Glyceride Structure of Vegetable Oils by Countercurrent Distribution. VI. Corn Oil[1]

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Corn oil has been fractionated in a 200-tube countercurrent distribution apparatus. Although this technique gives no information about the positional isomers of the glycerides, the fatty acid composition of the fractions and the amounts of the more unsaturated triglycerides are in agreement with an essentially random pattern.

COUNTERCURRENT DISTRIBUTION is an effective technique for the fractionation of glycerides of liquid oils. By this procedure the distribution of the fatty acids among the glyceride molecules of linseed, soybean, and safflower oils (1,2,3) has been shown to follow essentially a random pattern. This conclusion stands in conflict with the "rule of even distribution" (4), previously thought to constitute the generalized pattern for vegetable oils and fats. With a more saturated fat, cocoa butter, the previous conclusion of a deviation from random distribution has been confirmed (5), and a 1,3 random 2-olein pattern proposed (6).

Corn oil has been separated by Doerschuk and Daubert (7) into 19 fractions with a crystallization procedure. They concluded that the glyceride structure is described most closely by a "partial random" system. More recently Takeda *et al.* (8) measured the classes of saturated and unsaturated glycerides by the Kartha procedure. Their compositions corresponded closely to those calculated for a random distribution. The results of the countercurrent distribution described in this paper also indicate that corn oil, like other liquid oils examined in this series, approaches a random pattern.

Experimental

Fresh, wet, corn germ was dried at 45°C. in a forced-draft oven. Oil was pressed from the dried germ, and the press cake was extracted with pentane-hexane. High temperatures were avoided in preparing the oil. Since both the pressed and extracted oil had similar fatty acid composition, as determined by gas chromatography of their methyl esters, the two portions were combined. The combined fraction had the following fatty acid composition, as measured by gas chromatography: palmitic acid 10.0%, stearic acid 2.7%, oleic acid 26.7%, linoleic acid 60.3%, and linolenic acid 0.3%. These values were used in the subsequent calculations of theoretical glyceride composition. The Wijs iodine value (9) of the oil was 126.3. From this value and the alkali isomerization data (10) the fatty acid composition was calculated as: linoleic 58.1%, oleic acid 29.8%, and saturated acids 12.1%.

Ten grams of corn oil were fractionated in a 200-tube automatic countercurrent distribution apparatus, using pentane-hexane as the upper layer and furfural-nitroethane (1:1) as the lower layer. The distribution was carried out in the same way as previously described for safflower oil (3). Based upon the weight curve (Figure 1), the contents of collection tubes were combined in 20 fractions as shown in the upper half of Figure 1. These fractions were converted to methyl esters, using sodium methoxide catalyst. The fatty acid composition of these esters is also given in Figure 1.

