Distribution of Individual Fatty Acids in the Crystallization Fractions of Lard

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Abstract

Distribution of the individual fatty acids in the triglycerides of lard was determined by fractional crystallization, partial enzymatic hydrolysis with steapsin, and fatty acid analyses by GLC. It was found that none of the individual fatty acids corresponded to a random distribution in the crystallization fractions, but that the distribution of the total saturated and total unsaturated acids was very nearly random. The short chain fatty acids, C_{14} and C_{16}, both saturated and unsaturated, were found to be more predominant in the 2-position than in the 1- and 3-positions of the lard triglycerides. All of the C_{18} fatty acids were found to be more predominant in the 1- and 3-positions.

Introduction

A METHOD for the calculation of the amounts of the six glyceride types, SSS, SSU, SUS, SUU, USU, and UUU (S = saturated and U = unsaturated fatty acids) in a fat, from the total saturated fatty acid composition and the percentage of saturated acids in the 2-position, has been described by VanderWal (3). Random distribution and the same ratio of saturated and unsaturated acids in the 1- and 3-positions as in the 2-position were assumed. Youngs (5) presented a similar method together with experimental data for lard. Results based on both the Youngs data and the VanderWal calculation method indicate that the distribution of the saturated and unsaturated acids were random for the 2-position, and for the 1- and 3-positions of the six glyceride types in lard, even though the 2-position and the 1- and 3-position distributions were different. These results do not indicate whether the individual fatty acid distributions were random. The purpose of this investigation was to study the distribution of individual fatty acids in the glycerides of lard.

The study was made by fractionation of the lard by low temperature crystallization followed by enzymatic hydrolysis of the original lard and each of the crystallization fractions. The composition of the fatty acids liberated by the hydrolysis and the fatty acid compositions of the lard and each of the fractions were determined by gas-liquid chromatography of the fatty acid methyl esters.

TABLE I

Lard Crystallization Fractions

Fraction[a]	Wt in g	
	Before[b]	After[b]
P-3	4.22	1.57
P-6	2.46	5.08
F-6	10.80	6.43
F-5	5.01	5.86
F-4	2.51	6.07
Total	25.00	25.01

[a] P = precipitate; F = filtrate.
[b] Before and after molecular rearrangement.

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TABLE II

Glyceride Composition of the Fractions

Type[a]	Composition in mol percentages			
	Before[b]		After[b]	
	Exp.	Calc.[c]	Exp.	Calc.[c]
GS_3	3.4	7.1	4.7	9.5
GS_2U	29.6	30.1	33.9	34.0
GSU_2	54.7	42.6	49.1	40.4
GSU_3	12.3	20.1	12.3	16.0

[a] G = glycerol radical; S = saturated fatty acid; U = unsaturated fatty acid.
[b] Before and after rearrangement; experimental and calculated values.
[c] Calculated for random distribution on all possible sites, 1-, 2-, and 3-positions.

Experimental

Randomized lard was prepared by heating steam rendered lard with 0.2% sodium methylate for 2 hr at 120–150C. Samples of the natural lard and the rearranged lard were then both treated in the same manner through fractionation, hydrolysis, and fatty acid analyses. The fractional crystallization into five fractions followed the scheme of Luddy et al. (1), using dry ice for cooling.

A modification of Youngs' (5) enzymatic hydrolysis was used. Eighteen ml of 0.5M K_2HPO_4, adjusted to pH 8 by the addition of 0.5M NaH_2PO_4 and 30 mg

