Glyceride Structure of Vegetable Oils by Countercurrent Distribution. I. Linseed Oil[1]

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LINSEED OIL, like other seed fats, has been considered to conform to the general "rule of even distribution" (5); however with the application of improved methods of glyceride separation an increasing divergence has been noted. Thus Walker and Mills (9), using chromatographic adsorption analysis, obtained fractions of linseed oil with iodine values so high (246.5) as to indicate the presence of trilinolenin, a glyceride not permitted under the "strict even distribution pattern." While holding that linseed oil "follows the customary rule of even distribution," Hilditch and Seavell describe an improved fractional crystallization in a recent publication (6) and calculate from a fraction of iodine value 214 that as much as 5.1% of trilinolenin may be present in the whole oil.

The countercurrent-distribution technique has been shown to constitute a valuable tool for the study of the glyceride structure (4). Acquisition of an automatic 200-tube countercurrent-distribution instrument afforded an opportunity to apply the high resolving power of this instrument to the separation of the complex glyceride mixture of linseed oil. The present paper reports three fractionations obtained with this instrument.

Experimental Procedure

The countercurrent-distribution equipment[3,4] used is similar to the instrument described in detail by Craig, Hausmann, Ahrens, and Harfenist (2), being fully automatic in its solvent introduction, mixing, settling, transfer, and collection operations. It differs from that instrument in possessing 200 tubes with larger solvent capacity (80 ml. each) and in its improved solvent introduction and collection systems.

The linseed oil used for the distributions of Figures 1 and 2 was that one which was also analyzed by the collaborators on the Spectroscopy Committee of the American Oil Chemists' Society in 1948 (8). The present sample was stored at 0°F.(—18°C.) in a sealed glass bottle. It was a raw, winterized (*i.e.*, dewaxed) oil supplied by the Minnesota Linseed Oil Company. Iodine value of the oil at the time of its receipt was 182.2 as determined by the Analytical Unit of the Northern Utilization Research Branch. The polyunsaturated acid composition data of the committee report have been recalculated, using the revised constants for natural linoleic and linolenic acids determined by Brice, Swain, Nichols, and Riemenschneider (1). These recalculated values are 50.8% linolenic acid and 14.8% linoleic acid. Our iodine value and fat acid analyses, which are used in the calculations of this paper, are as follows: iodine value, 183.5; linolenic acid, 52.3%; linoleic acid, 14.9%; oleic acid, 24.3%; and saturated acids, 8.5%.

Pentane-hexane and furfural were selected as solvents for the distribution to be presented in Figure 1. The pentane-hexane (boiling range 35° to 60°C.) was distilled on a steam bath to exclude the possibility of a non-volatile residue. Furfural was distilled under vacuum, and 0.01% hydroquinone was added to inhibit oxidation and resultant discoloration.

Eight liters of each of these two solvents were placed in a 5-gal. jar and agitated with an air stirrer to attain equilibrium. After separation of the phases 40 ml. of the lower layer were introduced into each of the 200 tubes of the apparatus with the exception of tube 0. This tube was filled with solvents and linseed oil as described below. The upper-phase solvent was placed in the reservoir, and the pump, which dispenses the upper solvent into tube 0 at each decantation stage, was adjusted to deliver 2.5 ml. Prior to introduction of the solvents the apparatus was flushed with nitrogen.

Linseed oil was mixed with the hydrocarbon furfural solvents before introducing the system into tube 0 as follows: 15 g. of the oil were placed in a separatory funnel with 5 ml. of the upper solvent. The lower solvent was added with shaking until the volume of the lower phase equalled 40 ml. This immiscible furfural-hydrocarbon-oil system was placed in tube 0, and the automatic distribution process was begun. Because of the effect of high oil concentration on the mutual solubility of the hyper- and hypophasic solvents, the volume of the lower layer tended to increase in the lead tube as the distribution proceeded. This difficulty was counteracted by removing amounts of lower layer just ahead of the lead tube equal to the excess being developed. After a few initial transfers the concentration of oil dropped sufficiently low as not to affect the solvent levels in the tubes. When 200 transfers had been completed, the fraction collector was turned on and the combiner was set to unite eight effluent fractions in each of the collector tubes. Another 600 transfers were then completed. At this point all of the glycerides had passed out of the apparatus into the first 75 tubes of the fraction collector.

