Positional Distribution of the Fatty Acids in the Triglycerides of the Oil of Some African Peanut Varieties

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ABSTRACT

The distribution of fatty acids between the sn-1-, sn-2- and sn-3-positions of the triglycerides from the oils of eight African peanut varieties has been determined. The saturated fatty acids and eicosenoic acid occur almost exclusively at the sn-1- and sn-3-positions. The sn-1-position contained slightly more palmitic acid than the sn-3-position. The fatty acids with a chain length exceeding 18 carbons were preferentially incorporated in the sn-3-position. Linoleic acid was preferentially esterified at the sn-2-position, whereas oleic acid was equally distributed among the three positions. The amount of the saturated fatty acids, i.e., palmitic and stearic acid, and of oleic acid and linoleic acid incorporated in the sn-1-, sn-2- and sn-3-position, were each linearly related to their respective content in the triglycerides.

INTRODUCTION

The positional distribution of fatty acids in naturally occurring plant triglycerides is not random. Some regularities have been found concerning the incorporation of fatty acids in the sn-2-position. Saturated fatty acids, of which the chain length exceeds 14 carbon atoms, and monounsaturated fatty acids, with more than 18 carbon atoms, are almost exclusively esterified at the sn-1- and sn-3-position of the glycerol moiety of the triglycerides. Oleic, linoleic and linolenic acid are incorporated in the three different positions (1-3).

The composition of the fatty acids incorporated in the sn-1- and sn-3-position is known from only a restricted number of seed fat triglycerides so that general patterns for the distribution of the fatty acids between these positions cannot be established with certainty. It appears that there are small but distinct differences between the fatty acids incorporated in the sn-1- and sn-3-positions. It is not excluded that these differences are specific for each species (4).

We therefore determined the positional distribution of the fatty acids in the triglycerides of a single species – peanuts. From the analysis of the positional distribution of the fatty acids of the triglycerides of different fatty acid composition obtained from different peanut varieties, it became possible to demonstrate a regular pattern for the distribution of the fatty acids among the three hydroxyl groups of the glycerol moiety of the triglycerides.

EXPERIMENTAL PROCEDURES

Materials

Five peanut varieties (A65, A20, A1055, A1052 and P43) were obtained from the Agronomical Research Station of Inera at M'Vuazi (Republic of Zaire) and three other varieties (Bambey 73-33, Bambey 28-206, Bambey 55-437) from the Agronomical Research Station of Bambey (Senegal). The peanuts were grown at the respective stations; after harvest the fruits in the shell were dried in the sun. In the laboratory the dried seeds were shelled and stored at -18 C.

Methods

Extraction and isolation of triglycerides. The peanuts were crushed in a mortar in the presence of seasand. The ground nuts were extracted three times with a mixture of chloroform/methanol (2:1), and the combined extracts were washed with 0.9% NaCl as described by Folch et al. (5). Triglycerides were isolated from the crude oil by preparative thin layer chromatography on Silica Gel G with petroleum ether (40-60 C)/diethyl ether/acetic acid (70:30:1). The triglycerides were recovered from the Silica Gel G by extraction with chloroform, and the solvent was evaporated under reduced pressure.

Analysis of triglycerides; lipolysis of triglycerides (6). One hundred and fifty mg triglycerides liquified with 0.2 ml hexane were shaken with 1.8 ml Tris-HCl (1 M, pH 8.0), containing 20 mg Steapsin (Sigma, Chemical Co. St. Louis, MO, USA), purified with diethyl ether and acetone, and 2 mg $CaCl_2$, at room temperature for 20 min. The reaction was stopped by the addition of a drop of H_2SO_4 1 N and 5 ml diethyl ether. The lipids, recovered from the ether extraction, were separated by thin layer chromatography on Silica Gel G with petroleum ether (40-60 C)/diethyl ether/acetic acid (70:30:1). The different lipid classes were visualized with 2′,7′-dichlorofluorescein under U.V. Triglycerides, diglycerides, monoglycerides and fatty acids were

TABLE I

Fatty Acid Composition of the Triglycerides of the Oil of the Different Peanut Varieties

	Fatty acid (mole %)							
Peanut variety	16:0	18:0	18:1	18:2	20:0	20:1	22:0	24:0
Zaire								
A65	12.7	2.6	38.8	39.4	1.8	1.3	2.4	1.0
A20	11.9	3.8	39.8	37.1	2.4	1.5	2.6	0.9
A1052	10.8	4.4	42.7	35.6	2.4	1.0	2.7	1.0
A1055	10.7	4.7	40.6	36.0	2.7	1.7	2.9	0.7
P43	11.4	4.8	40.2	35.8	2.7	1.2	3.0	0.9
Sengal								
Bambey 55-437	11.3	3.0	49.5	31.0	1.2	0.8	2.6	0.6
Bambey 28-206	9.1	2.1	66.2	17.2	1.2	1.4	1.9	0.9
Bambey 73-33	10.6	2.2	58.9	22.9	1.4	1.2	1.9	0.9

