Varietal Differences in Peanut Triacylglycerol Structure[1]

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ABSTRACT

Stereospecific analysis of triacylglycerols from six peanut varieties showed diversity in percent fatty acid placement. Distribution of the fatty acids among the *sn*-1, -2 and -3 positions was clearly nonrandom. The percentages of palmitic and stearic acids, generally very low at the *sn*-2 position, were more predominant at the *sn*-1 than the *sn*-3 position. Long chain fatty acids were located almost exclusively at the *sn*-3 position. The *sn*-2 position of all varieties was high in unsaturated fatty acids. Triacylglycerols were sufficiently different to suggest that concentrations of specific triacylglycerol species may vary with variety.

INTRODUCTION

Stereospecific analyses of animal (1-5) and plant (4,6-9) triacylglycerols indicate that the distribution of fatty acids is not random, but that each position has a characteristic fatty acid pattern. Usually, the fatty acid compositions of *sn*-1 and -3 positions are similar, but none of the fats examined has exhibited a completely symmetrical distribution. Triacylglycerol composition and structure are important from the standpoint of nutrition (10), oil stability (11) and possible physiological effects (12).

Early studies of plant triacylglycerols were conducted on refined oil unidentified as to specific variety and probably representing genetically heterogeneous source material. Analyses of maize (7,8) and soybean (9) triacylglycerols from several specific varieties indicate that structure is not constant but is variable among varieties. Biochemical studies on heterogeneous material may be of little value especially if one of the goals of the study is to effect some eventual genetic change in the source material should the conclusions so indicate. The variation in fatty acid composition of oil from various peanut varieties is well documented (13) and is an indication of the variability found in the triacylglycerol fraction which routinely accounts for more than 90% of the total composition. de la Roche et al. (8) reported that the fatty acids at each position of corn oil triacylglycerols were influenced by the fatty acid concentration in the total triacylglycerol except for the saturates in the *sn*-2 position. This indicates that triacylglycerols with diversity in fatty acid composition might reasonably be expected to have diverse structures.

Stereospecific analysis of peanut oil has been reported by Brockerhoff and Yurkowski (6). Myher et al. (14) compared the triacylglycerol structures of native, rearranged, and simulated peanut oils and found that native oil, the most atherogenic in laboratory animals, contained a significantly greater proportion of certain triacylglycerol structures than the synthetic oils. Only dietary testing will determine whether those triacylglycerol structures are indeed associated with increased atherogenic potency (14). Hokes (15) determined the fatty acids attached to the *sn*-2 position of triacylglycerols from several peanut cultivars, but did not differentiate between fatty acids at the *sn*-1 and *sn*-3 positions.

This paper describes the variability that exists in the stereospecific structure of triacylglycerols from six peanut varieties.

MATERIALS AND METHODS

All of the peanut varieties were grown, harvested and cured using conventional methods in Headland, AL as part of the 1976 National Peanut Performance Trials. Peanuts were shipped to this laboratory where they were shelled. Seed riding a 0.635 x 1.905 cm shaker screen were sealed in plastic bags and stored at 4 C.

For lipid extraction, random 10 g samples of sound, mature, intact peanuts of each variety

TABLE I

Mean Differences between *sn*-3 Position Fatty Acid Percentages of Six Peanut Varieties Calculated by Two Methods

Fatty Acid	Difference
16:0	2.6 ± 1.2
18:0	0.9 ± 0.6
18:1	1.1 ± 1.2
18:2	3.1 ± 0.8
20:0	0.2 ± 0.1
20:1	0.5 ± 0.3
22:0	0.3 ± 0.1
24:0	0.3 ± 0.2

