

Glyceride Structure Variation in Soybean Varieties: I. Stereospecific Analysis¹

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ABSTRACT

Stereospecific analysis of soybeans and related species showed that there was little palmitic or stearic acid on the *sn*-2-position, and the *sn*-1-position is consistently richer in palmitic, stearic, and linolenic acids than the *sn*-3-position. The *sn*-3-position is enriched in oleic acid and the *sn*-2-position with linoleic. Plots of the percentage of fatty acids on the glycerol positions vs. the percentage in the whole oil revealed a soybean variety that had a deviant distribution that is probably genetically controlled.

INTRODUCTION

The glyceride structure of fats and oils may influence their stability towards oxidation (1-3). Weber and Alexander (4) have reported genetic differences in the glyceride structure of corn. The glyceride structure of soybean oil has been examined during maturation by silver ion chromatography (5), by pancreatic lipase analysis (5-9), and by stereospecific analysis (10), but only a limited number of varieties have been studied for glyceride composition by lipase and silver ion chromatography (11). This paper reports the variation in glyceride structure determined by stereospecific analysis of a number of soybean varieties and closely related species selected to embrace a wide range of fatty acid compositions.

¹Journal Paper No. J-8837 of the Iowa Agriculture and Home Economics Experiment Station, Ames IA. Project No. 2143.

TABLE I

Average Differences between the Percentage of Fatty Acids Determined on the *sn*-3-Position by Two Methods of Calculation and the Standard Deviation of the Differences

Fatty acid	Ave. diff.	S.D.
16:0+18:0	0.45	0.26
18:1	1.63	0.71
18:2	1.24	0.47
18:3	0.37	0.20

MATERIALS AND METHODS

Beans were obtained from the Agronomy Department of Iowa State University. These included soybeans, *Glycine max* (L.) Merr., which were commercial cultivars, lines produced in breeding programs to alter fatty acid composition (FA), and plant introductions (PI). In addition, one variety each of *Glycine soya* and *Glycine gracilis* were examined.

The soybeans (50 g) were ground in a Wiley mill and extracted with hexane. Triglycerides were isolated from the lipid extract by thin layer chromatography (12). Lipase hydrolysis was done according to Luddy et al. (13), and stereospecific analysis according to Christie and Moore (14). For fatty acid analyses, the glycerides were transesterified with 0.1 M sodium methoxide in methanol, and the methyl esters were separated on a Beckman GC5 instrument fitted with a 1.8 m x 3.3 mm column of 15% EGSSX on Chromosorb W and a hydrogen flame detector.

Data plots were fitted with straight lines by linear regression (15). Data for PI 68-788 and *G. soya* were omitted in the regression.

RESULTS

The stereospecific analysis yields two ways of calculating the composition of the *sn*-3-position of the triglycerides. The *sn*-3-position can be obtained by subtracting the composition of the *sn*-1- and *sn*-2-position from the composition of the whole triglycerides or by subtracting the composition of the *sn*-2-position from that of the *sn*-2,3-diacyl-phosphatides that are left unhydrolyzed by snake venom. The former is regarded as the more accurate, but the agreement of these two values is a general check on the reliability of the method (14). The average difference in the two values and the standard deviation of the differences for each fatty acid are given in Table I.

Amsoy and Corsoy cultivars grown in three separate years were analyzed to obtain an estimate of the size of seasonal effects of the positional distribution. These results are shown along with others in Table II. There are small year-to-year differences in the percentages of the fatty acids on the three positions of the glycerol, but these can mostly be attributed to the change in fatty acid composition from year

TABLE II
Stereospecific Analysis of Soybeans and Closely Related Species
(Results are in mol percent)

