

Fractionation and Structure of Soybean Glycerides¹

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NEW and extended uses for soybean oil are expected to result from its fractionation into "tailor-made" fats. Already commercial methods of fractionation into low and high iodine value oils have enhanced the usefulness of soybean oil in the edible and paint oil fields. Methods are also potentially available for the separation of valuable minor constituents of the oil such as sterols, phosphatides, pigments, and tocopherols; however fractionation of the glycerides, the major constituents of soybean oil, will probably constitute the critical problem in regard to increasing the volume of soybean oil utilized.

The success of any attempted glyceride fractionation must ultimately depend on the arrangement of fat acid radicals in the glycerides of the oil. Of the several patterns for the glyceride structure of soybean oil that can be conceived, the most readily distinguished are the monoacid triglyceride pattern, the even distribution pattern, and the random distribution pattern. The greatest degree of fractionation is possible theoretically if only one fat acid occurs in each triglyceride, e.g., the monoacid triglyceride pattern. Even and random patterns provide for an increasing number of individual glycerides but their segregation presents an increasingly difficult problem.

Agreement is complete on at least one point regarding which of these three alternative patterns of glyceride structure exists. Glycerides are known to occur "mixed" rather than arranged in the pure monoacid triglyceride pattern. The pattern of distribution of fat acids in the mixed glyceride is however a matter of considerable disagreement. Hilditch proposed and continues to maintain that the "rule of even distribution" applies qualitatively to both animal and plant fats (1). Soybean oil is described as one of those oils following the even pattern (9). Such an even pattern implies "biological specificity" in the synthesis of glyceride molecules (3). By contrast, the random pattern of arrangement, which Longenecker has considered to be followed in the case of animal fats, is based purely upon considerations of statistical probability (4). Norris and Mattill, and Riemenschneider, *et al.* have obtained data on animal fats which are best described by the concept of random distribution (3, 5). Daubert has recently shown that a partial random scheme accounts for the fat acid distribution in corn oil better than either even or random patterns (6).

At least a part of the disagreement over the pattern of distribution stems from the degree of strictness with which the random and the even patterns are considered. Hilditch would have the rule of even distribution applied as a generalization which covers "the general trend of the observed facts more or less adequately" (2). In defining the rule, the limits are so broadly drawn as to make evaluation of its

validity impossible in many instances, such as in the case of the following: "a minor component which forms much less than about a third of the total fatty acids (3 g., 15% or less), will not occur more than once in any glyceride molecule" (1). In a similar way, the proposal of certain random patterns may be said to obscure the contrasts. Allowed sufficient modifications of even and random rules, the difference between the two becomes experimentally indistinguishable. Therefore in the present paper only simple random distribution will be considered, and even distribution will be defined in strict stoichiometric algebraic terms. Another source of disagreement, as will be pointed out, is that present methods of glyceride separation are not highly effective and that conclusions as to what basic triglyceride pattern exists may depend on the efficiency of the fractionation techniques employed. For example, a randomly distributed oil may appear to be evenly distributed if it has been incompletely fractionated.

In the present paper fractionations of soybean oil, both original and published in the literature, are assembled. The results obtained by adsorption analysis, by countercurrent extraction, and by crystallization are compared with results predicted under monoacid, even, and random patterns in an attempt to throw further light on the structure of soybean glycerides and to assess the potentialities of soybean oil for industrial and food uses.

Calculation of Glyceride Composition

Distribution of fat acids within the glyceride molecules of soybean oil has been calculated in accordance with the monoacid, random, and even distribution patterns. Since an oil of the approximate fat acid composition of 9% linolenic, 53% linoleic, 23% oleic, and 15% saturates was used in much of the experimental work, these figures are taken for the present calculation. To effect a simplification, saturates are assumed to be stearic acid.

According to the monoacid pattern, soybean oil would consist of 9% trilinolenin, 53% trilinolein, 23% triolein, and 15% trisaturates. These figures are included in Table I, which summarizes the calculations for the three patterns.

