Stereospecific analyses of several vegetable fats

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ABSTRACT The distribution of fatty acids among the positions 1, 2, and 3 of triglycerides of seven vegetable fats has been determined. The fatty acid compositions in positions 1 and 3 are very similar in most cases, but in none of the fats is the distribution completely symmetrical.

KEY WORDS stereospecific · triglyceride · analysis · fatty acid distribution · vegetable oils · peanut · rapeseed · soybean · linseed · corn · olive · cacao butter

FOLLOWING THE DEMONSTRATION (1, 2) that pancreatic lipase hydrolyzes selectively the ester bonds at the α-position of triglycerides, numerous investigations have been carried out in which this enzyme has been used to determine the fatty acid distribution patterns of fats. In vegetable fats it appears that palmitic and stearic acid, as well as all acids of greater chain length, predominate in the α-positions (3) whereas unsaturated acids tend to occupy the β-position. It is not known whether there is any difference in the fatty acid compositions of the two α-positions,[1] 1 and 3, i.e., whether the distribution of fatty acids is symmetrical or not. A recently introduced method (4, 5) of stereospecific analysis of triglycerides permits an investigation of this problem, and in the present paper we present the stereospecific analyses of seven vegetable fats.

MATERIALS AND METHODS

Fats

Commercial vegetable fats were analyzed without prior purification. As tested by semiquantitative thin-layer chromatography none of the fats contained more than 1% free fatty acids or partial glycerides. The corn oil had been analyzed before (4), but at that time it had been purified by chromatography. On comparison it appears that a partial fractionation had occurred since the material examined earlier was richer in the saturated acids, 16:0 and 18:0. If this difference is taken into account the analyses agree very well, although by our present standards of accuracy the first analysis could no longer be accepted.

Soybean oil was randomized by adding 0.15 g of lithium *tert*-butylate dissolved in 3 ml of dimethylformamide to 30 g of oil and heating the mixture to 70° on a rotary evaporator for 1 hr, the last 10 min under high vacuum. The oil was then dissolved in petroleum ether–ether 9:1, and passed through a column containing 50 g of silicic acid (Mallinckrodt) which was then washed with 800 ml of the solvent. The eluate was passed through basic aluminum oxide to remove free fatty acids.

Method

An improved procedure of stereospecific analysis has been published (5), and we followed the directions given there, with the omissions suggested under "Results: Routine Analysis" (5), except for a modification of the lipolytic reaction, which was carried out without addition of hexane and with continuous titration of the liberated fatty acids.

One gram of vegetable fat (about 1.1 mmoles), 18 ml of M NaCl, 0.5 ml of 40% $CaCl_2$, 0.1 g of pancreatic lipase, and 2 drops of a mixed indicator (0.1% cresol red and 0.1% thymol blue) were shaken (horizontally at 120 strokes/min and 4 cm amplitude) in a water bath of 38° under an atmosphere of nitrogen. Through a polyethylene hose piercing the stopper, N aqueous NaOH was dispensed to keep the reaction mixture faintly purple (pH 7.7–8.0). When 0.7 ml of N NaOH had been consumed (10–15 min), 10 ml of ethanol and 2 drops of bromophenol blue solution were added and the mixture was adjusted to pH 4 (green-yellow) with HCl. All subsequent steps were carried out as described earlier (5). More than 0.7 mmole of fatty acid was always formed during lipolysis, usually about 1 mmole. Typical yields of monoglycerides and diglycerides were 0.3–0.4 mmole of each.

[1] 1, 2, and 3 refer to 1,2,3-triacyl L-glycerol.