TABLE II

Positional Distribution of the Fatty Acids in the Triglycerides of the Oil of the Different Peanut Varieties

Peanut variety	Position	Fatty acid (mole %) 16:0	18:0	18:1	18:2	20:0	20:1	22:0	24:0
Zaire									
A65	1	22.0	2.0	39.6	33.2	1.3	0.6	0.8	0.5
	2	0.9	0.2	30.3	68.3	---	0.3	---	---
	3	16.7	5.7	47.5	16.2	4.2	2.4	6.1	1.2
A20	1	20.4	6.3	37.2	31.5	1.6	0.8	1.7	0.5
	2	1.3	0.4	37.0	61.3	---	---	---	---
	3	15.0	5.8	43.6	18.3	5.8	3.1	6.2	2.2
A1052	1	17.6	7.0	39.6	30.8	1.7	0.7	1.9	0.7
	2	0.7	---	36.2	63.1	---	---	---	---
	3	14.4	6.8	48.3	12.5	6.1	2.7	6.9	2.3
A1055	1	17.6	7.3	38.2	31.3	2.1	1.0	1.9	0.6
	2	0.7	0.1	36.4	62.8	---	---	---	---
	3	13.5	7.4	45.6	13.6	6.5	3.6	7.3	2.5
P43	1	17.9	7.7	40.1	28.9	1.6	0.6	2.5	0.7
	2	1.2	0.2	36.5	62.1	---	---	---	---
	3	14.5	7.0	44.2	17.1	6.2	2.5	6.9	1.6
Senegal									
Bambey 55-437	1	15.4	3.4	52.4	25.6	0.6	0.4	2.0	0.2
	2	1.1	---	44.2	54.7	---	---	---	---
	3	15.6	4.6	53.0	15.8	3.0	1.1	5.8	1.1
Bambey 28-206	1	15.6	4.0	64.4	10.9	0.7	1.1	1.8	1.5
	2	1.3	0.2	69.2	29.3	---	---	---	---
	3	11.1	2.1	67.8	8.2	2.9	3.1	3.6	1.2
Bambey 73-33	1	15.6	3.4	59.9	18.1	0.5	0.9	1.2	0.4
	2	2.0	0.5	60.0	37.5	---	---	---	---
	3	14.0	3.4	58.7	12.3	3.4	2.6	3.8	1.8

methylated with 3% H_2SO_4 in methanol (7), and analyzed by gas liquid chromatography. The diglycerides were eluted with chloroform and the solvent was evaporated under reduced pressure.

Preparation of phosphatidylphenol. The diglycerides were converted to phosphatidylphenol with phenyldichlorophosphate as described by Brockerhoff (8). The phosphatidylphenol was purified by thin layer chromatography using a two step development:first with petroleum ether (40-60 C)/diethyl ether/formic acid (40:60:1); followed by diethylether/methanol/ammonia 25% (80:18:2).

Enzymatic hydrolysis (6). The synthetic phosphatidylphenol was hydrolyzed with phospholipase A_2 from the snake venom of *Crotalus atrox* (Sigma). The hydrolysis products were separated by thin layer chromatography as described for the purification of the phosphatidylphenol. The fatty acids, the lyso-sn-3-phosphatidylphenol and the sn-1-phosphatidylphenol were recovered from the silica gel with chloroform/methanol (1:1) and methylated with 3% H_2SO_4 in methanol.

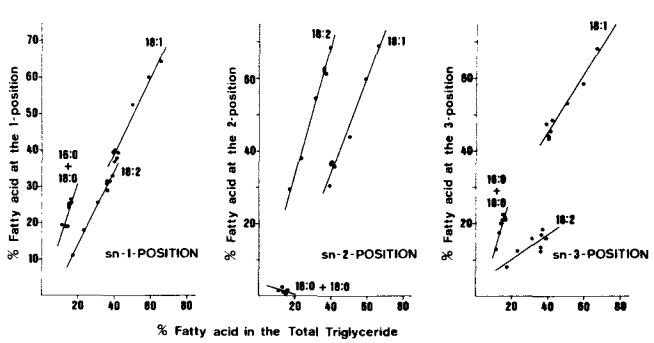

FIG. 1. Relationship between the positional distributions of the saturated fatty acids (palmitic and stearic), oleic and linoleic acid in the peanut triglycerides and the amount of the respective acids in the total peanut triglycerides. Linear regressions:

sn-1-position: 16:0 + 18:0, y = 1.60x - 0.29; 18:1, y = 1.03x - 2.17; 18:2, y = 0.999 x -5.59;

sn-2-position: 16:0 + 18:0, y = -0.16x + 3.67;.18:1, y = 1.31x - 17.68; 18:2, y = 1.77 x - 1.69;

sn-3-position: 16:0 + 18:0, y = 1.71x - 5.17; 18:1, y = 0.80 ox + 13.62; 18:2, y = 0.33x + 3.59.