According to the elementary concept of the even distribution theory, linolenic, oleic, and saturated acid groups can be present only singly in the triglycerides since these acids occur in the oil in percentages of less than 33⅓%. Following this rule, linoleic acid is present in every triglyceride molecule at least once; and in 59% of the molecules it must occur twice. Limited by these restrictions, only six constitutional glyceride structures are possible. They are a) stearo-oleo-linolein (SOL),* b) stearo-dilinolein (SLL), c) stearo-linolenol-linolein (SLeL), d) oleo-dilinolein (OLL), e) oleo-linolenol-linolein (OLeL), and f) linolenol-dilinolein (LeLL). Four linear equations can be written as described by Do-

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* Apparently through a typographical error this glyceride is given as saturated-oleo-linolenin by Hilditch, Meara, and Holmberg (9). Otherwise the proposed structures are in agreement.

TABLE I
 Iodine Value and Calculated Composition of Soybean Glycerides Under Monoacid, Even, and Random Distribution Patterns

Glyceride ¹	Fat acid per cent of glyceride				Iodine value	Monoacid per cent	Even per cent	Random per cent
	S	O	L	Le				
SSS.....	95.72				0.0	15.00		0.34
SSO.....	63.97	31.76			28.54			1.55
SOO.....	32.06	63.66			57.21			2.38
SSL.....	64.11		31.60		57.21			3.58
SLe.....	64.26			31.45	86.01			0.61
000.....		95.70			86.01	23.00		1.22
SOL(A).....	32.13	31.90	31.67		86.01		20.97	10.97
SOL.....	32.20	31.97		31.52	114.95			1.86
OLL.....		63.95	31.75		114.95			8.41
OLL(B).....	32.20		63.49		114.95		17.14	12.64
OLL(D).....		32.05	63.64		144.01		33.94	19.38
OLLe.....		64.09		31.59	144.01			1.43
SLe(C).....	32.28		31.82	31.59	144.01		7.02	4.29
SLe.....	32.35	32.12		63.32	173.21			0.36
OLLe(E).....			31.89	31.66	173.21		11.71	6.58
LLL.....		32.19	95.67		173.21	53.00		14.89
OLLe.....				63.47	202.54			0.56
LLLe(F).....			63.93	31.74	202.54		9.22	7.58
LLLe.....			32.04	63.62	232.00			1.29
LeLeLe.....				95.64	261.60	9.00		0.07

¹ S = Saturated; O = Oleic; L = Linoleic; Le = Linolenic.

erschuk and Daubert (6) relating the fat acid content of these six component glycerides to the fat acid content of the whole oil. A fifth equation states that the sum of these six glycerides equals 100% while the sixth equation relates the iodine value of the individual glycerides to the iodine value of the whole oil. The equations are given in Table II.

Unfortunately, the sum of the first four equations is equivalent to the fifth equation, and actually only five independent equations relate the six unknowns. An approximate method of solution must therefore be used instead of the precise methods of determinants or of simultaneous equations.†

Glyceride	Per cent acid available	First approximation of composition—%
LeLS.....	15	$\frac{15}{57.67} \times 27 = 7.02$
LeLO.....	23	$\frac{23}{57.67} \times 27 = 10.77$
LeLL.....	$\frac{19.67^1}{57.67}$	$\frac{19.67}{57.67} \times 27 = 9.21$

¹ 53.0—33.33=19.67. After further approximations the compositions obtained, as shown in Table II, are LeLS—7.02%, LeLO—11.71%, and LeLL—9.23%.

The structure of the six glycerides and the approximate solutions or the calculated percentages for the composition obtained from these equations are given in Table I. When these percentages are substituted in the six equations, the check values given in the last column of Table II are obtained, indicating the solution is sufficiently exact.

