stearic acid. The time-temperature curves were carefully studied to see if a second transition temperature, indicating compound formation, could be found, but none could be observed. Slight changes in composition gave abrupt changes in the solidification points.

Figure 2 shows no indication of compound formation between stearic acid and stearonitrile. A definite eutectic halt occurred at 38.5°, which corresponds to a composition of about 89 mole percentage stearonitrile.

The solidification points of mixtures of stearic acid and 1-octadecanol are plotted in Figure 3. In this case there was no indication of compound formation or of a definite eutectic halt. Mixtures close to the indicated eutectic composition supercooled greatly, even when the melt was seeded. Ralston (4) reported that the difficulty in obtaining eutectic halts in mixtures of saturated acids could be considered as evidence of solid-solution formation.

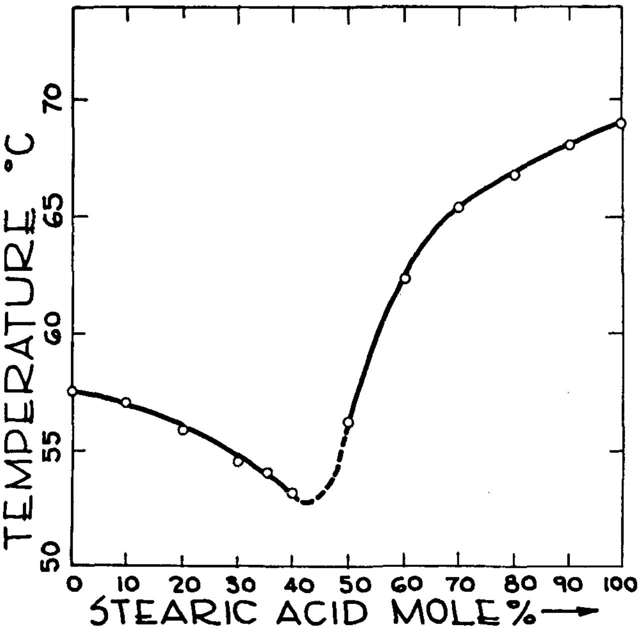

FIG. 3. Solidification Temperatures of Stearic acid—1-Octadecanol.

Figure 4, which shows the solidification points of mixtures of stearonitrile and stearamide, indicates the eutectic temperature to be 39.6°. This temperature corresponds to a composition of 2 mole percentage stearonitrile. No evidence of compound formation could be observed.

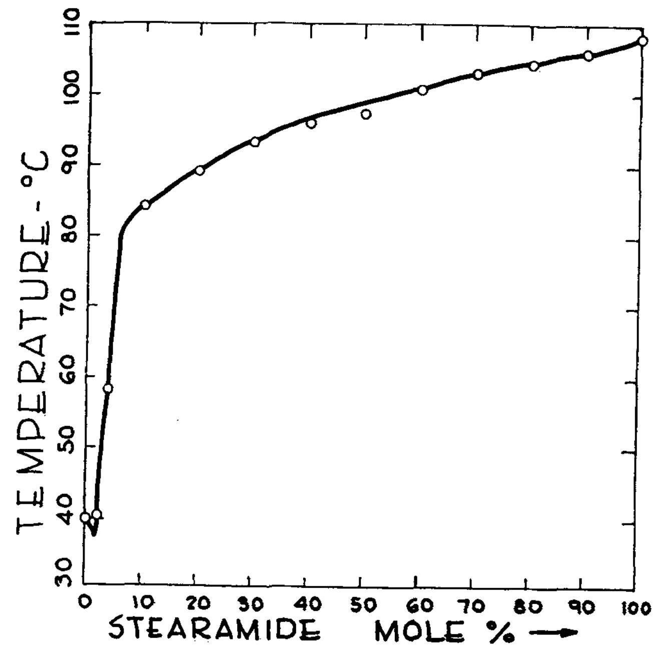

FIG. 4. Solidification Temperatures of Stearonitrile—Stearamide.

Summary

The solidification points of binary mixtures of stearic acid-stearamide, stearic acid-stearonitrile, stearic acid-1-octadecanol, and stearonitrile-stearamide were determined.

