The Pancreatic Hydrolysis of Natural Fats. IV. Some Exotic Seed Fats

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Abstract

Eight exotic seed fats (karanja, kusum, neem and aceituno oils, malabar tallow, kokum butter and mowrah and dika fats) have been subjected to pancreatic hydrolysis. From the original fatty acid compositions, and those of the monoglycerides produced by hydrolysis, the fatty acid distributions have been determined, and their probable glyceride compositions calculated.

The significance of the observed fatty acid distributions is discussed; and an expression relating the glyceride composition to the overall fatty acid composition is shown to give satisfactory agreement with the calculated values, for these, and other vegetable fats.

Introduction

THE HIGH DEGREE of positional specificity of pancreatic lipase (EC.3.1.1.3), in catalysing the hydrolysis of fatty acid residues esterified at the primary positions of triglycerides, has been established in a number of investigations (1). Following the suggestion of Mattson and Beck (2) and Savary and Desnuelle (3), a number of workers have employed pancreatic lipase to investigate the distribution of fatty acids in the triglycerides of natural fats (4–7).

VanderWal has suggested (8) that the acids of the primary positions of the triglycerides of a natural fat are randomly distributed throughout all of these positions. If this is so, it is possible to calculate the original triglyceride composition of a fat, from the data obtained by subjecting it to hydrolysis with pancreatic lipase. The results of such calculations (8–10) have provided much information, not previously available, on the structure of natural fats; and there are now a number of lines of evidence which point to the validity of VanderWal's theory (see below). The triglyceride compositions so calculated provide material for the examination of a number of theories of the distribution of fatty acids in natural fats, which have recently been advanced (6,11,12).

In a previous contribution (9) the results obtained by the application of pancreatic hydrolysis to a number of the commoner natural fats, have been described. In the present study eight less common, exotic seed fats have been examined by this method. They include karanja, kusum, neem and aceituno oils, malabar tallow, kokum butter and mowrah and dika fats.

Experimental

Six of the fats were obtained commercially in Bombay, through the courtesy of D. A. Shave; who also secured the sample of dika seeds from West Africa. The sample of aceituno oil (from San Salvador) was kindly supplied by Messrs. Loders and Nucoline Ltd. The ground dika seeds were extracted with chloroform in a Soxhlet extractor. All samples were subjected to a preliminary purification, by applying approximately 1 g to a 30 g column of silica gel, and eluting the triglyceride fraction with 200 ml of benzene (13).

A 50 mg sample of this fraction was set aside for determination of the overall fatty acid analysis. The remainder was hydrolysed in the the way previously described (9) at pH 8.5 and 37.5C, using a purified pork pancreatic lipase preparation (14), with the addition of Ca^{++} ions and bile salts. Hydrolysis times varied between 10 and 15 min. After extraction of the reaction products with ether, and removal of the free fatty acids with IR 400 "Amberlite" resin, the monoglycerides were isolated chromatographically (13). The monoglycerides, and the original triglyceride samples were then saponified in alcoholic KOH, the free acids liberated and extracted, and converted to methyl esters with diazomethane. The samples were analysed by gas-chromatography (GLC) on a 1.2 m x 4 mm column of 16% polyethylene glycol succinate on "Embacel," at 196C. The detector used was a gas-density balance. Peak areas were determined as the product of peak height and the width at half height: the weight percentages so obtained being converted to mole percentages.

The lipolysis and subsequent analysis was performed twice on each fat.

Results

The duplicate fatty acid analyses were in good agreement for both the original triglycerides and the monoglycerides produced by hydrolysis. The average values are given in Tables I and II; in no case did the mole percentage of any constituent differ from these by more than 2%.

The triglyceride compositions were calculated from these values, in accordance with VanderWal's theory, in the way previously described (9). Tables II and III give the results of these calculations, in which

TABLE I

Fatty Acid Compositions of 7 Seed Fats, and of the Monoglycerides Produced by Hydrolysis

Fat	Glyc.	Sat. acid content (M%)	Fatty acid composition (M%)						
			16	18	18:1	18:2	18:3	20	Others
Karanja oil	T	26.6	14.6	6.8	51.1	17.3	5.0	1.0	4.2[a]
	M	4.4	2.5	1.2	57.9	32.5	5.2	0.7	....
Kusum oil	T	27.4	8.7	1.5	47.3	7.3	....	17.2	1.5[b] 16.5[c]
	M	3.4	1.0	....	83.7	12.9	....	2.4	....
Neem oil	T	40.5	20.1	19.2	44.4	16.3	t	....	....
	M	4.7	2.9	1.8	68.8	26.1	0.4	....	....
Aceituno oil	T	41.1	11.6	27.7	55.0	3.9	....	1.8	....
	M	4.4	0.7	3.7	90.2	5.4	....	....	....
Mowrah fat	T	45.7	23.7	22.0	38.5	15.8	t	....	....
	M	6.6	3.2	3.4	57.0	35.0	1.4	....	....
Malabar tallow	T	57.3	9.0	46.9	41.4	1.3	t	1.4	....
	M	5.0	1.1	3.9	90.7	3.9	0.4	....	....
Kokum butter	T	62.7	1.7	61.0	36.8	0.5	....	....	....
	M	4.3	...	4.3	94.4	1.3	....	....	....