Variety	Compound or position	16:0	18:0	18:1	18:2	18:3
Amsoy 73	TG	11.14	3.95	21.84	53.92	9.15
	1	20.72	6.78	19.66	42.89	9.94
	2	0.69	---	19.03	71.74	8.54
	3	11.85	5.00	26.83	47.13	8.97
Amsoy 74	TG	10.99	3.65	23.73	52.56	9.07
	1	21.11	6.58	20.71	42.44	10.16
	2	0.60	0.11	21.16	69.15	8.99
	3	12.15	4.22	29.33	46.07	8.07
Amsoy 75	TG	11.53	3.49	25.32	51.86	7.80
	1	21.34	5.98	22.46	41.29	8.93
	2	0.63	---	23.54	68.39	7.44
	3	12.44	4.45	29.96	45.89	7.04
Corsoy 73	TG	11.63	3.68	23.19	52.81	8.68
	1	21.15	5.80	18.99	44.38	9.68
	2	1.03	0.27	19.76	70.63	8.32
	3	12.55	4.93	30.83	43.44	8.04
Corsoy 74	TG	11.54	3.71	22.99	53.43	8.33
	1	20.99	6.34	18.96	43.58	10.09
	2	0.61	---	19.29	72.31	7.79
	3	12.84	4.74	30.73	44.39	7.09
Corsoy 75	TG	12.13	3.34	23.27	53.89	7.37
	1	21.93	5.87	19.79	43.64	8.78
	2	0.60	---	21.64	70.94	6.82
	3	13.69	4.09	28.36	47.09	6.54
Wells	TG	11.07	3.78	20.09	57.07	7.98
	1	19.52	6.30	19.24	44.58	10.37
	2	1.02	0.30	19.31	72.68	6.86
	3	12.54	4.70	21.89	53.97	6.71
PI 68-543	TG	12.30	4.21	22.92	53.73	6.85
	1	21.38	6.92	20.33	43.09	8.28
	2	0.99	0.30	22.75	69.82	6.14
	3	14.35	5.34	25.68	48.27	6.13
PI 70-091	TG	11.94	3.98	24.32	53.00	6.75
	1	21.99	6.85	20.89	42.87	7.40
	2	0.85	0.21	23.90	68.77	6.28
	3	12.82	4.83	28.17	47.37	6.58
PI 68-788	TG	13.32	4.15	28.59	47.18	6.77
	1	21.52	6.20	22.67	42.10	7.51
	2	0.87	---	24.74	67.69	6.71
	3	17.38	6.19	38.26	31.76	6.08
FA 3171-2	TG	9.21	3.00	51.34	31.89	4.56
	1	16.75	4.56	46.47	27.39	4.82
	2	0.60	0.10	52.74	41.78	4.77
	3	10.16	4.30	54.79	26.51	4.09
FA 3043-2	TG	10.98	3.35	39.77	40.03	5.86
	1	19.19	5.48	35.75	32.99	6.59
	2	0.76	0.18	39.92	53.52	5.62
	3	12.85	4.36	43.66	33.57	5.38
FA 3017-2	TG	9.14	2.79	53.00	30.21	4.86
	1	16.16	5.00	48.24	25.31	5.28
	2	0.84	0.18	54.66	39.39	4.93
	3	10.33	3.16	56.11	25.91	4.35
FACO 2-8203	TG	11.49	4.17	33.57	44.85	5.91
	1	19.64	6.45	29.86	37.38	6.66
	2	0.99	---	33.86	59.72	5.43
	3	13.70	6.02	37.00	37.45	5.63
FACO 2-8256	TG	10.24	3.84	44.93	35.69	5.29
	1	16.95	5.82	41.34	30.09	5.79
	2	0.88	---	48.86	45.71	4.54
	3	12.76	5.65	44.59	31.28	5.55
PI 65-549 (<i>G. soya</i>)	TG	13.38	3.28	12.64	55.34	15.36
	1	23.84	5.61	11.28	43.39	15.87
	2	0.69	---	10.67	73.49	15.16
	3	15.39	4.17	15.96	49.15	15.05
PI 135-589 (<i>G. gracilis</i>)	TG	14.35	3.56	15.33	55.17	11.58
	1	25.77	5.73	13.25	41.38	13.88
	2	1.12	0.22	13.72	75.11	9.83
	3	15.92	4.68	19.00	49.06	11.03

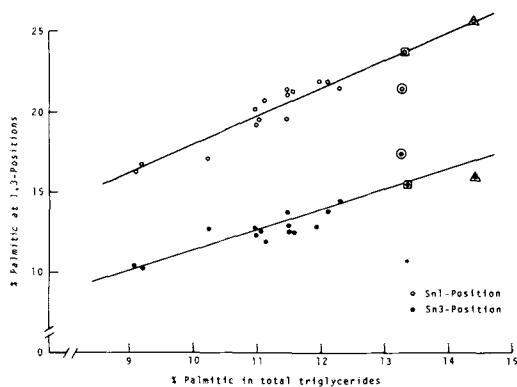


FIG. 1. Percentage of palmitic acid on the *sn*-1 and *sn*-3-positions of glycerol vs. the percentage of palmitic acid in the triglyceride. (○) PI 68-788, (□) *G. soya*, (Δ) *G. gracilis*.