The underlying assumption of random distribution is that there is no directional force such as "biological specificity" to influence the plant's synthesis of triglycerides. Under random distribution the reaction of fat acids with glycerol is considered to be purely a problem of statistical probability involving the combinations of 4 fat acids, 3 at a time, yielding 20 combinations

$$\frac{n^3 + 3n^2 + 2n}{6} = 20(6)$$

Equations describing this pattern have been pub-

†As a first approximation, for example, the linoleo-linolein which composes 27% of the glycerides may have stearic, oleic, or a second linoleic acid group as a third component acid. The assumption is made that these three glycerides will occur in amounts proportional to the stearic, oleic, and linoleic acids available for reaction. Thus:

lished (6, 11) and have been used to calculate the random distribution given in Table I.

A theory of partial random distribution has been proposed by Bernstein (12) which purports to consider both the relative reactivities of the primary and secondary hydroxyl groups of glycerol and the reactivity of the individual fat acids. Since it is impossible at present to evaluate these factors, this theory is not considered further. Such an approach to the constitution of vegetable oils appears theoretically more valid than the simple random pattern. However this must remain a field for future investigation.

Experimental Fractionations

A ready index of the efficiency of any glyceride fractionation process is given by the spread of iodine values between the high and low unsaturated fractions obtained, for example, as between extract and raffinate fractions in systems of immiscible solvents or between solid phase and solution in the case of crystallization. To compare efficiencies of separation, iodine values and the proportions of each fraction for even and random distribution patterns may be arranged as is done in Figures 1, 2, and 4. These curves are calculated from the data of Table I by repeatedly dividing the glycerides into a high and a low iodine value group and by calculating the percentage of the total that each group represents and the average iodine value of each group. The lowest and highest curves are the limits of fractionation imposed by the random distribution pattern. The next higher and next lower are the curves for the fractionation limits according to even distribution. Between these theoretical separation curves are the

 TABLE II
 Equations Describing Even Distribution

	Check values
1. Linolenic acid: 0% A + 0% B + 31.59% C + 0% D + 31.66% E + 31.74% F = .956 × 900 = 860.4.....	884.2
2. Linoleic acid: 31.67% A + 63.49% B + 31.82% C + 63.64% D + 31.89% E + 63.93% F = .956 × 5300 = 5068.....	5099.2
3. Oleic acid: 31.90% A + 0% B + 0% C + 32.05% D + 32.12% E + 0% F = .956 × 2300 = 2198.....	2132.9
4. Saturated acids: 32.13% A + 32.20% B + 32.28% C + 0% D + 0% E + 0% F = 1500 × .956 = 1434.....	1452.3
5. Triglycerides: % A + % B + % C + % D + % E + % F = 100.....	100
6. Iodine value: 86.01% A + 114.9% B + 144.0% C + 144.0% D + 173.2% E + 202.5% F = 13502.....	13567

TABLE III

Isolation of High and Low Iodine Value Glycerides from Soybean Oil Raffinates and Extracts by Adsorption Analysis

Sample	Iodine value	Linolenic acid	Per cent of crude oil
Crude soybean oil.....	135	9.0	100
Raffinate			
Before adsorption.....	96.85	2.34	21.3
After first adsorption, fractions			
4.....	88.01	1.52	4.01
5.....	90.49	1.67	3.78
6.....	91.97	1.87	3.64
7.....	96.05	2.25	1.98
8.....	98.27	2.67	1.27
Combined fractions.....	91.60	1.84	14.68
After second adsorption, fractions			
9.....	78.79		
10.....	77.28	0.67	
11.....	78.95	0.85	
Combined fractions.....	78.20		5.20
Extract			
Before adsorption.....	170.97	19.15	24.0
After adsorption, fractions			
15-16.....	195.48	35.28	0.50
17-20.....	202.69	38.43	0.53
Combined fractions.....	199.12	36.90	1.03

curves for actual fractionations obtained by adsorption analysis (Figure 1), countercurrent extraction (Figure 2), and low-temperature crystallization (Figure 4).