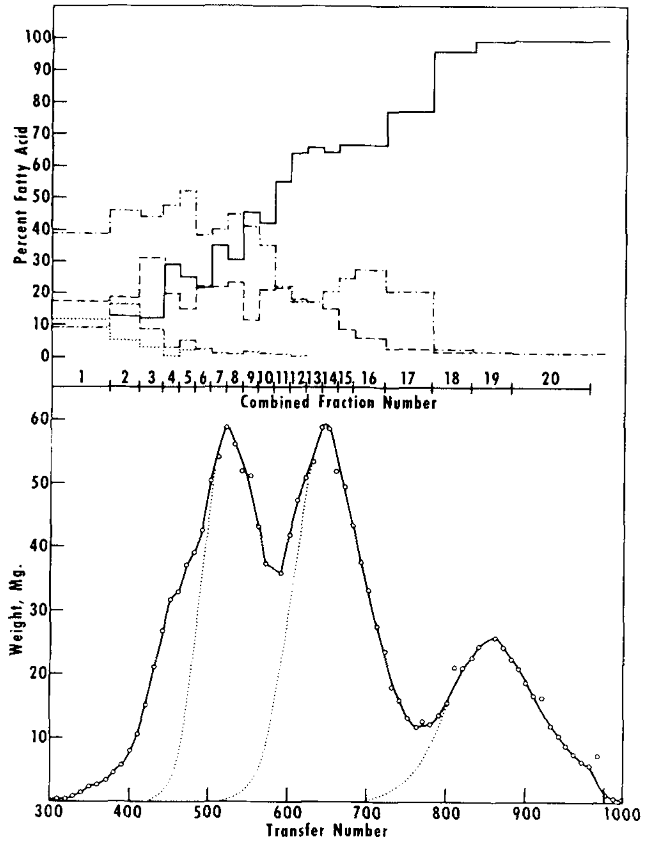

FIG. 1. Countercurrent fractionation of corn oil glycerides with a pentane-hexane, furfural-nitroethane solvent system. Lower half: weight curve. Upper half: fatty acid analysis of combined fractions. Dotted line represents C_{20} saturated acids; diagonal dash-dot, stearic acid; dash, palmitic acid; dash-dot, oleic acid; and solid line, linoleic acid.

Discussion

In Table I are shown the amounts of the different triglycerides calculated for a random distribution. The glycerides are grouped in the order of their partition coefficients. This order corresponds to the order of their appearance in the fraction collector. Palmito-glycerides have only slightly higher partition

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coefficients than the corresponding oleo-glycerides (3), and they are found in the same fractions.

TABLE I

Glyceride Composition of Corn Oil Under a Random Distribution

Group	Glyceride	%	Group	Glyceride	%
0	SSS	0.00	5	PPLe	0.01
				POLe	0.04
1	SSP	0.02		PLL	9.97
	SSO	0.06		OLL	30.08
				OOLe	0.07
2	SPP	0.07		SLLe	0.03
	SPO	0.41			
	SOO	0.64	6	PLLe	0.10
	SSL	0.14		LLL	22.43
				OLLe	0.30
3	PPP	0.07		SLeLe	0.00
	PPO	0.66			
	POO	1.99	7	PLeLe	0.00
	PSL	0.91		OLeLe	0.00
	OOO	2.00		LLLe	0.33
	SOL	2.74			
	SSLe	0.00	8	LLeLe	0.00
4	PPL	1.48	9	LeLeLe	0.00
	POL	8.92			
	PSLe	0.01			
	OOL	13.44			
	SLL	3.07			
	SOLe	0.01			

As seen in Figure 1, the weight curve for the corn oil sample consists of three maxima corresponding to glycerides containing 6, 5, and 4 double bonds and of a shoulder on the side of the Group 4 maximum corresponding to the more saturated glycerides. The areas under these maxima are divided in the same way as was done previously for safflower oil, *i.e.*, the areas are divided by drawing the theoretical curves down the left side from the peaks by dotted lines under the weight curve. These areas correspond to 20.8% under the Group 6 maximum, 37.3% under Group 5, and 29.7% under Group 4. These values are quite similar to those calculated for a random distribution: Group 6, 22.8%; Group 5, 40.2%; and Group 4, 29.9%.