Total weight and iodine values were determined for the glyceride fractions in the collector. Since furfural dissolved in the oil-hydrocarbon layer would interfere with evaporations and spectro-analyses, it was first removed by washing the pentane-hexane solutions three times with equal volumes of 75% ethanol. The pentane-hexane solutions were then placed in 25-ml. volumetric flasks and diluted to volume with pentane-hexane. Portions (10-ml.) were transferred to tared 50-ml. Erlenmeyer flasks. After removal of the pentane-hexane under reduced pressure the glycerides in the flasks were obtained by weighing. Iodine values were determined for the glyceride fractions by the 1-hr. Wijs method (7). Since the sample sizes were generally determined by amount in the Erlenmeyer flasks, the volume of reagents was proportionately scaled down to allow proper excesses of Wijs reagent.

At selected points in the distribution curve where the tubes contained sufficient weight of material, the

[1] Presented at the meeting of the American Oil Chemists' Society, Minneapolis, Minn., Oct. 11–13, 1954.
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[3] The countercurrent-distribution apparatus was constructed by the H. O. Post Scientific Instrument Company, Maspeth, N. Y.
[4] The mention of firm names or trade products does not imply that they are endorsed or recommended by the U. S. Department of Agriculture over other firms or similar products not mentioned.

remaining 15 ml. of solution were used for the determination for fatty acid composition of the glyceride fractions. Polyunsaturated acids were determined by the 45-min. isomerization method of Brice *et al.* (1), and calculations were made, using their revised constants. Results are reported as percentages of linolenin and linolein. Olein was calculated by combining spectrophotometric and iodine value data. Saturated glycerides were estimated as the difference between the total glycerides and the summation of the unsaturated glycerides.

The data of Figure 2 were obtained upon the same sample of linseed oil but with the use of a solvent system in which linseed oil glycerides have lower partition coefficients than in the pentane-hexane-furfural system and in which improved resolution of the more saturated glycerides was observed. This solvent system consisted of a mixture of pentane-hexane-furfural-nitroethane in a 2:1:1 ratio. Except for the difference in solvent system and the use of 5 ml. of upper phase solvent, the procedure of this distribution was similar. In this experiment however two aliquots were combined for analysis instead of eight. The portion of the curve corresponding to eight and nine double bond glycerides was only cursorily checked in this experiment, and results are not included in the figure. The results of spectrophotometric analyses on samples of this experiment are represented in Figure 3.

A second sample of linseed oil (I.V. 184.1) was studied for comparative purposes. It was obtained by the cold pressing of flaxseed (Variety, Marine grown in the St. Paul, Minnesota, area and obtained through the courtesy of J. O. Culbertson). The expressed oil was fractionated with pentane-hexane furfural system and analyzed for eight and nine double bond glycerides as described in connection with the data of Figure 1.

Results

Weight and iodine value data are plotted *versus* transfer number in Figure 1. The total weight curve is made up of four component curves, two of which are well resolved, a third of which is partially resolved, and a fourth, large unresolved curve. As indicated by the iodine values, the bell-shaped curve on the left consists of glycerides possessing nine double bonds per glyceride molecule. The next curve to the right must represent glycerides with eight double bonds, and the next peak, Curve 3, must represent glycerides with seven double bonds. The remaining curve represents all of the other glycerides, which have a lesser degree of unsaturation.