Adsorption Analysis: A fractionation of soybean oil by adsorption analysis has been published (13).

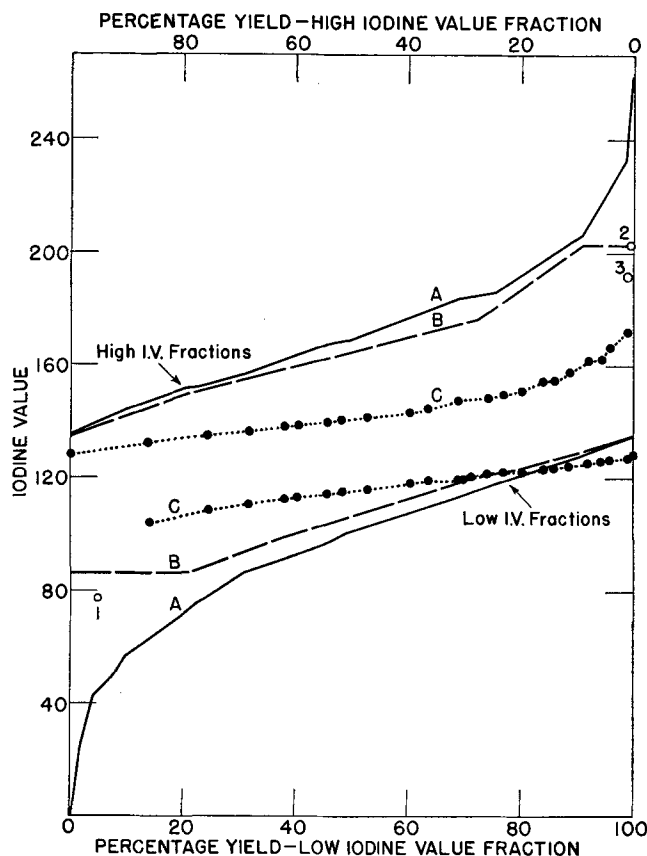


FIG. 1. Experimental fractionation by adsorption analysis and theoretical separations of soybean glycerides.

- A. Random pattern.
- B. Even pattern.
- C. Adsorption analysis.

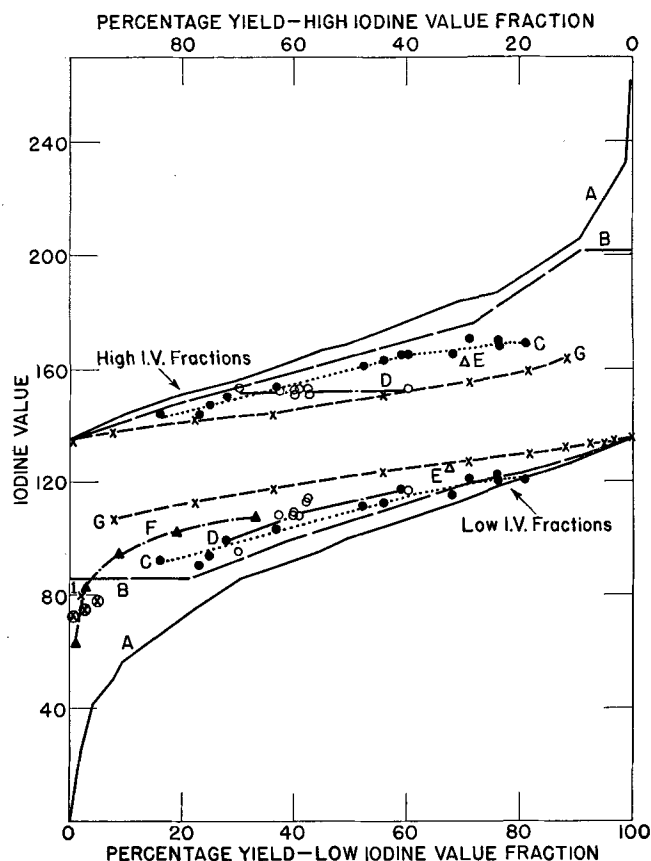


FIG. 2. Experimental fractionations by countercurrent extraction and theoretical separations of soybean glycerides.