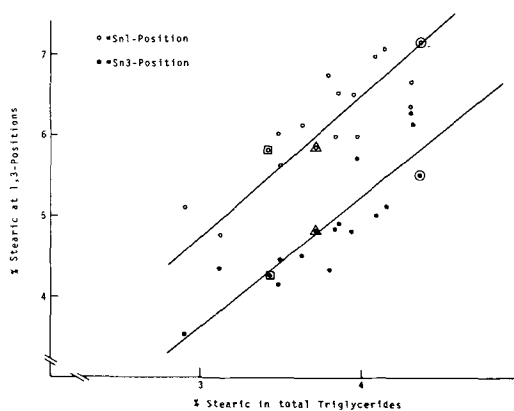


FIG. 2. Percentage of stearic acid on the *sn*-1 and *sn*-3-positions of glycerol vs. the percentage of stearic acid in the triglyceride. (○) PI 68-788, (□) *G. soya*, (Δ) *G. gracilis*.

to year. The good agreement among the cultivars grown in different years is a further indication of the reproducibility of the analyses.

The results of Table II are generally in close agreement with those previously reported on soybean oil which were determined by pancreatic lipase (5-9,11) and a single stereospecific analysis (10). There is very little stearic or palmitic acid on the *sn*-2-position, and the *sn*-1-position is consistently richer in palmitic, stearic, and linolenic acids than is the *sn*-3-position. The *sn*-3-position is richer in oleic acid than is the *sn*-1-position, and the *sn*-2-position is enriched in linoleic and oleic acid.

If the percentage of a fatty acid at one of the positions of the glycerol is plotted vs. the percentage of that fatty acid in the whole fat (Fig. 1-5), one generally gets a straight line. This has been reported for other fats and oils

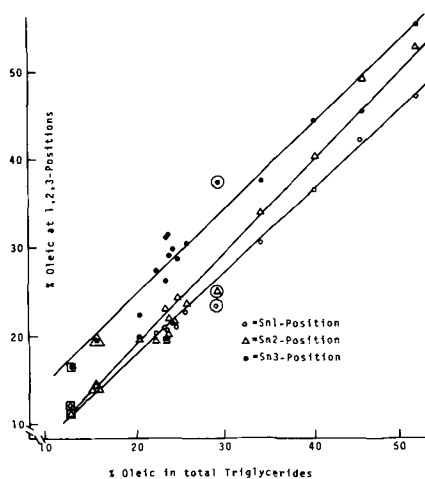


FIG. 3. Percentage of oleic acid on the *sn*-1-, *sn*-2-, and *sn*-3-positions of glycerol vs. the percentage of oleic acid in the triglyceride. (○) PI 68-788, (□) *G. soya*, (Δ) *G. gracilis*.

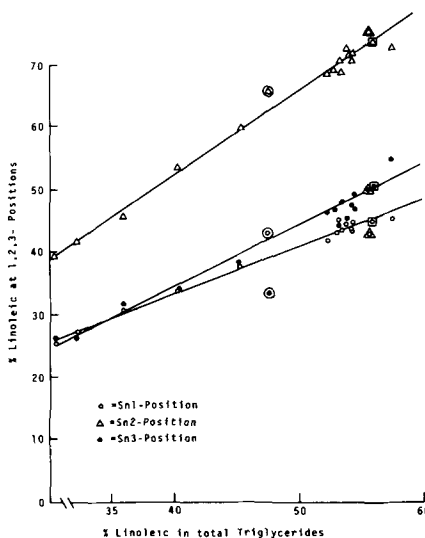


FIG. 4. Percentage of linoleic acid on the *sn*-1, *sn*-2-, and *sn*-3-positions of glycerol vs. the percentage of linoleic acid in the triglyceride. (○) PI 68-788, (□) *G. soya*, (Δ) *G. gracilis*.