[1]Mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

TABLE II

Stereospecific Analyses of Triacylglycerols from Six Peanut Varieties

Variety	Compound or position	Fatty acid distribution, mole %[a]							
		16:0	18:0	18:1	18:2	20:0	20:1	22:0	24:0
Florigiant	TG	10.8	2.9	53.1	27.3	1.8	1.0	1.9	1.1
	1	20.1	4.9	50.7	22.6	0.5	0.7	0.4	0.3
	2	2.2	0.7	51.5	45.3	0.1	0.3	0.1	—
	3	10.3	3.2	57.2	14.0	4.8	2.0	5.3	3.0
Early Bunch	TG	13.3	2.6	42.0	36.3	1.5	0.9	2.4	1.1
	1	24.7	4.4	38.3	31.2	0.3	0.4	0.5	0.2
	2	3.5	1.5	37.2	57.4	0.1	0.2	—	—
	3	11.8	1.9	50.6	20.3	4.0	1.9	6.5	2.9
Florunner	TG	11.4	2.1	50.9	29.1	1.6	1.1	2.4	1.3
	1	20.7	3.5	49.5	24.4	0.3	0.7	0.5	0.5
	2	2.1	0.6	47.8	48.8	0.1	0.4	0.1	0.1
	3	11.4	2.3	55.5	14.1	4.4	2.3	6.5	3.4
Tifrun	TG	12.6	2.4	42.4	36.8	1.5	0.9	2.6	0.8
	1	22.7	4.2	38.8	32.7	0.3	0.4	0.6	0.2
	2	3.2	1.2	36.1	58.8	0.2	0.2	0.1	—
	3	11.9	1.7	52.2	18.9	4.0	2.0	7.2	2.2
Starr	TG	14.2	3.3	43.3	33.0	1.8	1.1	2.7	0.7
	1	24.2	4.9	40.4	28.4	0.4	0.6	0.7	0.3
	2	2.4	0.8	39.5	56.9	0.1	0.2	0.1	—
	3	16.0	4.2	50.0	13.8	4.8	2.4	7.3	1.8
Spancross	TG	13.5	2.9	44.1	33.4	1.9	1.1	2.2	0.9
	1	24.2	5.2	40.3	28.4	0.3	0.5	0.6	0.4
	2	2.6	0.9	40.2	56.2	0.1	—	—	—
	3	13.5	2.8	51.8	15.6	5.2	2.5	6.5	2.3

[a]Each value is the mean of 3 replications.

were blended in 100 ml petroleum ether (B.P. 35-60 C). The homogenates were filtered and the solvent was removed on a rotary evaporator. Triacylglycerols were separated by thin layer chromatography (TLC) on Silica Gel G (.05 mm)(Brinkmann) with a developing solvent of petroleum ether/diethyl ether/acetic acid (80:20:1). Careful attention was given to prevention of autoxidation. All thin layer plates were developed in a nitrogen atmosphere and sprayed with 0.02% butylated hydroxytoluene (BHT) in petroleum ether. BHT (ca. 10 μl of 0.1% solution) was added in each step of the isolation procedure and stereospecific analysis.

Methyl esters were prepared with boron trifluoride-methanol (14% w/v, Applied Science Laboratories, Inc.) according to a modified Morrison and Smith (16) procedure. Benzene was replaced with toluene in the methylation mixture of methyl alcohol/benzene/boron trifluoride-methanol (11:4:5). Lipids were transmethylated in 9 ml vials sealed with teflon cap liner and tape. The vials were heated in an oven at 100 C for 20-30 min, depending on the lipid type. Water (1 ml) was added to the cooled mixture, and the methyl esters were extracted with two 3 ml portions of hexane; then they were analyzed by GLC. The gas chromatograph, equipped with a FID and a 6.35 mm x 1.83 m stainless steel column that was packed with 10% EGSS-X on 100/120 Gas-Chrom P (Applied Science Laboratories, Inc.), was operated isothermally at 210 C. Carrier gas was helium at 100 ml/min. Fatty acid percentages were determined by digital integration and normalization of peak areas. Accuracy of the system was verified by analysis of National Heart Institute-type fatty acid standard KD. Fatty acids were identified by comparison with known standards.

Stereospecific analysis was conducted essentially according to Brockerhoff (17) as modified by Weber et al. (7). The method for preparing of phosphatidyl phenols was modified such that the diacylglycerols in no more than 0.5 ml diethyl ether were added slowly with shaking to 2 ml pyridine (spectrophotometric grade, Aldrich Chemical Company) and 0.12 ml phenyl dichlorophosphate (Aldrich Chemical Company) to prevent precipitate formation.

RESULTS AND DISCUSSION

Two determinations provide an indication of the accuracy of stereospecific analysis (4,7).