- A. Random pattern.
- B. Even pattern.
- C. N.R.E.L. pilot plant.
- D. P.P.G. pilot and commercial plants.
- E. "Solexol" pilot plant.
- F. Laboratory nitroparaffin-petroleum ether countercurrent distribution.
- G. Laboratory furfural-hexane countercurrent distribution.

Iodine values of fractions recovered after passage of a crude soybean oil through a single column packed with alumina ranged from 104 to 178. These fractions have now been arranged in an order to give progressively increasing iodine values. The percentage yields vs. iodine values of the fractions have been calculated and are plotted in Figure 1. Comparison of these curves with the theoretical separation curves shows that the degree of fractionation given by passage through a single adsorption column is insufficient to permit any conclusion concerning the pattern of fat acid distribution. However the sharp inflections at the high iodine value end of the upper curve and at the low iodine value end of the lower curve (Figure 1) are suggestive of random distribution. Since 17.6% of the glycerides, and preferentially the more unsaturated glycerides were not recovered from the column, the iodine value of the recovered oil is 128 rather than 135. This loss also causes the low iodine value fraction curve to drop below the theoretical limits on the right of the graph.

The two points (1 and 2) of Figure 1 representing the highest and lowest iodine values were obtained by the adsorption analysis of raffinate and extract fractions from the pilot plant extraction column employing the furfural-heptane system. The raffinate comprised 21.3% of the original crude soybean oil

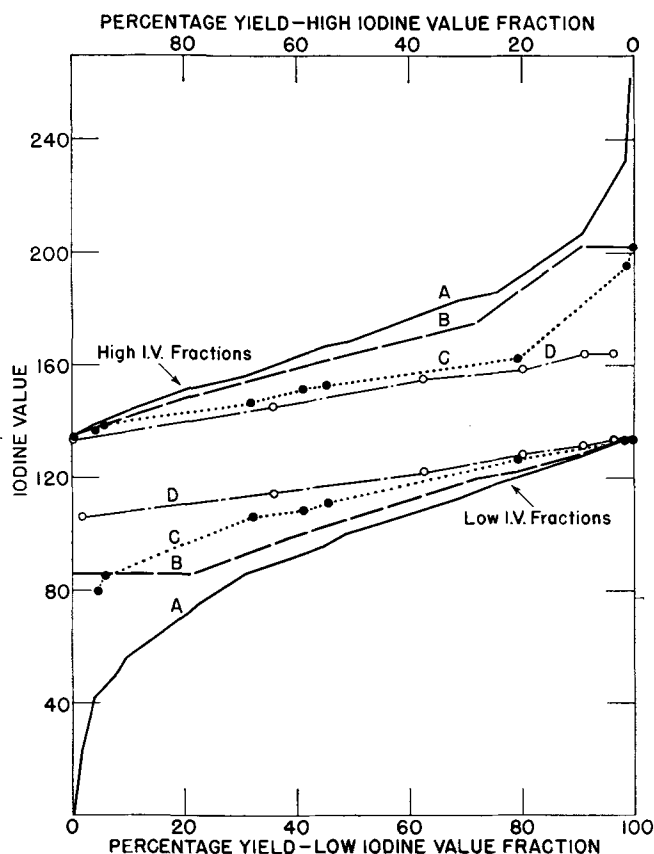


FIG. 4. Experimental fractionations by crystallization and theoretical separations of soybean glycerides.