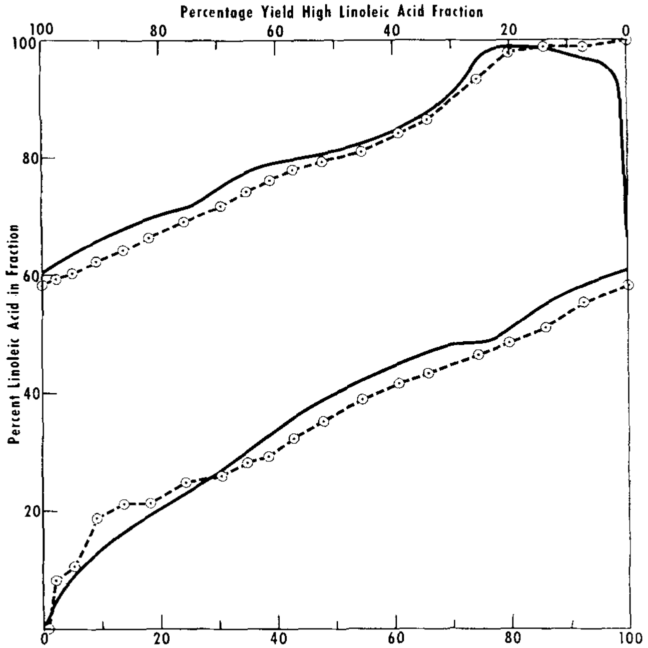

FIG. 2. Linoleic acid percentage yield representation for random pattern (solid line) and for countercurrent distribution data (dash line) of Figure 1.

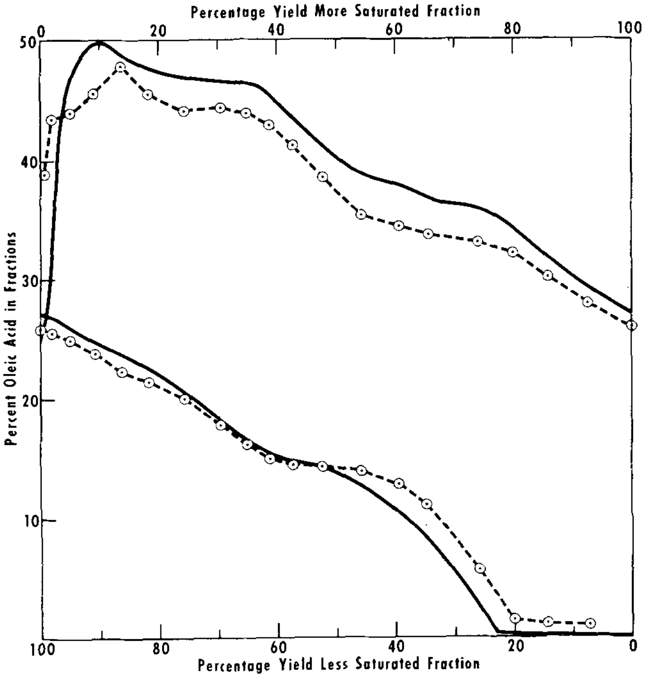

FIG. 3. Oleic acid percentage yield representation for random pattern (solid line) and for countercurrent distribution data (dash line) of Figure 1.

The amounts of individual glycerides in the 20 combined fractions may be calculated from the fatty acid composition in simpler cases. In Fractions 17 through 20 (upper section of Figure 1) if the oleic and palmitic acids are assigned to LLO and LLP, 22.0% of LLL remains. Again, this percentage is similar to the amount expected for a random distribution.

Calculating the individual glycerides in Group 5 is more difficult, especially in the fractions where Group 5 and 4 glycerides overlap. Stearic acid glycerides are present, and the number of glycerides is so large that it has not been practical to calculate the individual glycerides even by approximate methods. However an estimate may be made, based upon the composition of the fractions near the maximum, as was done with safflower oil. Based upon the composition of the combined Fractions 13, 14, 15, and 16, the oil is estimated to contain 24.4% OLL and 13.1% PLL. The accuracy of this estimate is limited because there is some fractionation of palmito- and oleo-glycerides and the fatty acid composition of the fractions chosen may not be representative of the total Group 5 glycerides.

Another method of comparing fatty acid composition with that expected under a calculated pattern is shown in Figures 2, 3, and 4. It consists of dividing the oil into more and less saturated fractions and in plotting the fatty acid composition of these fractions against the proportion they represent of the whole oil. A similar method was used with soybean and linseed oil fractions, using iodine value instead of fatty acid composition (1,2). The curves for linoleic and oleic acids in corn oil follow closely those calculated for a random distribution. Because the amount of palmitic acid is smaller, there are larger errors in its measurement. For this reason the difference is larger between the amount recovered in the combined fractions and the amount in the original