Calculation of the positional distributions (6). The composition of the fatty acids at the position 1 was obtained from the composition of the lyso-sn-3-phosphatidylphenol. Position 2 was given by the monoglyceride fraction obtained by the degradation of the triglycerides with Steapsin. Position 3 was calculated as 2 x sn-1-phosphatidylphenol-monoglycerides and from 3 x triglycerides – monoglycerides – lyso-sn-3-phosphatidylphenol.

Gas liquid chromatography. A Hewlett-Packard F&M 5750 gas chromatograph with a dual flame ionization detector and stainless steel columns (6 ft x 3 mm ID) packed with 10% SP-2330 on 100/200 mesh Chromosorb W AW, was used for the separation of the fatty acid methyl esters. The following conditions of flow and temperature were used: nitrogen, 15 ml/min; hydrogen, 24 ml/min; air, 400 ml/min; injection port and detector temperature, 250 C; initial oven temperature, 190 C; linear temperature programming, 8 C/min up to 250 C, starting 3 min after injection. Peak areas and retention times were determined by a Hewlett-Packard Model 3370A integrator. Linolenic and eicosenoic acid are measured together under these conditions.

RESULTS AND DISCUSSION

The fatty acid composition of the triglycerides of the oil of the different peanut varieties and the positional distribution of the fatty acids in the peanut triglycerides are presented in Tables I and II. As has been found for other seed fats, the saturated fatty acids palmitic, stearic, arachidic, behenic and lignoceric acid, are almost totally incorporated in the sn-1- and sn-3-position of the peanut triglycerides. The distribution of palmitic acid between the sn-1- and sn-3-position is slightly asymmetric in the Zairese peanut triglycerides. This asymmetry is not so clear for the

TABLE III

Comparison between the Positional Distribution of the Fatty Acids in Peanut Triglycerides Reported by Brockerhoff (4) (†) and the Hypothetical Positional Distribution as Derived from our Regression Equations (‡)

	Fatty acid (mole %)						
Position	16:0 + 18:0	18:1	18:2	20:0	20:1	22:0	24:0
1†	13.6	59.2	18.5	0.7	1.1	1.3	0.7
1‡	13.6	57.6	16.8				
2†	1.6	58.5	38.6	---	0.3	0.2	0.5
2‡	1.7	58.7	38.0				
3†	11.0	57.3	10.0	4.0	2.7	5.7	2.8
3‡	9.7	60.3	11.1				

Senegalese peanut triglycerides. The preferential incorporation of arachidic, behenic and lignoceric acid at the sn-3-position, resulting in a marked asymmetric distribution, confirms the positional fatty acid distribution within peanut triglycerides as reported by Brockerhoff (4). Eicosenoic acid occurs mainly at the sn-3-position as do the long chain saturated fatty acids. This is in contrast with the incorporation of eicosenoic acid in rapeseed triglycerides, where this acid is equally distributed between the sn-1- and sn-3-position (4). It has to be noted that eicosenoic acid is determined together with linolenic acid. This has probably no influence on the determination of eicosenoic acid because peanut triglycerides contain less than 0.13% of linolenic acid (9). About 57% of the total linoleic acid is incorporated in the sn-2-position, whereas oleic acid is rather equally distributed among the three positions.

From Figure 1 it appears that the incorporation of the major fatty acids at the different positions is linearly related to the total amount of the respective fatty acids in the total triglycerides. The positional distribution of palmitic, stearic, oleic and linoleic acid in the triglycerides of a single peanut variety, reported by Brockerhoff (4) agreed very well with the values predicted by our regression equations (Table III). From these relations can also be seen that the symmetric or the asymmetric distribution of certain fatty acids between the sn-1- and sn-3-position depends on their respective content in total triglycerides. Oleic acid, for example, is more symmetrically distributed when present in large amounts in the triglycerides. The incorporation of linoleic acid, on the other hand, becomes more asymmetric when present in large amounts. Similar conclusions can be drawn from the data reported by de la Roche et al. (10) for the positional distribution of the fatty acids in maize triglycerides, obtained from different genotypes.

From these findings it follows that the incorporation of the fatty acids in the triglycerides proceeds in a regular and predictable manner. It is clear that this is a consequence of the selective enzymatic acylation of glycerolphosphate. However, experimental proof for the specific acylation of glycerolphosphate in the biosynthesis of plant triglycerides is lacking for the moment. Therefore, a satisfactory explanation of the positional distribution of the fatty acids in the triglycerides has to await a better knowledge on triglyceride biosynthesis.

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