- A. Random pattern.
- B. Even pattern.
- C. Golubic, Martin, and Daubert (7).
- D. Hilditch, Meara, and Holmberg (9).

and had an iodine value of 96.85. Fifteen grams of this fraction were adsorbed on an alumina column by use of the procedure previously described (13), except for a developing solvent which consisted of 30% diethyl ether in hexane. After the 100-ml. fractions were eluted, weighed, and analyzed, the lowest iodine value fractions (data in Table III) were combined to give a composite fraction with an iodine value of 92.33. A 5-gm. aliquot was readsorbed on an alumina column of one-third the cross sectional area but of length equal to the first column. From this second adsorption, three fractions comprising 5.2% of the crude soybean oil were isolated. Their combined iodine value was 78.2. This iodine value (Point 1, Figure 1) is less than 86.01, the lowest iodine value permitted under the even distribution pattern. These three low iodine value fractions were also analyzed spectrophotometrically for linolenic and linoleic acids. The saturated acid content on a molar basis (palmitic-stearic 3:1) of each fraction is also calculated with the use of the iodine value, and, while these figures possess inherently large error, they do exceed 33 $\frac{1}{3}$ % and indicate the presence of disaturates which should not occur under the even distribution pattern. The presence of some linolenic acid (Table III) even in these low iodine value fractions is of practical importance in any scheme proposing to produce a linolenic-acid-free fraction from soybean oil. Point 2 in Figure 1 is based upon a similar adsorption analysis but of an extract fraction from the pilot plant column. The extract with an iodine number of 170.89

comprised 24% of the original oil. Adsorption analysis of 15 grams of this extract gave the high iodine value fractions listed in the lower section of Table III. In this instance the highest iodine value, 202, is at the upper limit which could occur under even distribution and therefore cannot be cited in favor of either the even or the random theory of distribution. Spectrophotometric data however do indicate that linolenic acid is present in two fractions in excess of 33 $\frac{1}{3}$ %. No dilinolenin is permitted under the even distribution arrangement, and linolenic acid should never account for more than one-third of any fraction. Point 3 represents a fractionation of another extract but of lower initial iodine value.

Countercurrent Extraction: Much work has been done on liquid-liquid extraction of soybean oil (8, 10, 14, 15, 16). At present, soybean oil is fractionated on a commercial scale with the use of furfural and hydrocarbon solvents (8). Countercurrent extraction with propane also presents a potential method for the fractionation of soybean oil (10). Figure 2 shows published pilot-plant data for furfural and propane extraction processes. In addition, pilot-plant data from the Northern Regional Research Laboratory are included. Over the range of extract yields (19-83%) these latter data compare very favorably with any fractionation yet reported for soybean oil, laboratory operations not excluded.

Fractionations in this laboratory's pilot plant were carried out on a solvent-extracted, degummed soybean oil (iodine value 133.9). The extraction tower was 18 feet 4 inches tall, 2.4 inches inside diameter, and packed with one-quarter inch Raschig rings. It was operated at 120°F., with furfural entering near the top, oil at the midpoint, and heptane near the bottom of the tower. Twenty parts of furfural to one of oil were used. The heptane to oil ratio was increased from 1.9 to 3.0 to give extract yields ranging from 19 to 83%. Solvents were removed from the oil fractions in a series of conventional evaporators and stripping stills.

The Craig countercurrent distribution apparatus is of particular interest for laboratory-scale countercurrent extraction studies. Experiments can readily be repeated, and operating conditions are easily varied. An added advantage is that the apparatus provides a definite and constant number of extraction stages. A general description of and operating methods for this equipment have been published in detail (17).

In the furfural-hexane fractionation reported here, 2 grams of crude soybean oil were distributed between hexane and furfural in a 25-tube model (one-half inch tube diameter). Each tube contained hexane and furfural in a 1 to 4 ratio except tube 0 to which the oil sample was added in place of the hexane. After a 24-stage distribution the contents of each tube were removed from the apparatus and the furfural layer extracted three times with pentane-hexane. The petroleum ether extract was then water-washed successively three times and the solvent removed under vacuum. The oil fractions were weighed and iodine values determined where size permitted. Figure 3 shows tube number plotted against iodine value and fraction weight. In Figure 2 iodine value is plotted against the percentage yield along with results of the pilot-plant countercurrent extractions. Conditions

of operation for the Craig machine make comparisons with pilot-plant data unwarranted except to note that the fractionation made with the pilot-plant equipment is superior to that made in a 25-tube Craig distribution apparatus.