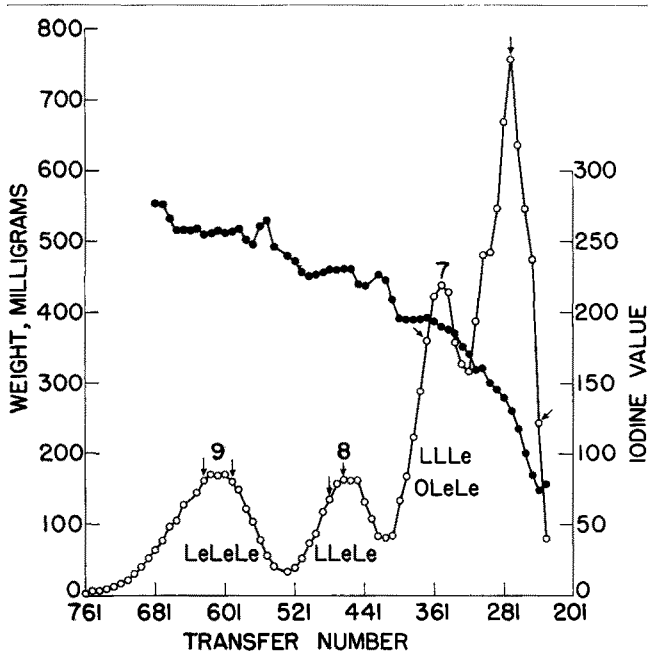

FIG. 1. Countercurrent fractionation of linseed oil glycerides with the pentane-hexane, furfural solvent system.
Open circles—weight curve.
Closed circles—iodine value curve.

Table I lists the results of the analyses performed on the fractions indicated by the arrows on the total weight curve of Figure 1. Spectrophotometric analysis of glycerides in transfer numbers 591–599 and 625–633 from Curve number 9 shows that it contains essentially trilinolenin. This conclusion is confirmed by iodine values of 255.1 and 257.9, which approach the theoretical value of 261.6. Spectrophotometric analysis of glycerides in transfer numbers 465–473 and 481–488 from Curve 8 are calculated within the limits of error of the spectro-iodine value method to be roughly two-thirds linolenin and one-third linolein. The experimental iodine values of 233.0 and 230.5 compare very well with the theoretical iodine value of 232.0 for linoleo-dilinolenin. Spectrophotometric analysis of the glycerides of numbers 369–377 from Curve 7 shows 26.2% olein, 11.3% linolein, and 59.5% linolenin. There are two possible glycerides which contain seven double bonds indicated by the experimental iodine value of 197.7 (theoretical 202.5), namely, dilinoleo-linolenin and oleo-dilinolenin. Calculation of the proportions of these two glycerides which would account for the compositional data are 17.2% dilinoleo-linolenin and 82.8% oleo-dilinolenin. The analyses for the glycerides of transfer numbers 241–249 and 273–281 from the remaining curve show the tendency of the fractions toward the more pentane-hexane soluble portion of the curve to contain less of the highly unsaturated glycerides and more of the saturated types.

TABLE I
Analyses of Fractions from the Countercurrent Distribution of Linseed Oil Corresponding to Figure 1

Curve number	Transfer number	Iodine value	Spectro-iodine value analyses				Triglyceride
			S	O	L	Le	
			%	%	%	%	
9	625-633	255.1	....	8.8	—0.5	94.9	LeLeLe
	591-599	257.9	....	....	—0.8	102.3	
8	481-488	233.0	—1.4	3.7	31.7	66.0	LLeLe
	465-473	230.5	—3.7	10.9	26.2	66.6	
7	369-377	197.7	2.8	26.2	11.3	59.5	LLLe OLeLe
Unresolved	273-281	134.0	13.8	45.5	14.5	26.2	
	241-249	73.7	30.5	59.0	5.8	4.7	

Table II lists the chemically distinguishable glycerides possible by combinations of the four fat acids and the corresponding iodine values. Stearic and palmitic acids are considered together as saturates in the calculations. The percentages of glycerides possible under the monoacid, even, and random patterns of distribution of fatty acids are also listed. Under the monoacid pattern, glyceride composition is identical with the fatty acid composition and allows only four glycerides. The "strict even pattern" distributes the fatty acid in the widest possible manner in the triglycerides. It permits six triglycerides, whose percentages are calculated as previously described (4).