Acknowledgment

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Glyceride Structure of Vegetable Oils by Countercurrent Distribution. II. Soybean Oil[1]

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SOYBEAN OIL has been considered to conform to a general rule of even distribution although, as Eckey (5) has stated, some studies "indicate that the arrangement cannot be strictly that which would conform with pure even distribution." Hilditch, Meara, and Holmberg (9) fractionated soybean oil by crystallization from acetone, and their data indicate "that the individual fatty acids are distributed extremely widely or evenly throughout all the triglycerides in the oil." On the other hand, Hashi (6) has isolated oleo-dipalmitin from soybean oil, and Hashi (7) and Suzuki and Yokayama (12)

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have isolated as bromo derivatives several other glycerides which are not permitted under a strict even distribution. Dutton, Lancaster, and Brekke (3) have reported fractions with iodine values lower than those permitted under the rule of even distribution. Their data also indicate the presence of disaturates and of dilinolenin. Venkatasubramanian (13) has reported 14.6% of disaturated glycerides in soybean oil. This value is higher than would be found even under a random distribution.

A major cause of difficulty in the study of glyceride structure is that methods of glyceride separation are not highly effective, and a randomly distributed oil may appear to be evenly distributed if it has been inadequately fractionated. At this laboratory countercurrent distribution has been shown to be a valuable tool for the study of glyceride structure. By the use of an automatic 200-tube countercurrent distribution apparatus it was shown in the first paper of this series (2) that linseed oil glycerides follow essentially a random pattern of distribution. By a similar technique it is shown in this paper that the glycerides of soybean oil also appear to follow a random distribution pattern.

Experimental Procedure

Hawkeye soybeans, grown at Urbana, Illinois, in 1954, were obtained from the U. S. Regional Soybean Laboratory. The beans were flaked and extracted with pentane-hexane in a Soxhlet extractor. Since crude oils tend to emulsify in the countercurrent distribution apparatus, the oil was degummed by shaking with a small amount of water and centrifuging. The oil was then dried under vacuum with the addition of small amounts of acetone to aid in the removal of water. The iodine value and fatty acid analyses which are used in the calculations of this paper are as follows: iodine value, 128.7; linolenic acid, 6.7%; linoleic acid, 49.1%; oleic acid, 30.5%; saturated acids, 13.7%.

The countercurrent distribution procedure was similar to that employed with linseed oil (2). The solvent system was prepared by mixing 10 liters of pentane-hexane, 4 liters of furfural, and 4 liters of nitroethane. Then 40-ml. portions of the lower phase were introduced into each of the tubes with the exception of the first five. The upper-phase solvent was placed in the reservoir, and the pump was adjusted to deliver 5 ml. into tube 0 at each decantation stage. A 9-g. sample of the soybean oil was dissolved in a small portion of the solvent system, and both upper- and lower-phase solvents were added so that the total volume was 25 ml. of upper layer and 200 ml. of lower layer. This was introduced into the first 5 tubes with 5 ml. of upper layer and 40 ml. of lower layer in each.

During the first few transfers the volume of the lower layer in the lead tubes tended to increase because of the high oil concentration. This was corrected by removing a small amount of lower layer just ahead of the lead tube and replacing it with upper layer. After a few initial transfers the oil concentration was low enough so that this was no longer necessary.

When 200 transfers had been completed, the fraction collector was started and set to combine two effluent fractions in each collector tube. Another 800 transfers were then applied, at which point all the glycerides had passed out of the instrument into the 400 collector tubes.

The solutions in selected collector tubes were washed three times with equal volumes of 75% ethanol to remove small amounts of non-volatile solvent. These solutions were then transferred to tared 50-ml. Erlenmeyer flasks, and volatile solvent was removed under vacuum at room temperature. The weight of the fractions is plotted *versus* transfer number in Figure 1. Iodine values and polyunsaturated acids were determined either upon the material in the flasks or, in the case of very small samples, upon material from a combination of several flasks. Iodine values were determined by the 1-hr. Wijs method (11), which was scaled down to allow for the small samples available. Polyunsaturated acids were determined by the 45-min. isomerization method of Brice *et al.* (1), and calculations were made by using their revised constants. The microtubes described by Herb and Riemenschneider (8) were used with 6 ml. of glycol solution in order to use smaller samples.