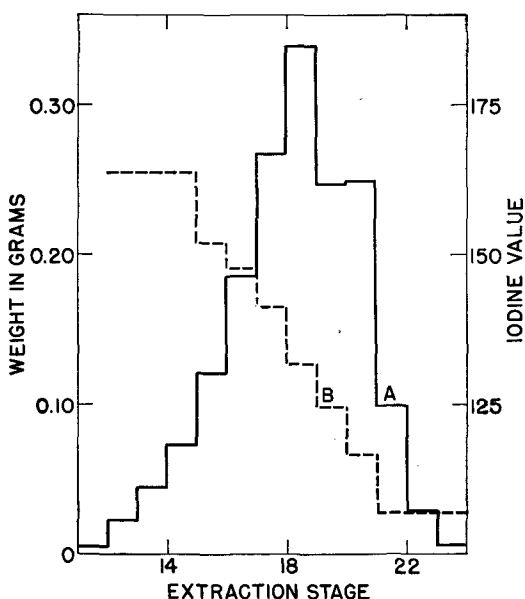


Fig. 3. Countercurrent distribution of soybean glycerides between hexane and nitroparaffins.

A. Weight.
B. Iodine value.

The data indicated by point No. 1 on the graph (Figure 2) were obtained by a separatory funnel extraction of the pilot-plant raffinate (iodine value 96.85) described above. A 100-gram portion of the raffinate dissolved in 100 ml. of hexane was extracted 50 times with 400 ml. portions of hexane-equilibrated furfural. After evaporation of the hexane, the fraction was analyzed for iodine value and saturates. The iodine value of the glycerides was 80.96 and the iodine value of the free acids, 83.60. The saturated acid content, determined by crystallization (18) was found to be 34.2% of the acids on a weight of acids basis or 36.15% on a molar basis, assuming a 3:1 ratio of palmitic to stearic acids.

In repeating this experiment, two funnels were employed in a countercurrent flow system. After 48 equilibrations, the iodine values of the two hexane layers were 74.38 and 76.75 and comprised 0.81 and 3.15% respectively of the original soybean oil. These data are also plotted in Figure 2 as circled crosses. The fat acids of the combined fractions had an iodine value of 76.06 and the saturated acid content, as determined by crystallization, was 35.51%. Calculated from iodine value and spectrophotometric data, the saturated acid percentage was 38.06. Assuming a 3:1 ratio of palmitic to stearic acid, the molar fraction of saturated acids was 37.53% by crystallization and 40.2% by the I.V.-spectro method. The unsaponifiable content of the combined fractions was 1.3%.

Also reported in Figure 2 is an experiment in which 20 grams of oil were distributed between equal volumes of petroleum ether and nitroparaffin solvents (20% nitromethane, 80% nitroethane) in a 29-tube model (1 inch tube diameter). After a 28-stage distribution the contents of each tube (169 ml.) were

removed from the apparatus and evaporated under vacuum. The oil fractions were weighed and iodine values were determined. It is apparent that the iodine values of fractions obtained were lower than those permitted under the even pattern. Unfortunately in this instance the low iodine value fractions were too small to permit both iodine value and saturated acid analysis, but the saturate content may be assumed to be higher than 37%.