TABLE II
Iodine Value and Calculated Composition of Linseed Oil Glycerides Under Monacid, Even, and Random Distribution Patterns

Glyceride	Number double bonds	Iodine value	Monoacid	Even	Random
			%	%	%
SSS	0	0	8.5		0.1
SSO	1	28.54			0.5
SOO	2	57.21			1.5
SSL	2	57.21			0.3
SSLe	3	86.01			1.1
OOO	3	86.01	24.3		1.4
SOL	3	86.01			1.9
SOLe	4	114.95		10.6	6.5
OOL	4	114.95			2.6
SLL	4	114.95			0.4
OLL	5	144.01			1.6
OOLe	5	144.01			9.3
SLLe	5	144.01		6.5	4.0
SLeLe	6	173.21		8.3	7.0
OLLE	6	173.21		25.2	11.4
LLL	6	173.21	14.9		0.3
OLeLe	7	202.54		36.5	19.9
LLLe	7	202.54			3.5
LLeLe	8	232.00		12.9	12.2
LeLeLe	9	261.60	52.3		14.3

The random pattern is that calculated by statistical probability, accounting for the proportions of the fatty acids present in linseed oil. Under this pattern the number of constitutionally different combinations of four acids is

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

Trilinolenin represented by the area under Curve 9 of the total weight curve corresponds to 18.2% of the total glycerides. Linoleo-dilinolenin represented as the area under Curve 8 is 12.28% of the total glycerides. Curve 7 is estimated to represent 23.6% of the total glycerides. Based on the spectrophotometric analyses for linolenin and linolein in Table I, these glycerides are dilinoleo-linolenin, 4.1%, and oleo-dilinolenin, 19.5%. Table III compares the experimentally determined percentages for certain of the more unsaturated glycerides with those calculated under even and random patterns. Since the difference between the content of linoleo-dilinolenin found experimentally and that calculated by either random or even pattern is less than the experimental error, no conclusion can be drawn from these data as to the type of distribution. However trilinolenin and dilinoleo-linolenin, which are not permitted under the even pattern, were found to be present in amounts close to those calculated by the random pattern. Oleo-dilinolenin, which is permitted under both patterns, was isolated in an amount which compares more closely with the random pattern than with the even pattern.

TABLE III
Comparison of the Experimentally Determined Percentages of Highly Unsaturated Glycerides of Linseed Oil With Those Allowed Under Even and Random Distribution Patterns

Distribution pattern	Trilinolenin	Linoleodilinolenin	Dilinoleolinolenin	Oleo dilinolenin
Even	....	12.9	....	36.5
Random	14.3	12.2	3.5	19.9
Experimental	18.2	12.3	4.1	19.5

The data of Figure 2 obtained with the use of pentane-hexane-furfural-nitroethane solvent system are essentially a further separation of the unresolved portion of Figure 1. If as indicated, the pattern of distribution for linseed oil is random, the unresolved curve of Figure 2 should be comprised of the 16 remaining constitutional glyceride types. While the resolution of these glycerides is still incomplete in the data of Figure 3, certain observations may be made by considering the weight curve, the iodine value curve, and the iodine value of individual glycerides given in Table II. No evidence for glycerides having less than two double bonds is apparent, *i.e.*, iodine values less than 57. According to random theory, these glycerides should comprise only 0.6% of the oil. Failure to detect them therefore is not surprising. The curve composed of glycerides having two double bonds is largely superimposed upon the larger curve for glycerides having three unsaturated groups per glyceride. Glycerides with four double bonds appear as a shoulder of the major peak for glycerides having five double bonds. The curve composed of glycerides having six unsaturated groups is incompletely resolved from the curve for glycerides with seven double bonds.