Results

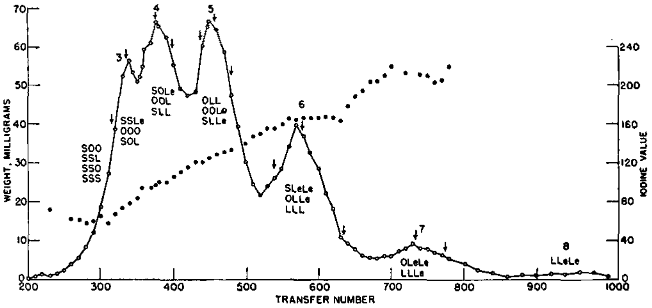

FIG. 1. Countercurrent fractionation of soybean oil glycerides with a pentane-hexane, furfural-nitroethane solvent system.
Open circles—weight curve.
Closed circles—iodine value curve.

Weight and iodine value data for the fractions are plotted *versus* transfer number in Figure 1. As with linseed oil, it appears from the iodine values that the oil has been fractionated according to the number of double bonds in the glyceride molecule. The total weight curve is made up of six component curves, including the very small curve 8 and two other fairly well resolved curves. At the left are 3 partially resolved curves. Table I lists the results of the analyses performed on the fractions indicated

TABLE I
Analyses of Fractions from the Countercurrent Distribution of Hawkeye Soybean Oil Illustrated in Figure 1

Curve number	Transfer number	Iodine value	Spectro-iodine value analyses			
			Linolenic acid %	Linoleic acid %	Oleic acid %	Saturated acids %
7	772–778	213.1	34.1	59.2	24.1	....
	732–738	199.1	32.9	55.7	19.4	....
6	634–638	171.7	11.1	67.0	30.1	....
	576–578	165.9	8.5	75.2	14.4	1.9
	538	155.9	12.9	66.7	6.1	14.3
5	478	133.6	4.3	52.2	34.9	8.6
	458	126.4	4.5	50.1	31.8	13.6
	438	121.2	4.7	48.0	29.9	17.4
4	398	106.4	2.8	29.0	56.0	12.2
	376	97.0	1.2	27.8	52.3	18.7
3	338	75.8	1.2	12.9	55.7	30.2
	316–318	66.9	1.0	12.0	48.9	38.1

by the arrows on the total weight curve of Figure 1.

The area under curve 8 is estimated as only 1.3% of the area under the total weight curve. Because of the extremely small amount of material no analytical data were obtained in this region. From its position on the curve this fraction is believed to contain linoleo-dilinolenin, the only possible glyceride with 8 double bonds. However this fraction also appears to contain some minor non-glyceride impurity. As is shown in Table II, the weight of material is greater than that expected for linoleo-dilinolenin, and in other similar distributions where analytical data were obtained, iodine values and linoleic and linolenic acid content always drop below those calculated for linoleo-dilinolenin.

TABLE II

Composition of Soybean Oil Glycerides Compared with Composition Under Random and Even Distribution Patterns

Glyceride	Number double bonds	Even %	Random %	Experimental %
SSS	0	......	0.26	......
SSO	1	......	1.72	......
SOO	2	......	3.82	......
SSL		......	2.76	......
SSLe	3	......	0.38	......
OOO		......	2.83	......
SOL		33.9	12.31	......
SOLe	4	......	1.68	......
OOL		......	13.70	......
SLL		4.7	9.91	......
OLL	5	41.0	22.06	25.2
OOLe		......	1.87	3.6
SLLe		2.2	2.70	1.6
SLeLe	6	......	0.18	1.3
OLLe		15.8	6.02	9.2
LLL		......	11.84	13.7
OLeLe	7	......	0.41	0.5
LLLe		2.4	4.85	5.2
LLeLe	8	......	0.66	1.3
LeLeLe	9	......	0.03	......