[a] Behenic.
[b] Palmitoleic.
[c] Eicosenoic.
T, Triglycerides.
M, Monoglycerides.

TABLE II

Fatty Acid Compositions of Triglycerides and Monoglycerides of Dika Fat; and Calculated Triglyceride Composition (VanderWal's Theory)

Fatty acid composition (M%)			Triglyceride composition			
Fatty acid	Triglyc.	Mono-glyc.	Glyceride	M%	Glyceride	M%
10	0.8	....	LLL	2.9	MMP	4.8
12	35.5	12.1	LLM	5.3	LPL	2.7
12:1	0.9	1.2	MLM	2.4	LPM	4.9
14	54.7	76.3	LML	18.2	MPM	2.2
16	5.7	6.9	LMM	32.7	PLP	0.1
16:1	t	0.5	MMM	14.7	PMP	0.4
18	0.4	0.2	LLP	0.9	LPP	0.8
18:1	2.0	1.8	MLP	0.8	MPP	0.7
18:2	t	1.0	LMP	5.4	PPP	0.1

L—Lauric, including a little capric and stearic.
M—Myristic.
P—Palmitic, including some unsaturated acids.

the fatty acids have been grouped as "palmitic," "stearic" and "unsaturated" (following Hilditch [15]); except for kusum oil and dika fat where the fatty acid compositions and distributions suggested more appropriate groupings.

For the purpose of comparison with earlier determinations, the results have also been grouped as the proportions of the four main triglyceride classes, tri-, di-, and mono-saturated and tri-unsaturated: and these are given in Table IV. It will be seen that there is general agreement between the present and previous results. Some of the differences (e.g. for kusum oil) are no doubt due to differences in the fatty acid compositions of the two samples examined: others may well be due to the incomplete resolution of triglyceride classes afforded by the older methods of crystallisation and oxidation, used in the previous analyses. The fatty acid compositions of the triglycerides of aceituno oil, and of the monoglycerides produced, agree well with values reported by Mattson and Volpenhein (4).

Discussion

It is convenient to consider the seven fats listed in Table I together; the case of dika fat will be discussed separately, below.

The validity of VanderWal's fatty acid distribution theory, and of the triglyceride compositions of natural fats calculated with its aid, rests on several lines of evidence. Firstly there is the general agreement between compositions so calculated, and the experimental results obtained by the older methods of oxidation and fractional crystallisation (1,9), and the more recent counter-current distribution between solvents (1). Secondly, there is the agreement between such results and those obtained by thin-layer chromatography (TLC) on $AgNO_3$-impregnated silica gel (16). Thirdly, there is similar agreement with the results obtained by various combination methods; lipolysis with oxidation (10); lipolysis with TLC (16,17) and GLC (18); oxidation and GLC (19); column chromatography, oxidation and GLC (20). It is important to note that the combination methods constitute different ways of segregating the triglycerides; and in general give a unique answer for the proportions of the various triglycerides present. Thus, providing the original fat does not contain too many unsaturated acids, the combination of TLC and lipolysis can give a complete picture of the distribution of unsaturated acids, within and between the triglycerides present. Similarly the combination of oxidation and GLC yields a detailed picture of the distribution of saturated acids.

The good agreement between these results and those calculated from lipolysis data with VanderWal's theory, constitute formidable evidence in favour of the theory; whilst the independence of the monoglyceride composition of the extent of hydrolysis (21) is also consistent with the theory, although not conclusive evidence (22) in its favour.

