Present results			Coleman	Privett (S = sat'd)	Quimby	Riemenschneider
		Run 1	Run 2[b]	Calc[b]				
GS_3	S_3	....	....	0.2	SSS 0.2			
	PS_2	2.2	1.6	2.4	PSS 0.1			
	P_2S	1.8	1.7	2.2	SPS 2.4	S_3 7.0	2.4	1.9
	P_3	0.5	0.5	0.4	PPS 1.7			
	MP_2	0.2	0.2	0.2	PSP 0.2			
					PPP 0.3			
GS_2U	S_2O	1.4	1.6	1.9	SSU 1.2			
	S_2L	....	....	0.1	SUS 1.1			
	PSO	15.6	15.5	15.5	SPU 18.9	S_2O 23.6	28.0	25.9
	PSL	2.2	2.4	4.0	PSU 0.4	S_2L 11.9		
	PSLi	0.3	0.1	....	PUS 0.7			
	P_2O	5.4	6.0	6.2	PPU 6.5			
	P_2L	1.2	1.2	1.5	PUP 0.1			
	P_2Li	0.1	0.2	....				
	M_2O	0.1	0.2	0.1				
	MPO	0.8	1.1	0.5				
GSU_2	SO_2	8.7	8.9	9.1	SUU 8.3			
	SL_2	0.8	0.6	0.4	USU 2.4			
	SOL	4.0	3.2	2.7		SO_2 19.4	40.1	54.6
	PO_2	24.3	24.3	23.3	PUU 2.9	SOL 23.5		
	PL_2	2.4	2.2	1.6	UPU 36.5			
	POL	11.2	11.0	12.1				
	MO_2	1.3	1.4	1.4				
	ML_2	0.4	0.3	0.1				
	MOL	0.6	1.0	0.5				
GU_3	O_3	7.8	8.9	7.9				
	O_2L	6.7	5.9	5.7	UUU 16.1	O_3 14.6	29.5	17.6

[a] M—myristic, P—palmitic, S—stearic, O—oleic, L—linoleic, Li—linolenic.
[b] Values calculated according to Vander Wal from lipase hydrolysis data.

and only saturated glycerides were recorded. Figure 2C depicts the compounds present in fraction 2 (1 double bond group). The combination of fatty acids possible in this group are those of oleic acid with 2 saturated acid groups. The predominant peak in this chart is that of PSO which represents nearly 65% of the fraction. Figure 2D gives the separation of fraction 3 (2 double bonds). Here we can expect combinations of 2 oleic acid groups with 1 saturated or 1 linoleic with 2 saturated acid groups. Palmitodiolein represents nearly 65% of this fraction. In 2E we see the separation of fraction 4 (3 double bonds). The glycerides in this group contain either 3 oleic acid groups or 1 linoleic, 1 oleic and a saturated acid group. Trace amounts of glycerides of linolenic acid with 2 saturated acid groups were also detected in this fraction. Triolein and POL are the main peaks in this group. Figure 2F represents separation of fraction 5 (4 double bonds). Combinations of fatty acids in this group are 2 linoleics with 1 saturated or 2 oleics with 1 linoleic. Linoleodiolein represents 65% of this fraction. As ether was used to elute this fraction, glycerides containing more than 4 double bonds may also be included. However, the fatty acid composition of this fraction would indicate that such materials, if present, occur in very small amounts. From Figure 2 it becomes apparent that there is clear cut separation of fractions according to the number of double bonds. On the basis of the above analysis, lard has been shown to consist of 24 glycerides in the proportion given in Table II. The triglyceride type distribution determined by GLC for whole lard agreed well with those obtained for the fractions. The agreement of any individual triglyceride peak of whole lard and the total from the fractions was 95% or better. The fatty acid composition calculated from the glyceride composition agrees closely with that of the original to within 1% (Table V). As palmitoleic acid gives azelaic acid on oxidation, it will be included along with oleic acid when the values for monoethenoid acids are calculated from the glyceride composition. In Table I, percentage

TABLE IV

Comparison of Glyceride Composition of Cocoa Butter

		Present results		Coleman	Privett (S = sat'd)	Dutton	Hammond	Meara	Hilditch	Scholfield
		Run 1	Calc[a]							
GS_3	S_3	0.3	1.0	SSS 0.3						
	PS_2	0.6	2.6	SSP 0.4						
	P_2S	0.4	2.2	SPS 0.6	2.0	0.9		3.0	2.8	0.16
	P_3	0.1	0.7	PPS 0.9						
				PSP 0.2						
				PPP 0.3						
GS_2U	S_2O	20.5	19.0	SSU 0.1						
	S_2L	0.3	1.4	SUS 27.4						
	S_2Li	0.2	0.1							
	SPO	37.8	32.7	SPU 0.2	S_2O 74.7	S_2O 26.5	S_2O 17.0	S_2O 22.0	S_2O 18.4	S_2O 22
	SPL	1.1	2.5	PSU 0.1	S_2L 6.3	PSO 38.7	PSO 46.6	PSO 57.0	PSO 51.9	PSO 41
	SPLi	0.4	0.1	PUS 39.3		P_2O 15.1	P_2O 21.0	P_2O 3.7	P_2O 6.5	P_2O 12
	P_2O	15.5	14.0	PUP 14.1						
	P_2L	1.2	1.0	PPU 0.1						
	P_2Li	0.2	0.1							
GSU_2	SO_2	8.8	9.0							
	SOL	2.1	1.1	SUU 8.9						
	SL_2	....	1.5							
	PO_2	6.7	7.6		SO_2 17.0		SO_2 6.6	SO_2 5.8	SO_2 12.0	
	POL	2.0	1.5	PUU 6.4			PO_2 8.8	PO_2 7.4	PO_2 8.4	
	PL_2	....	0.7							
GU_3	O_3	1.8	1.2	UUU 0.7				1.1		

[a] Values calculated according to Vander Wal from lipase hydrolysis data.