TABLE 1 STEREOSPECIFIC ANALYSIS OF LINSEED OIL, WITH ANALYSES OF INTERMEDIATES

	Compound*	Positions of Triglyceride	Fatty Acid 16:0	16:1	18:0	18:1	18:2	18:3
			moles %					
I	Triglyceride	1, 2, 3	6.0	0.2	3.5	16.0	16.4	57.9
III	Diglyceride	1, 2; 2, 3	4.8	0.1	2.7	16.1	18.4	58.0
III_c	Diglyceride, calc.	1, 2; 2, 3	4.9	0.2	3.8	16.2	17.6	58.4
IV	Monoglyceride	2	1.6	0.1	0.7	16.3	21.3	59.8
V	Triglyceride, recovered	1, 2, 3	5.8	0.2	3.6	16.6	16.9	57.2
VII	Lysophosphatide	1	10.1	0.2	5.6	15.3	15.6	53.2
VIII	β-Fatty acid	2	1.6	0.2	0.8	17.1	22.3	58.1
IX	D-Phosphatide	2, 3	3.6	0.1	2.3	17.0	17.7	59.0
	I–IV–VII†	3	6.3	0.3	4.2	16.4	12.3	60.7
	IX–IV‡	3	5.6	0.1	3.9	17.7	14.1	58.2

* Reference 5 should be consulted for interpretation.
† Calculated from 3 × I (triglyceride, positions 1, 2, and 3) minus IV (monoglyceride, position 2) minus VII (lysophosphatide, position 1).
‡ Calculated from 2 × IX (D-phosphatide, positions 2 and 3) minus IV (monoglyceride, position 2).

Adjunctive procedures, gas–liquid chromatography, and calculations are described in earlier publications (4, 5), which also contain critical discussions of the method. For one fat, linseed oil, we give again a complete analysis (5) in Table 1; for interpretation, the previous paper (5) should be consulted. Attention should be directed toward the agreement between the monoglyceride and the β-fatty acid released from the L-phosphatide (IV and VIII), both representing position 2, and between the two calculations for position 3 (Table 1).

Accuracy

The two calculated fatty acid compositions of position 3 shown in Table 1 give a picture of the accuracy achieved with the present method. The first of these compositions is obtained by subtracting the data for position 1 (VII, lysophosphatide) and position 2 (IV, monoglyceride) from the triglyceride (I, positions 1, 2, and 3). The second analysis is derived from IX (D-phosphatide, positions 2 and 3) minus IV (monoglyceride, position 2). As discussed at length in a previous paper (5), errors occurring during the course of the stereospecific analysis will be reflected in discrepancies between these two sets of calculations. Conversely, the degree of agreement is a measure of the accuracy of the analysis. The percentages given for the 3-positions of the fats in Table 3 are the averages of the two independent calculations. We accepted analyses only if the discrepancies were not larger than 2% (absolute) for any minor acid (<10%) or 3% for any major acid, and 4% for acids occurring in molar fractions of 40% and higher. The analyses can therefore be considered accurate to ±1.5% or ±2% for major fatty acids. The only vegetable fat of those fats tested that did not yield an acceptable analysis was Malabar tallow (gift of Dr. M. H. Coleman), a fat extremely rich in stearic acid; for this acid, the discrepancy between the two calculated values for position 3 was 8%.

TABLE 2 STEREOSPECIFIC ANALYSIS OF RANDOMIZED SOYBEAN OIL

Position in Triglyceride	Fatty Acid 16:0	18:0	18:1	18:2	18:3
	moles %				
1	9.5	4.2	23.6	54.9	7.8
2	11.1	4.4	23.3	52.9	8.3
3	10.6	4.6	25.1	51.2	8.5

Analysis of Randomized Soybean Oil

The validity of the method was again (5) tested on a triglyceride mixture of known fatty acid distribution. Randomized soybean oil was chosen; the results, in Table 2, were within the required limits of accuracy.

RESULTS AND DISCUSSION

The distribution of fatty acids between positions 1 and 3 (Table 3) is nearly symmetrical for most fatty acids in most fats. Any asymmetric distribution of C_{16} and C_{18} acids is less pronounced than it is in many animals (6). However, none of the fats is completely symmetrical; there are in all fats differences between positions 1 and 3 that are clearly beyond the possible experimental errors. Contrary to the findings in mammalian fats (6), there appears to be no consistent pattern of asymmetry. It is to be hoped that a meaningful discussion of the present data will be possible when it becomes known through which pathways the fats of the different fruits and seeds are synthesized.