TABLE III

Triglyceride Compositions Calculated from Hydrolysis Data (VanderWal's Theory)

Glyc.	Karanja oil[a,b]	Neem oil	Aceituno oil[a]	Mowrah fat	Malabar tallow[a]	Kokum butter[c]	Kusum oil
PPP	0.1	0.2	....	0.4	....	....	....
PPSt	0.2	0.5	0.1	0.7	0.2	....	....
StPSt	0.1	0.2	0.1	0.3	0.6	....	....
PStP	0.1	0.2	0.1	0.4	0.1	....	....
PStSt	0.1	0.3	0.5	0.7	0.7	....	....
StStSt	0.1	0.2	0.7	0.3	1.9	3.6	....
PPU	0.6	0.7	0.1	0.8	0.1	....	....
StPU	0.5	0.7	0.2	0.7	0.3	....	....
PStU	0.5	0.5	0.5	0.8	0.2	....	....
StStU	0.4	0.4	1.3	0.7	0.9	0.6	....
PUP	4.1	7.9	2.8	10.7	1.6	....	....
PUSt	6.7	15.3	13.8	19.8	17.3	....	....
StUSt	2.8	7.3	17.2	9.2	47.1	80.9	....
UPU	1.0	0.6	0.1	0.4	....	....	....
UStU	0.7	0.3	0.6	0.4	0.1	....	....
PUU	24.6	23.7	13.2	22.0	4.1	....	....
StUU	20.3	23.1	32.9	20.4	22.2	14.3	....
UUU	37.1	17.9	15.8	11.3	2.6	0.6	....
SSS	....	....	....	....	....	....	0.5
SSU	....	....	....	....	....	....	1.0
SSE	....	....	....	....	....	....	0.7
SUS	....	....	....	....	....	....	15.0
SUU	....	....	....	....	....	....	27.4
SUE	....	....	....	....	....	....	18.7
USU	....	....	....	....	....	....	0.4
USE	....	....	....	....	....	....	0.6
ESE	....	....	....	....	....	....	0.2
UUU	....	....	....	....	....	....	12.5
UUE	....	....	....	....	....	....	17.2
EUE	....	....	....	....	....	....	5.8

E—Eicosenoic.
P—Palmitic.
St—Stearic.
U—Unsaturated.
S—Saturated.
Stearic includes a little—[a] archidic; [b] behenic; [c] palmitic.

Some investigators have reported examples of fats which do not appear to conform to the fatty acid distribution envisaged by VanderWal. It is necessary however to distinguish between real departures from VanderWal's theory, and those calculated from lipolysis data for fats which are unsuitable for investigation by pancreatic hydrolysis. Of the former, only the results of Blank et al. (23) on rat liver triglycerides show major departures from VanderWal's theory. Small discrepancies have been noted for palm oil by Subbararam and Youngs (24) and Jurriens and Kroesen (18); and for human depot fat (24). One investigation (18) reports small departures for lard, whilst the other does not (24); and similarly for cocoa butter, small differences are noted in one (24), but not in the other (18). Departures from VanderWal's theory which are probably due to difficulties associated with the lipolysis, include the fat from bitter gourd seed (25) and bayberry tallow (26). It is interesting to note that castor oil has given results (27) in agreement with the

TABLE IV

Comparison of Present with Previous Results

Fat	Sat. acid content (M%)	Triglyceride composition (M%)			
		S_3	S_2U	SU_2	U_3
Karanja oil	26.6	0.7	15.6	46.6	37.1
Ref. 11	26	....	15	48	37
Kusum oil	27.4	0.5	16.7	47.3	35.5
Ref. 30	41.3	1	37	47	15
Neem oil	40.5	1.6	32.8	47.7	17.9
Ref. 11	40	....	39	41	20
Mowrah fat	45.7	2.8	42.7	43.2	11.3
Ref. 11	43	t	47	36	17
Malabar tallow	57.3	3.5	67.5	26.4	2.6
Ref. 31	57.3	1	73	22	4
Kokum butter	62.7	3.6	81.5	14.3	0.6
Ref. 32	59.2	2	76	20	2

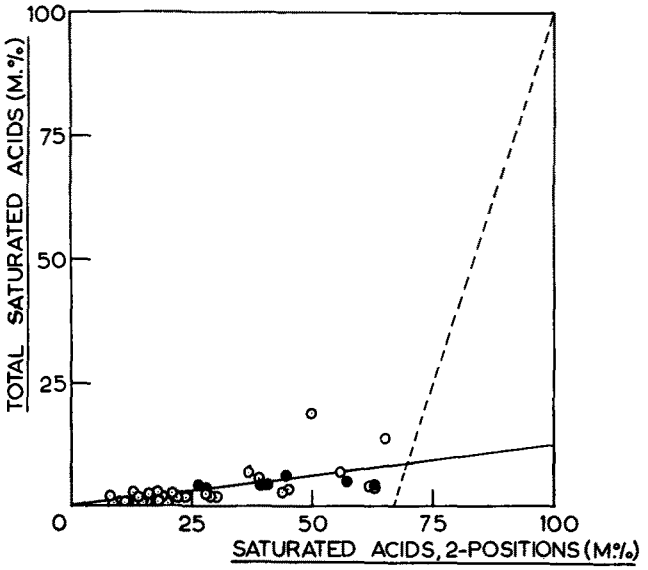

FIG. 1.—The relationship of the saturated acid content of the 2-positions to that in the whole fat, for 48 vegetable fats. ○—Data from refs. 4, 5 and 9. ●—Present results. (Above 66.7 M% additional saturated acids must enter 2-positions—indicated by dotted line.)

theory, in spite of the presence of about 90 M% of hydroxy-acids.