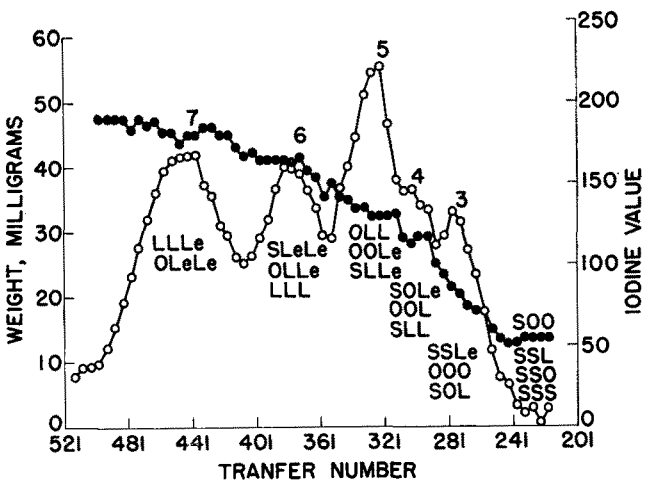

FIG. 2. Countercurrent fractionation of linseed oil glycerides with the pentane-hexane, furfural-nitroethane solvent system.
Open circles—weight curve.
Closed circles—iodine value curve.

The analyses of individual fractions for their fatty acid content are given in Figure 3. Qualitatively the changes observed in fatty acid composition are compatible with the anticipated glyceride compositions

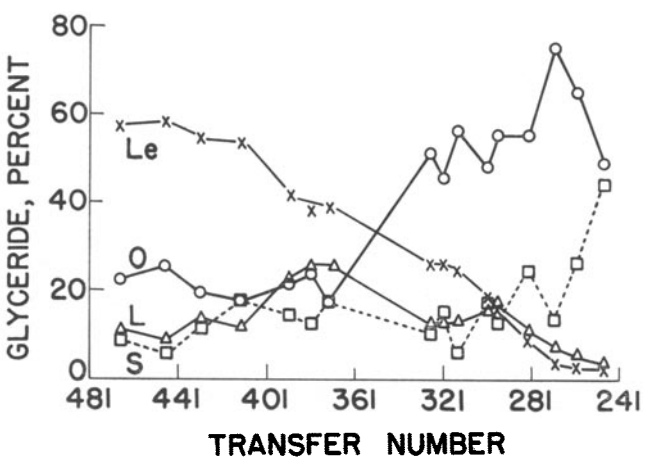

FIG. 3. Fat acid composition of fractions from the countercurrent distribution shown in Figure 2.
S—saturate, O—oleic, L—linoleic, and
Le—linolenic

TABLE IV
Comparison of Experimental Analyses for Fatty Acids with Values Based on the Random Pattern of Distribution

Acid	9 Double bonds		8 Double bonds		7 Double bonds			6 Double bonds		5 Double bonds		4 Double bonds	
	Theory	Exp. 1	Theory	Exp. 1	Theory	Exp. 1	Exp. 2	Theory	Exp. 2	Theory	Exp. 2	Theory	Exp. 2
Saturates	....	....	....	—2.5	....	2.8	11.3	12.7	15.0	9.1	10.4	24.5	14.8
Oleic	....	4.4	....	7.3	28.6	26.2	21.5	20.5	21.0	45.5	51.4	41.2	52.0
Linoleic	....	—0.6	33.3	28.9	10.0	11.3	11.1	21.9	24.4	16.0	12.7	11.9	16.0
Linolenic	100.0	98.4	66.7	66.3	61.4	59.5	55.9	45.0	39.6	29.5	25.5	22.6	17.2

under random pattern. However the incomplete resolution of glyceride types do not justify the attempt to make quantitative calculations of glyceride compositions from the fat acid data, such as was done in Table III. In Table IV are included the spectro-iodine value data for fatty acid composition averaged for the regions of the curve corresponding to 7, 6, 5, and 4 double bond glycerides. Also included are the values calculated from the random distribution pattern data of Table II. Agreement for these fractions of the theoretical and experimental data (within the limits of experimental error) supports the random distribution hypothesis for this sample of linseed oil.