Crystallization: Several publications on the low-temperature crystallization of soybean oil are found in the literature (7, 9, 19, 20, 21). When these are compared by plotting in graphical form the fraction percentage against iodine value, the crystallizations of Golumbic, Martin, and Daubert (7) and of Hilditch, Meara, and Holmberg (9) appear to have been the most efficient. These data, shown in Figure 4, are compared to the theoretical separation curves. It is apparent that a wide divergence exists between these fractionations. Although Hilditch's data are not inconsistent with the theory of even distribution, the fractionation appears less efficient than that of Golumbic's. The shape of the graph for Golumbic's data is not indicative of even distribution, and his isolation of 5% of the oil with an iodine value of 79 is not compatible with the even distribution pattern. Further, this fraction is reported to have 56.2% of saturated acids.

Discussion

Certain observations pertinent to the fractionation of soybean oil can be made based on calculations for the even and the random distribution patterns such as has been done here. In a recent paper Goss (22) predicted that "roughly the maximum difference is 70 iodine number units" between raffinates and extracts for random and even patterns. Over the range of 25% to 80% yield of raffinates or over the range of the practical operating conditions this generalization is confirmed in the present calculations. However at the left and right extremes of the curves, the differences are 135 and 129 respectively under the random pattern. Under the even pattern the corresponding differences in iodine values are 49 and 67.

Although a random-type distribution provides for more individual glyceride molecules and thus increases the difficulties of fractionation of soybean oil, oil with this basic pattern would have greater potentialities as a source of glycerides of diverse properties once efficient fractionation methods were achieved. As many as 20 constitutional glycerides, including the monoacid triglycerides, may exist as compared to 6 under even distribution.

Many attempts have been made to eliminate linolenic acid glycerides from the oil by fractionation since linolenic acid has long been suspected of being the precursor of off-flavors in soybean oil. If even distribution exists, then linolenic acid should occur theoretically only in glyceride components having an iodine value of 144 or higher (Table I). Assuming that separations are made on the basis of iodine value, it should be possible theoretically to obtain 72.1% of the glycerides of soybean oil, free of linolenic acid with an iodine value of 120, while the linolenic-acid-containing fraction with an iodine value of 175 would comprise the remaining 27.9% of the oil (Table IV). If the random pattern exists, however, linolenic acid would occur in glycerides possessing an iodine value of 86 and over. In this case a glyceride fraction

TABLE IV
Theoretical Separations Permitted Under Monoacid, Even,
and Random Distribution Patterns

Distribution pattern	Linolenic acid free fraction		Linolenic acid containing fraction	
	Per cent yield	Iodine value	Per cent yield	Iodine value
Monoacid.....	91.0	122.5	9.0	261.6
Even.....	72.1	120.0	27.9	175
Random.....	7.9	49.0	92.1	142.4

having an iodine value of 49 and comprising 7.9% of the oil could be obtained free of linolenic acid. While elimination from soybean oil of the glycerides containing linolenic acid appears to be a theoretical possibility under even distribution, total removal of all linolenic acid from soybean oil with present fractionation methods is impractical, if not impossible, under a random-type distribution.

Data compiled here indicate that at present it is the inefficiency of current fractionation methods rather than the basic triglyceride pattern which limits the degree of separation obtained on soybean oil. However when fractionation procedures improve sufficiently, the basic pattern of distribution of fat acids within the triglycerides will limit the amount of fractionation obtainable and will thus determine the ultimate usefulness of soybean oil.

Summary

The glyceride composition of soybean oil has been calculated according to even and random distribution patterns and has been compared with experimental fractionations obtained by adsorption analysis, liquid-liquid extraction, and crystallization. Evidence is presented which indicates that soybean oil does not follow the elementary conception of even distribution. Fractions have been isolated by these three methods with iodine values lower than that permitted under the rule of even distribution. Further data indicate the occurrence of di-saturates and dilinolenin, neither of which is permitted under the rule of even distribution.

The ultimate proof of the distribution pattern existing in soybean oil, whether random or partial ran-

dom, must await the development and application of more efficient methods of fractionation. At the present time separation of soybean triglycerides appears limited by the efficiency of fractionation procedures rather than by the pattern of fat acid distribution.

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