In the area under curve 7 much more material is available. The area under this curve is estimated to be 6.27% of the area under the total weight curve. As indicated by iodine values, the material in this region consists of glycerides possessing 7 double bonds in each molecule (theoretical iodine value 202.5). There are two possible glycerides which contain 7 double bonds, dilinoleo-linolenin, and oleo-dilinolenin. The linoleic and linolenic acid content found for transfers 732–738 correspond to 5.2% dilinoleo-linolenin and 0.5% oleo-dilinolenin.

The area under curve 6 is estimated to be 21.8% of the total weight. The iodine value of fractions in this region indicates that it consists of material with 6 double bonds per molecule (theoretical iodine value 173.2). There are three possible glycerides which contain 6 double bonds, stearo-dilinolenin, oleo-linoleo-linolenin, and trilinolein. Based on the linoleic, oleic, and saturated acid content of transfers 576–578, the oil is calculated to contain 13.7% trilinolein, 9.2% oleo-linoleo-linolenin, and 1.3% stearo-dilinolenin.

As the less completely separated curves 3, 4, and 5 are considered it is evident from the iodine values that this portion of the weight curve is made up of the partially separated glycerides with 5 double bonds or less per molecule. In this region it is more difficult to determine the area to assign to each curve, but it is estimated that the 5 double bond glycerides make up 34% of the oil. There are three possible glycerides which contain 5 double bonds, oleo-dilinolein, dioleo-linolenin, and stearo-linoleo-linolenin. Based upon the linolenic, linoleic, and oleic acid content of transfer 458, the oil is estimated to contain 25.2% oleo-dilinolein, 3.6% dioleo-linolenin, and 1.6% stearo-linoleo-linolenin.

Table II lists the chemically distinguishable glycerides possible by combinations of the four fatty acids. The experimentally determined percentages of these glycerides are also listed together with the composition calculated under the even and random patterns of distribution. The even and random distribution patterns were calculated as in previous papers (2, 3). For the major components the experimentally determined glyceride composition corresponds much more closely to a random than to an even distribution. There is 13.7% of trilinolein, which is not permitted under the even distribution pattern. The amounts of oleo-dilinolein, oleo-linoleo-linolenin, and dilineo-linolenin are much closer to those predicted for a random than for an even distribution. Among the glycerides present in very small amounts the differences between the experimental values and those calculated under either the random or even pattern are less than experimental error.

In the distribution described above, no attempt was made to calculate the amounts of the glycerides containing less than 5 double bonds. In order to obtain greater resolution of the more saturated glycerides, material corresponding to curve 3 (transfer 200–350) from two other similar distributions was combined and redistributed. With this more nearly homogeneous material it was possible to recycle the fractions through the instrument until 525 transfers had been applied. Fractions were then removed from the instrument and collected with two fractions in each collection tube as before. The resulting weight curve and iodine values of the fractions are shown in Figure 2. The weight curve with its shoulder on the left side can be considered as made up of two partially separated components represented by the dotted curves. From its position and from the iodine value the component represented by curve A might be expected to be composed of glycerides with 2 or less double bonds. This component represents 3.84% of the oil. As can be seen from Table II under a random distribution, the sum of glycerides with 2

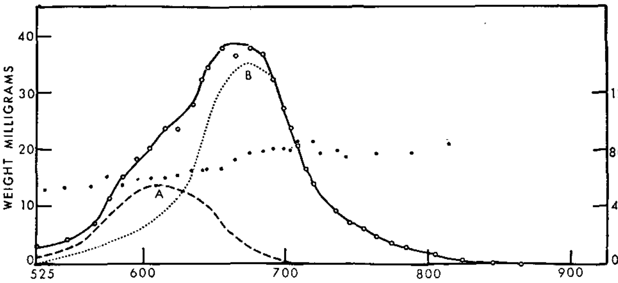

FIG. 2. Redistribution of more saturated glyceride fraction corresponding to component 3 of Figure 1.
Open circles—weight curve.
Closed circles—iodine value curve.