Inspection of Table I shows that the seven fats listed exhibit the same preponderance of C_{18} unsaturated acids in the 2-positions, which has been observed for vegetable fats in general (4,5,7,9). Gunstone has advanced a theory of fatty acid distribution in vegetable oils (12) which requires that the 2-positions are preferentially esterified with C_{18} unsaturated acids, as far as the composition allows, and thereafter the remaining acids are distributed randomly amongst the unoccupied positions.

The monoglyceride compositions given in Table I show that the present results agree fairly closely with this theory; but not completely so, since each contains about 5% of the saturated acids. According to Gunstone's theory, other acids should not appear in the 2-positions until the C_{18} unsaturated acid content falls below one-third of the acids of the whole fat; a restriction which does not apply to any of the fats examined here. It may be argued that the presence of saturated acids in the monoglycerides arises from a lack of complete specificity in the hydrolysis, or isomerisation of the partial glycerides produced. But against this must be set the fact that trisaturated triglycerides have been detected by a variety of methods in fats containing more than one-third of C_{18} unsaturated acids (10,16–20,28).

From these considerations it would seem appropriate to introduce a modification into Gunstone's theory, to permit the prediction of triglyceride composition from a knowledge of the fatty acid composition of a vegetable fat. Mattson and Volpenhein (5) have already indicated one such extension of Gunstone's theory, in which they suggest that the proportion of any one C_{18} unsaturated ("Category II") acid, considered as the mole percentage of acids of this kind, is the same in the 2-positions, as in the whole fat; i.e., these acids follow Gunstone's theory individually, as well as collectively. However, they qualify this by observing that there is a tendency for linoleic acid to exceed the expected proportion by about 10%, with a corresponding reduction in oleic acid.

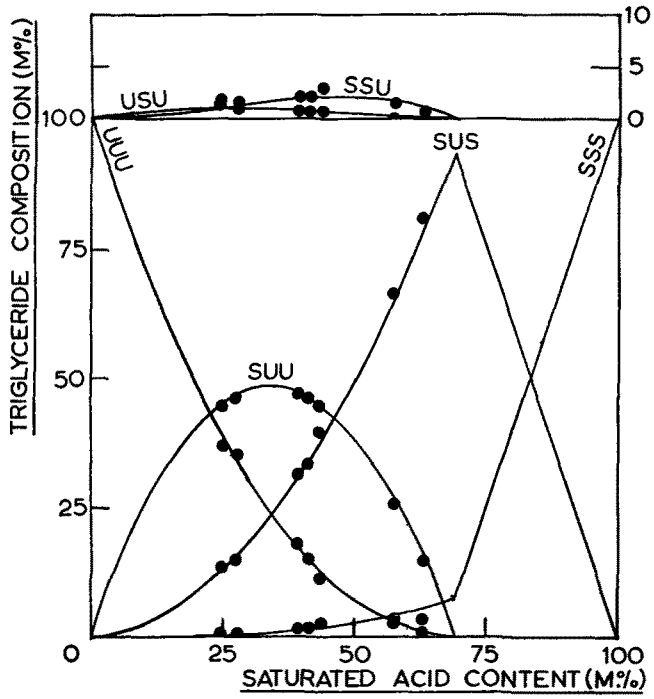

FIG. 2.—The triglyceride composition of vegetable fats. Continued lines calculated from expressions given in text. ●—Data from Table III. (Upper portion gives the proportion of USU and SSU, absent from Gunstone's theory.)