TABLE 3 STEREOSPECIFIC ANALYSES OF VEGETABLE FATS

Oil or Fat	Position	Fatty Acid 16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	22:0	22:1	24:0
		moles %										
Peanut	1	13.6	0.3	4.6	59.2	18.5		0.7	1.1*	1.3		0.7
	2	1.6	0.1	0.3	58.5	38.6		—	0.3*	0.2		0.5
	3	11.0	0.3	5.1	57.3	10.0		4.0	2.7*	5.7		2.8
Rapeseed	1	4.1	0.3	2.2	23.1	11.1	6.4		16.4	1.4	34.9	
	2	0.6	0.2	—	37.3	36.1	20.3		2.0	—	3.6	
	3	4.3	0.3	3.0	16.6	4.0	2.6		17.3	1.2	51.0	
Soybean	1	13.8		5.9	22.9	48.4	9.1					
	2	0.9		0.3	21.5	69.7	7.1					
	3	13.1		5.6	28.0	45.2	8.4					
Linseed	1	10.1	0.2	5.6	15.3	15.6	53.2					
	2	1.6	0.1	0.7	16.3	21.3	59.8					
	3	6.0	0.3	4.0	17.0	13.2	59.4					
Corn	1	17.9	0.3	3.2	27.5	49.8	1.2					
	2	2.3	0.1	0.2	26.5	70.3	0.7					
	3	13.5	0.1	2.8	30.6	51.6	1.0					
Olive	1	13.1	0.9	2.6	71.8	9.8	0.6					
	2	1.4	0.7	—	82.9	14.0	0.8					
	3	16.9	0.8	4.2	73.9	5.1	1.3					
Cacao butter	1	34.0	0.6	50.4	12.3	1.3		1.0				
	2	1.7	0.2	2.1	87.4	8.6		0.0				
	3	36.5	0.3	52.8	8.6	0.4		2.3				

*Together with 18:3.

A partial stereospecific analysis of one of the vegetable fats analyzed here has been reported by Schlenk (7), who isolated palmito-oleo-stearin from cacao butter and showed by physical methods that it was a racemate. Our analysis cannot offer a conclusive confirmation of his results, since we have been concerned with the over-all distribution of fatty acids only and have not isolated individual triglycerides. According to the data in Table 3 any palmito-oleo-stearin present in cacao butter could still be asymmetric, since over-all symmetry of fatty acid distribution does not preclude asymmetry of individual triglycerides, although over-all asymmetry precludes complete symmetry of every individual. This consideration applies to all fats, and the problem can be solved only by separating a fat into the individual triglycerides, and performing a stereospecific analysis on each of them.

Our data on cacao butter are, however, compatible with Schlenk's results. In general, looking at the largely symmetrical distribution of fatty acids in the fats listed in Table 3, one would be surprised if future investigations should discover large percentages of nonracemic triglycerides, although in some cases these must be present. A minimum of nonracemic or "optically active" species would result from *a positionally restricted random distribution*, which demands that there is intermolecular random distribution of fatty acids 1, 2, and 3, within these positions. [This would be an extension of Vander Wal's concept (8) of random intermolecular distribution of α- and β-fatty acids.] In fats with completely symmetrical fatty acid distribution all triglyceride species would then be racemates. In soybean oil, for instance (Table 3), the 1-palmitoyl 2,3-dilinoleoyl L-glycerol (L-PLL) would occur in 13.8 × 69.7 × 45.2 or 4.4 moles %; its antipode, L-LLP, in 48.8 × 69.7 × 13.1 or 4.5 moles %. In an asymmetric fat there would be incomplete racemization of individual triglycerides. In rapeseed oil, for instance, the 1,2-dioleoyl 3-erucoyl L-glycerol (L-OOE) would be present in 23.1 × 37.3 × 51.0 or 4.4 moles %; its antipode, L-EOO, in 16.6 × 37.3 × 34.9 or 2.2 moles %. Isolation would then yield a triglyceride which would be two-thirds racemate, the remaining third being the excess of one enantiomorph.

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