(12). The data in Table II were fitted by linear regression, and the slopes and intercepts found are given in Table III. The fits were highly significant, and most of the variance was accounted for by the linear regression. The linearity of these plots indicates that the mechanism responsible for the placement of the fatty acids on the three positions retains its specificity over the range of compositions investigated. The

slopes of the lines can range from 3 down. Slopes of 1 would be obtained for all instances if the distribution were random, and the slopes of all three positions for an acid should average 1. Slopes higher than 1 indicate that the placement of the fatty acids were favored on that position, and the higher it is, the more the placement is favored. The intercepts would be 0 if the plots could be extrapolated to zero. This is not found in Table III, but the algebraic sum of the intercepts is near 0 as would be required for a good fit over the range examined.

These plots are helpful in looking for genetic variation in the mechanism of distribution of the fatty acids on the positions of glycerol. If points for a fatty acid fall away from the linear plots, some change in mechanism of placement is indicated. Examination of the data indicates that points for PI 68-788 fell well off the plots for linoleic acid and were among the most deviant points on the oleic acid plots. This variety seems to have less linoleic acid on the *sn*-3-position and more on the *sn*-1- and 2-positions than would be expected from its fatty acid composition. We believe that this deviation is genetically controlled. Likewise, the values for PI 65-549, the *G. soya* variety tested, fall off the plots for linolenic acid. In this species, the linolenic acid is more evenly distributed on the three positions of the glycerol.

The work of Raghuvver and Hammond (1) suggested that glycerides with unsaturated fatty acids on the *sn*-2-positions and saturated fatty acids on the *sn*-1- and 3-positions should be more stable to autoxidation. Rates of autoxidation are believed to increase with the number of double bonds in fatty acids (16-17). This suggests that the most stable oil would be one in which the linolenic and linoleic acids are concentrated on the *sn*-2-position. The two deviants found are in the right direction, but it is not clear if the deviations are enough to have a significant effect on oil stability. *G. Soya*, unfortunately, is very rich in linolenic acid. It will cross with *G. max*, and *G. gracilis* is thought to be such a cross. The distribution of

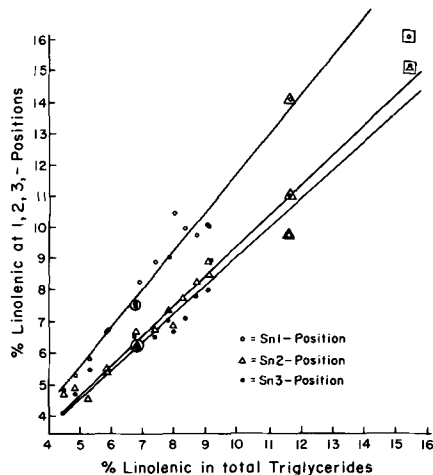


FIG. 5. Percentage of linolenic acid on the *sn*-1-, *sn*-2-, and *sn*-3-positions of glycerol vs. the percentage of linolenic acid in the triglyceride. (○) PI 68-788, (□) *G. soya*, (Δ) *G. gracilis*.

G. gracilis seems to follow the *G. max* distribution in the instance tested.

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TABLE III

The Slopes and Intercepts of the Linear Regression of the Percentage of a Fatty Acid at a Particular *sn*-Position vs. the Percentage of that Fatty Acid in the Whole Fat

Position	16:0		18:0		18:1		18:2		18:3	
	Slope	Inter	Slope	Inter	Slope	Inter	Slope	Inter	Slope	Inter
1	1.87	-0.86	1.49	0.61	0.94	-1.41	0.72	4.21	1.24	-0.63
2	—	—	—	—	1.14	-4.78	1.34	-0.84	0.84	0.67
3	1.05	0.86	1.48	-0.68	0.93	6.23	0.94	-3.45	0.92	0.00
Ave.	0.97		0.99		1.00		1.00		1.00	
Sum		0.00		-0.07		-0.04		-0.08		0.04

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[Received June 14, 1977]