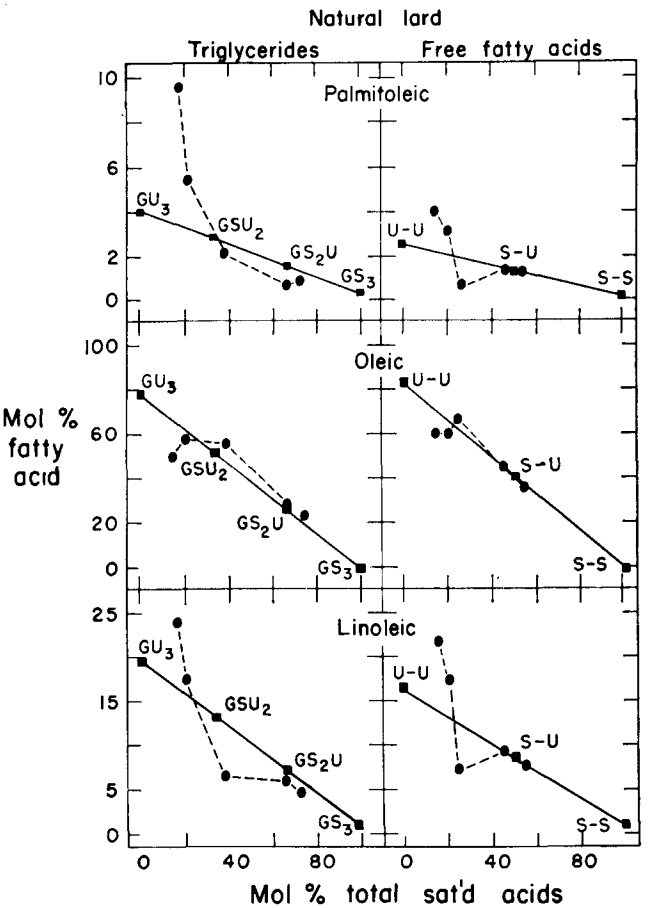

FIG. 1. Distribution of palmitoleic, oleic, and linoleic acids in lard.

TABLE III
Fatty Acid Composition of Lard Fractions
(In mol percentages)

		Lard		Crystallization fraction									
				P-3		P-6		F-6		F-5		F-4	
		B	A	B	A	B	A	B	A	B	A	B	A
Myristic	TG	1.8	2.0	0.7	2.3	2.0	1.7	2.0	2.0	2.1	1.9	2.5	1.9
	FFA	0.6	2.0	0.4	2.1	0.4	1.3	1.0	1.8	0.8	1.2	0.4	1.6
Palmitic	TG	27.8	30.7	37.3	57.3	41.0	42.6	30.1	32.1	15.9	25.2	13.6	12.7
	FFA	9.1	27.9	11.9	43.6	16.9	41.0	12.6	32.9	12.4	29.4	9.1	13.4
Palmitoleic	TG	2.4	3.5	0.8	0.3	0.8	1.9	2.1	3.0	5.4	4.6	9.5	7.6
	FFA	1.8	3.2	1.1	1.5	1.2	2.8	0.6	2.2	3.0	3.2	3.8	4.7
Stearic	TG	11.8	13.0	35.5	31.7	23.6	20.9	6.1	14.1	2.6	8.0	1.7	1.7
	FFA	14.3	14.2	43.2	36.0	28.4	18.7	11.9	19.8	7.2	11.9	5.7	4.0
Oleic	TG	45.5	45.3	22.2	8.4	27.9	29.7	54.1	44.2	57.7	53.2	49.9	66.9
	FFA	62.5	48.7	36.9	15.6	45.0	32.2	67.8	38.9	59.9	48.5	60.3	66.9
Linoleic	TG	10.7	5.5	3.6	Trace	4.8	3.2	5.6	4.7	16.2	7.1	22.9	9.2
	FFA	11.6	4.0	6.6	1.2	8.1	4.1	6.2	4.5	16.6	5.8	20.8	9.4
Mol % S	TG	41.4	45.7	73.4	91.3	66.6	65.2	38.2	48.1	20.7	35.1	17.7	16.3
	FFA	24.0	44.1	55.5	81.7	45.7	61.0	25.5	54.5	20.4	42.5	15.2	19.0
Calcd I.V.	TG	61.5	53.1	26.8	7.9	34.2	34.0	60.0	50.3	84.0	63.9	92.9	81.8
	FFA	79.6	55.5	46.3	20.9	56.9	40.3	73.4	46.6	87.5	58.6	96.1	82.7

Abbreviations: P = precipitate; F = filtrate; B = before rearrangement; A = after rearrangement; TG = triglyceride; FFA = fre fatty acid; S = saturated; I.V. = iodine value.