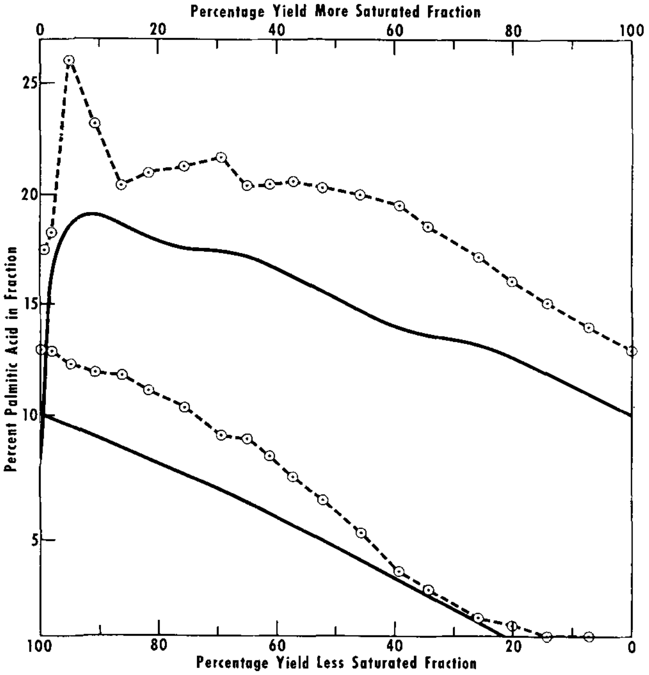

FIG. 4. Palmitic acid percentage yield representation for random pattern (solid line) and for countercurrent distribution data (dash line) of Figure 1.

oil. This difficulty would be even greater with stearic acid, and the data are not calculated.

The largest deviations from the calculated random distribution curves are in the more saturated fractions. These are the fractions which pass through the countercurrent instrument most rapidly and receive the least fractionation. Although there may be some variation from random distribution in the more saturated fractions, the data from these figures do not provide evidence for it. It is quite possible that the deviations from the random curves are caused by insufficient fractionation in this region.

Recent work (11), using selective hydrolysis of 1 and 3 position fatty acids by pancreatic lipase, has shown that in a number of vegetable oils the arrangement of the fatty acids in the individual glyceride molecules is not random. In general, the greatest departure from randomness was found in the location of the saturated acids. Although corn oil was not included in this study, it may be pointed out that, in liquid oils which contain only small amounts of saturated acids, the amounts of the more highly unsaturated, constitutionally different glycerides may approach quite closely those calculated for a strict random distribution. Further it has been pointed out by Vander Wal that, in a random fat, "long-chain saturated and unsaturated groups become associated by chance in those triglyceride molecules which are formed but are not distributed within each molecule at random" (12).

The departure of our corn oil analysis from the partial random distribution pattern suggested by Doerschuk and Daubert (7) is most clearly evident in the trilinolein content. The fatty acid compositions of the two corn oils investigated here and by Daubert are similar. Under a partial random distribution we should find a value near their calculated figure of 0.8% for trilinolein. Actually 22.0% was found, which is comparable to the value of 22.8% calculated for a random distribution.

This difference in results is attributed to the merits of countercurrent distribution and its inherently high resolving power for this type of problem. Even in a precise and exhaustive solvent crystallization, such as Doerschuk and Daubert carried out, the choice of crystallization conditions is empirical; there is no simple predictable relationship among the fractions, and uncertain assumptions must be made as to the glycerides present in the individual crystallization fractions. With countercurrent distribution there is a much larger number of equilibration stages and of fractions obtained, and the mathematical relationships between partition coefficients and fractions are an invaluable aid in the interpretation of results.

It should be emphasized however that, despite the high resolving power of countercurrent distribution, this technique does not separate glyceride isomers with respect to the position of the attachment of fatty acids on the glycerol molecule. Indeed there is good evidence that saturated acids in soybean, olive, peanut, and cottonseed oils are specifically positioned within the glyceride molecule (11). The application of selective enzymatic hydrolysis to the compositionally distinguishable glycerides separated by countercurrent distribution constitutes a promising area for future research.

Acknowledgment

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