TABLE III

Linear Regression Analyses and Correlation Coefficients for the Relationship of Fatty Acids in Total Triacylglycerols and Fatty Acids at Each Position

Fatty acid	Position	Slope	y Intercept	r[a]
16:0	1	1.43	4.68	0.95[b]
	2	—	—	0.43[d]
	3	1.32	-4.23	0.86[c]
18:0	1	1.28	1.03	0.88[c]
	2	—	—	0.09[d]
	3	1.82	-2.23	0.83[c]
18:1	1	1.16	-10.33	0.99[b]
	2	1.28	-16.87	0.99[b]
	3	0.56	26.84	0.95[b]
18:2	1	1.01	-5.07	0.99[b]
	2	1.38	8.83	0.95[b]
	3	0.60	-3.75	0.82[c]
20:0	3	2.78	-0.15	0.99[b]
20:1	3	2.31	-0.16	0.91[c]
22:0	3	2.39	0.88	0.96[b]
24:0	3	2.65	-0.01	0.99[b]

[a]r = Correlation coefficient.
[b]Significant at 1% level.
[c]Significant at 5% level.
[d]Not significant.

The mixed 1,2(2,3)-diacylglycerols used to make the phosphatidyl phenols must be representative of the triacylglycerols; therefore, they must agree in composition to that calculated for diacylglycerols. Differences in calculated and analyzed diacylglycerols of the six varieties were less than 2% for any fatty acid. Data obtained by the two methods of determining the fatty acid composition of the *sn*-3 position should always be compared. Analyses with minor differences (<5%) for major components are generally considered acceptable (7) although agreement should be closer. The mean differences between the *sn*-3 fatty acid percentages calculated by two methods for each fatty acid of all varieties are presented in Table I. Composition calculated by subtracting the *sn*-1 and *sn*-2 position fatty acid percentage from the percentage composition of the whole triacylglycerol is regarded as more accurate; however, agreement of the two methods indicates overall accuracy.

The results shown in Table II indicate a nonrandom distribution of fatty acids among the *sn*-1, -2 and -3 positions of the triacylglycerols. The percentages of palmitic and stearic acids were generally very low for the *sn*-2 position, higher for *sn*-3, and highest for *sn*-1. The long chain (20-24) fatty acids were located almost exclusively at the *sn*-3 position. The *sn*-2 position of triacylglycerols from all the varieties was high in unsaturated fatty acids. The general patterns of fatty acids found at the *sn*-1 and *sn*-3 positions were similar for all varieties, although the mole percentages of each acid at the two positions frequently differed widely. Mole percentages of palmitic, stearic and linoleic acids were always higher for the *sn*-1 than for the *sn*-3 position, while those of oleic acid were consistently higher for the *sn*-3 position. The patterns of fatty acid distribution at *sn*-2 differed not only from those at *sn*-1 and -3, but with variety as well. On the *sn*-2 position, the percentage of oleic acid was higher than that of linoleic acid in Florigiant, but the percentages were about the same in Florunner. In the other four varieties, there was more linoleic acid esterified at the *sn*-2 position than oleic acid. Florigiant and Florunner triacylglycerols contained more oleic acid and less linoleic acid than the other varieties examined; and the concentration effect, as reported by de la Roche et al. (8), probably was reflected by the fatty acid placement in the molecule.

The stereospecific analyses previously reported (6,14) are similar to the assay of Florigiant triacylglycerol (Table II), which showed more oleic acid than linoleic acid at the *sn*-2 position. The general fatty acid patterns at *sn*-1 and *sn*-3 positions were similar in all analyses. The stereospecific analyses of two commercially available peanut oils (data not presented) were very similar to the analysis of Florigiant triacylglycerols shown here.

Linear regression equations and correlation coefficients were calculated for the plots of the percentage of a fatty acid in the total triacylglycerol vs. the percentage of that fatty acid at one of the positions of the triacylglycerol (Table III). Significant correlations indicate that the total fatty acid present influenced placement of that fatty acid on the triacylglycerol (8). de la Roche et al. (8) found that major saturated, monoene and diene fatty acids of corn triacylglycerols exhibited a concentration effect in all cases except for saturated acids in the *sn*-2 position. Peanut triacylglycerols exhibited this same pattern, and the low concentrations of the long chain fatty acids in the triacylglycerol were significantly correlated with percentages found at the *sn*-3 position only. This may be due to the general restriction of the saturated acids (16:0 and 18:0) from the *sn*-2 position and the long chain acids from the *sn*-1 and *sn*-2 positions. Fatemi and Hammond (9) attribute any substantial deviation from the regression line to a change in the mechanism of fatty acid distribution and suggest genetic control of the deviation. No substantial deviation of any fatty acid from the regression lines was detected in the six peanut varieties examined. Although concentration effects were similar for the varieties, the variation in percentage of a fatty acid at any position is sufficient to indicate possible concentration differences in the various triacylglycerol species found in the total triacylglycerol fraction.

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