Another manner of representing glyceride composition data and of comparing theoretical glyceride patterns with experimental data is shown in Figure 4. It consists of calculating iodine value 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. It is apparent that the combined experimental data of Figures 1 and 2 follow closely the limits imposed by the random pattern but exceed the limits imposed by the strict even distribution pattern. In fact, the deviation of the experimental data from the random pattern lines appears to be wholly accounted for by the fact that the summed and averaged iodine values for the fractions recovered is 176 rather than 183.5 determined on the whole oil.

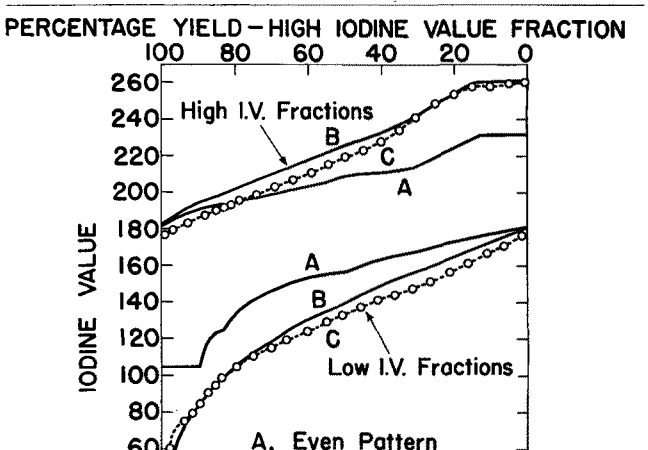

FIG. 4. Iodine value—percent yield representation for A. Even pattern; B. Random pattern; C. Counter-distribution data of Figures 1 and 2.

Evidence for the generalization that linseed oil is randomly constructed is to be found in the counter-current-distribution data on the expressed sample of linseed oil. From this oil of iodine value 184.1, 19.6% of trilinolenin was isolated. Consideration of the freshness of this oil sample, the low temperature of the process for expressing the oil, and the slow rate of the uncatalyzed interesterification reaction at room temperature leads to the conclusion that the random distribution pattern found in the data of Figures 1 and 2 are not an artifact but describe the structure of linseed oil glycerides in the natural and native state.

Summary

Linseed oil has been fractionated in a 200-tube countercurrent-distribution apparatus. Iodine values of fractions ranged from 51 to 261. As determined by the weight distribution curve, iodine values and spectrophotometric analyses, 18.2% trilinolenin, 12.3% linoleo-dilinolenin, and 19.5% oleo-dilinolenin combined with 4.1% dilinoleo-linolenin were isolated. Based upon this type of data and upon several methods of analysis and collation of the data, it is concluded that linseed oil glycerides follow essentially the random pattern of distribution.

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Letter to the Editor

September 5, 1955.

For further information on *Acrocomia Totai* (the Mbocaya palm) which K. S. Markley has reported so correctly in the July, 1955 edition of the Journal, I would like to add my experience with these products in Paraguay, where I worked under contract during the years 1939 and 1940 for one of the largest local factories and where I had the opportunity of processing thousands of tons of this seed.

The reader may be interested to know that the nature of the "pulp" is such that, contrary to expectations, processing in continuous presses of the Anderson and Rosedown type was more effective and gave a higher yield without the addition of hull than with it. When the hull was used, with or without ground mixtures, a lower yield was obtained and the oil was redder and contained more free fatty acid. At the second pressing, by either the continuous or hydraulic process, the residual oil in the cakes was never under 8%. The "kernel" was very easily handled, and notwithstanding its high oil content (sometimes up to 65%) the oil content in the cake after