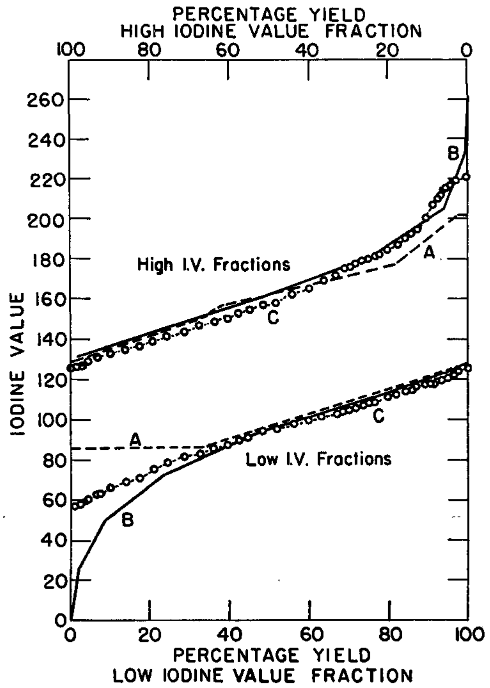

FIG. 3. Iodine value—percentage yield representation for A. even pattern; B. random pattern; C. countercurrent distribution data of Figure 1.

double bonds or less is 8.56%. Interpretation of the data in this region however is complicated by the fact that part of the saturated acids are palmitic instead of stearic. Palmitoyl glycerides during distribution move through the apparatus at a more rapid rate than do stearoyl glycerides. Such evidence as is available suggests that they move at approximately the same rate as oleyl glycerides. This complication has very little effect upon the distribution of the more unsaturated fractions, but it becomes progressively more important as the more saturated glycerides are studied.

The palmitic acid content of the oil was determined by first preparing the saturated acids by the procedure of Earle and Milner (4). Palmitic acid in the saturated acids was then measured by a modification of Nijkamp's (10) chromatographic procedure. Palmitic acid was found to make up 8.5% of the fatty acids.

Then, assuming that palmitic acid behaves in the same way as oleic acid during the countercurrent separation, component A under a random distribution would make up 3.11% of the oil. The fatty acid composition of fractions for this distribution, which are shown in Table III, is also more compatible with a random distribution. If the distribution were even, the most saturated glyceride possible would be stearo-oleo-linolein with an iodine value of 86.0 and approximately 33% each of linoleic, oleic, and saturated acids.

TABLE III
Analyses of Fractions from the Redistribution of Component 3 of Figure 1

Transfer number	Iodine value	Spectro-iodine value analyses			
		Linolenic acid %	Linoleic acid %	Oleic acid %	Saturated acids %
583–587..........	54.1	0.2	10.1	41.2	48.5
623–625..........	61.9	0.3	9.1	51.1	39.5
649–651					
655..........	66.5	1.1	16.9	40.2	41.8
675..........	76.8	0.2	11.7	63.4	24.7
703–705..........	79.6	0.4	10.2	65.9	23.5
755–773..........	77.7	1.0	25.2	33.1	40.7

Another method of representing glyceride composition data and of comparing theoretical glyceride patterns with experimental data is shown in Figure 3. It consists of calculating iodine values for combinations of high iodine value and low iodine value fractions and plotting this averaged and weighted iodine value against the percentage which that fraction represents of the whole oil. As can be seen, the experimental data follow more closely the random pattern, and iodine values of both high and low iodine value fractions lie outside the limits required by an even distribution.

A countercurrent distribution was also carried out on oil obtained from Clark soybeans. The weight and iodine value curves were quite similar to those shown for Hawkeye oil in Figure 1. Because of the similarity these results are not presented here.

Summary

Soybean oil has been fractionated in a 200-tube countercurrent distribution apparatus. Fractions have been obtained with iodine values both too high and too low to conform to an even distribution. From the weight distribution curve, iodine value, and spectrophotometric analyses the oil is estimated to contain 5.2% dilinoleo-linolenin, 13.7% trilinolein, 9.2% oleo-linoleo-linolenin, and 25.2% oleo-dilinolein. This composition is in agreement with a random distribution pattern. Also the fatty acid content of the more saturated fractions indicates the presence of disaturates and dioleins which are not permitted under an even distribution. Based upon this type of information, it is concluded that the fatty acids in soybean oil glycerides approach a random type of distribution.

Acknowledgment

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