The modification of Gunstone's theory proposed here seeks to accommodate the saturated acids found in the monoglycerides of fats containing less than two-thirds of saturated acids. Figure 1 illustrates the relationship between the saturated acid content of the whole fat, and that found in the 2-monoglycerides by hydrolysis, for 48 vegetable fats in which palmitic, stearic and the C_{18} unsaturated acids constitute more than 90% of the acids present. With few exceptions the saturated acid content of the 2-positions is approximately 10% of that in the whole fat. This value may be used to modify Gunstone's theory: for simplicity of illustration, a fat may be supposed to consist of 's' M% of a saturated acid (S) and (100—s) M% of a C_{18} unsaturated acid (U). The proportions of the six possible triglycerides will then be given by:

$$SSS = 0.1s\ (1.45s)^2 \div 10^4$$
$$SSU = 2[0.1s \times 1.45s\ (100 - 1.45s) \div 10^4]$$
$$SUS = (100 - 0.1s)(1.45s)^2 \div 10^4$$
$$SUU = 2[1.45s\ (100 - 0.1s)(100 - 1.45s) \div 10^4]$$
$$USU = 0.1s\ (100 - 1.45s)^2 \div 10^4$$
$$UUU = (100 - 0.1s)(100 - 1.45s)^2 \div 10^4$$

The results for all values of 's' are illustrated graphically in Figure 2; and the values of the results listed in Table III are plotted to show the agreement.

The fatty acid analysis for dika fat is similar to that described by Meara and Patel (29); and the triglyceride compositions are comparable (Table II). Myristic comprises three-quarters of the acids found in the 2-positions, whilst lauric and myristic, in roughly equal amounts form the great majority of the acids of the 1 and 3 positions. It is possible that differences in rates of hydrolysis may affect lipolysis data for a fat containing so much of the shorter chain acids.

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Semiquantitative Structural Analysis of Fats by Thin-Layer Chromatography of the Allyl Esters of the Products of von Rudloff Oxidation[1]

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Abstract

Each unsaturated acyl group in a fat molecule retards its migration on a silica-silver nitrate TLC plate to a degree dependent on its number of double bonds. Thus S_2U in which U is linoleic would migrate with SU_2 in which U is oleic. Therefore the molecular families S_3, S_2U, SU_2 and U_3 cannot be separated, as such, by this variety of TLC.

Oxidation of fats by the von Rudloff technique converts the glyceryl esters of the original unsaturated acids into the glyceryl half esters of dicarboxylic saturated acids, such as azelaic. Subsequent formation of allyl esters at the free carboxyls, in effect replaces each mono- or polyunsaturated acyl group with another group containing only a single double bond.

Fats so altered can be separated, at least semiquantitatively, into the famlies S_3, S_2U, SU_2, and U_3 where U is monounsaturated and represents, but is not identical with, the original acyl group. These results can be translated directly into the percentages of the original molecular families. Results of determinations are given.

Procedures for assigning spots to specific molecular families are (1) comparison of $R_f \times 100$ values with given experimental ranges, (2) comparison of the percent of S theoretically present after tentative assignment of spots, with the percent of S found by GLC analysis of the sample, (3) use of internal standards, and (4) miscellaneous.

Introduction

THIN-LAYER CHROMATOGRAPHY of fats on silica-silver nitrate plates effects separations of molecular varieties largely on the basis of total molecular unsaturation. For instance, the molecule SSU in which U is represented by oleate, with a single double bond, will migrate farther than the corresponding SSU in which U is linoleate, with two double bonds. On the other hand SUU in which U is oleate will migrate with SSU in which U is linoleate, because both molecules have a total of two double bonds. Thus, except for S_3, the four molecular types S_3, S_2U, SU_2 and U_3, and the isomers SUS—SSU, and USU—UUS are not generally segregated, as such, on silica-silver nitrate thin layer plates.

By means of the permanganate-periodate technique of von Rudloff (1) the unsaturated acyl groups in fat molecules may be oxidized at the double bond nearest the carboxyl to produce the corresponding saturated dicarboxylic acid residues.

If the free carboxyls are then esterified with a monoene, such as allyl alcohol, a new triglyceride molecule is produced, in which each original unsaturated acyl group, regardless of its degree of unsaturation, is represented by a new group containing only a single double bond.

Fats so altered can be separated by thin-layer chromatography into spots representing the original S_3, S_2U, SU_2, and U_3. By measurement of the spots the percentages of the orginial S_3, S_2U, SU_2 and U_3 can be determined semiquantitatively. There is usually some degree of separation of the isomers SUS—SSU and USU—UUS and sometimes this is semiquantitative. It is possible that improved technique or column separations would give quantitative results for both the types and the isomers.

Experimental

I. Outline of the Method

The fat to be analysed is subjected to von Rudloff oxidation, whereby all unsaturated acyl groups are converted into the corresponding dicarboxylic acid residues. The acidic residues are then converted into the silver salts and these in turn into the allyl esters by reaction with 3-iodopropene.

These allyl esters, along with the unaltered fully saturated components, are chromatographed on specially prepared silica-silver nitrate plates. The plates

[1] Presented in part at the AOCS meeting Houston, April, 1965.