bile salts, were added to 120 mg fat samples. The mixture was stirred at 40C until emulsion formed. After the addition of 48 mg of steapsin the hydrolysis continued for 15 min with constant stirring. Next 18 ml of 1*N* aqueous HCl, and sufficient NaCl to saturate the solution, were added. The hydrolyzed fat was extracted with ethyl ether and then treated with excess ethereal diazomethane. After standing with occasional stirring for 1 hr at room temperature the excess diazomethane and ether were evaporated and portions of the residue were injected into the gas chromatograph to obtain the compositions of the fatty acid methyl esters. Methyl esters of the lards and lard fractions were prepared by refluxing the samples with 5% anhydrous HCl-CH_3OH by the method of Vogel (4). The chromatograph was an F and M Model 500 with 1 MV Brown recorder, disc integrator, and a copper tube column packed with 20% ethylene glycol succinate (EGS) on 60/80 mesh Chromosorb. Column temperature was 215C. Part of the methyl ester analyses were also made in a column of 5% butanediol succinate (BDS) on 80/100 mesh aqua regia treated C-22 firebrick at 220C (6).

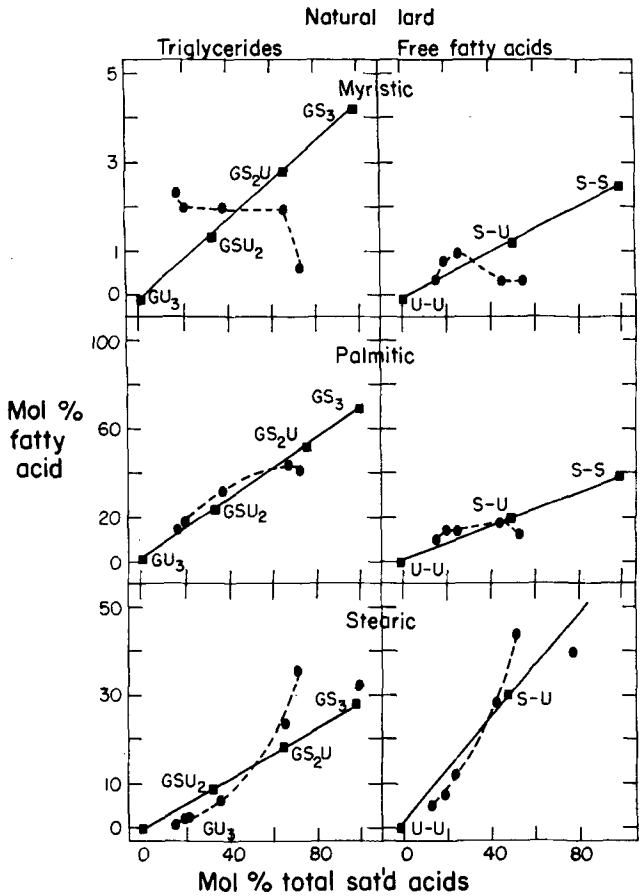

FIG. 2. Distribution of myristic, palmitic, and stearic acids in lard.

Data for the crystallization fractions are summarized in Tables I, II, and III. The crystallization fractions from the lard before and after rearrangement are shown in Table I. The glyceride compositions of the lards calculated from the fraction weights and the fatty acid compositions of the fractions and based upon the assumption that each fraction contained no more than two of the four major types of glycerides, GS_3, GS_2U, GSU_2, and GU_3 are shown in Table II. Table III shows the fatty acids liberated during hydrolysis, and the iodine values calculated from these fatty acid compositions.

The results agree in general with previous work such as that of Quimby et al. (2). The data in Figures 1 and 2 show that in the natural lard each of the individual fatty acid distributions deviate from random both for the combined 1-, 2-, and 3-position fatty acids (TG data), and the 1- and 3-position fatty acids (FFA data). The short chain fatty acids were more predominant in the 2-position than in the 1- and 3-positions. All of the C_{18} fatty acids were found to predominate in the 1- and 3-positions.

The samples after rearrangement corresponded very closely to a random distribution of the individual fatty acids in the lard fractions. The distribution of both saturated fatty and unsaturated acids among each of the six glyceride types, SSS, SSU, SUS, SUU, USU, and UUU, appeared to be random even though none of the individual saturated acids showed random distribution in these glycerides. The deviations of the individual saturated fatty acids from random were in general larger for the saturated fractions. The deviations of the individual unsaturated acids were larger for the unsaturated fractions and very nearly random for the saturated fractions.

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[Received November 26, 1962—Accepted April 12, 1963]