TABLE V

Fatty Acid Composition of Lard and Cocoa Butter

	(Mole %)					
	14:0	16:0	18:0	18:1	18:2	18:3
Lard						
Calc[a]	1.3	26.4	13.0	48.6	10.5	0.2
Original	1.5	26.0	12.5	48.5[b]	11.0	0.5
1:3 position[c]	0.7	8.2	18.9	56.8	15.4	.....
Cocoa butter						
Calc[a]		28.4	31.2	37.8	2.3	0.3
Original		28.1	32.7	36.0	3.0	0.2
1:3 position[c]		40.1	46.8	11.8	1.3	.....

[a] Values calculated from Tables II and IV.
[b] Includes palmitoleic acid 3%.
[c] From pancreatic lipase hydrolysis.

composition of the fractions separated on the column are given in wt per cent, while those in Tables II, III and IV are given in mole per cent. When values in Table I were converted on a molar basis, the differences were negligible and mole per cents were not included.

Cocoa butter was fractionated into 4 groups and the fractions were analysed in the same manner (Fig. 3 and Tables I,IV,V). The results show the presence of fully saturated glycerides in fraction 1, disaturated oleins in fraction 2, disaturated linoleins and monosaturated dioleins in fraction 3, and traces of disaturated linolenin, mono-saturated oleolinolein and triolein in the last fraction. Here again the calculated fatty acid and glyceride compositions agreed with the original composition of the fat.

In Tables III and IV, results of glyceride analysis of lard and cocoa butter are compared with those calculated according to Vander Wal's theory (13) and with data reported by other investigators. In the present investigation no attempt has been made to distinguish isomers; for example, POP and PPO are given as P_2O. Data calculated according to Vander Wal (13) from pancreatic lipase analysis for lard (Table III) and similar data given by Coleman (2) agree closely with the present results. Figures obtained by Privett and Blank (9) by TLC of the products of reductive ozonolysis of triglycerides appear to be somewhat different from the present results. While values for S_2L and SOL are higher by 8 and 7% respectively, that for SO_2 is lower by 15% as compared with the present results. Riemenschneider et al. (11), using low-temp crystallization for the determination of glyceride composition of lard, obtained almost the same results as reported here. Quimby et al. (10) who also used low-temp crystallization have reported 40.1 and 29.5%, respectively, for GSU_2 and GU_3. These values vary from the present results. As far as we know this is the first time that the presence of 24 glycerides has been reported for lard.

A comparison of results for cocoa butter (Table IV) shows that, in general, there is agreement of the values obtained for the 4 general classes of glycerides. Just as with lard there was close agreement between the present values and those calculated from lipase hydrolysis data. Values calculated by Coleman (2) from his pancreatic lipase data, are closer to the results of the present investigation than those calculated by us from our pancreatic lipase data. Jones and Hammond (7), Hilditch and Stainsby (5) and Meara (8) have reported values of 47,52 and 57% for PSO as compared to 37.8% obtained in the present investigation. This figure compares favourably with values ranging from 38–41 reported by Dutton et al. (4,12).

The method described in this investigation provides a new basis for the evaluation of glyceride types in natural fats. At present it has been used for the analysis of fats of relatively simple fatty acid composition. However, as a majority of fats fall into this group, the method should prove valuable to study their glyceride composition. Fats containing a large proportion of polyunsaturated or conjugated fatty acids have a different elution pattern on the silicic acid-silver nitrate column. Glycerides containing conjugated fatty acids behave more like those containing saturated acids. Here, modifications will have to be effected in the method used for column separations. Some of these aspects are being investigated in this laboratory.

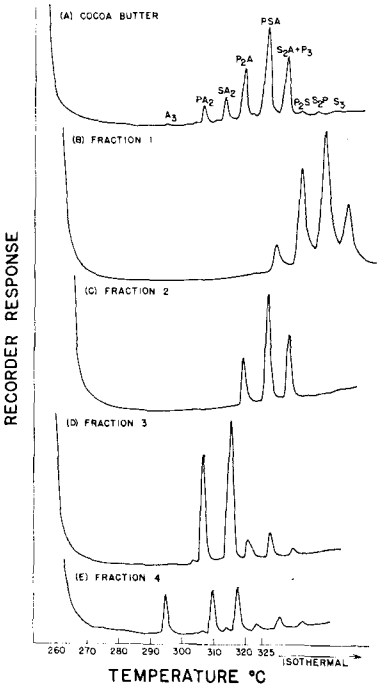

FIG. 3. GLC charts of oxidized methylated glycerides of cocoa butter.

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Packing—2% SE30 on Anakrom A.B.S.
Helium flow rate—100 ml/min
Hydrogen flow rate—30 ml/min
Air flow rate—400 ml/min
Temp program—260–340C at 3°/min
Inj. port temp—385C
Block temp—355C
Attenuation—800
Chart speed—2 min/in

ACKNOWLEDGMENTS

Technical assistance throughout the investigation by D. L. McPhee; synthetic glycerides, courtesy of Canada Packers Ltd.

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[Received November 20, 1963—Accepted March 9, 1964]