# Low-Temperature Solvent Fractionation of Corn Oil and Calculation of Its Glyceride Structure[1,2]

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## Introduction

A QUANTITATIVE knowledge of the composition of fats and glyceride oils is one of the basic essentials fundamental to an understanding of the behavior of this group of naturally occurring substances. Such analytical data, when complemented by independently determined properties of the several constituents, can serve to predict the behavior and properties of the natural fat or glyceride-oil mixture. Adequate tools for the determination of fatty-acid compositions exist (1); the fact, however, that fats and oils are composed of glycerides rather than fatty acids imposes important limitations on the value of fatty-acid analyses as a means of understanding the properties of fats and oils.

In recent years the problem of the separation and identification of the component glycerides of naturally occurring fats and oils has been investigated, and a number of techniques have been applied. Stepwise partial-hydrogenation experiments and oxidation studies of the Bertram type have yielded considerable information as to the presence of certain classes of glycerides (2). Molecular distillation has been found inadequate (3); a more promising approach is embodied in the precipitation of the glyceride components from a dilute oil solution over a temperature range extending to the temperature of dry ice (4). Riemenschneider (5, 6, 7), Jack and Henderson (8, 9, 10), Hilditch (2, 11, 12, 13), and others have applied various modifications of this procedure to a number of fats and oils and have accomplished a very considerable fractionation of the glyceride components.

To ascertain further the efficacy of low-temperature fractionation procedures for the determination of the fatty-acid distribution in vegetable oils, corn oil, because of its relatively simple fatty-acid content,[3] was subjected to precise low-temperature fractionation. The purpose of this paper, therefore, is three-fold: 1. to study the effect of low-temperature solvent fractionation on corn oil and to characterize the fractions obtained; 2. to elaborate a scheme that will describe the manner in which the various glycerides in the oil are distributed among the fractions and that will restrict the composition of the individual fractions sufficiently to allow a calculation of the glyceride structure directly from the fatty-acid composition of the fractions without the postulation of a mode of fatty-acid distribution; 3. to compare the glyceride structure of the total oil so obtained to the structures calculated by means of the various fatty-acid distribution schemes.

[1] The generous financial assistance of the Corn Products Refining Company, Argo, Illinois, is gratefully acknowledged.

[2] Contribution from the Department of Chemistry, University of Pittsburgh.

[3] Detailed determinations of the fatty acid composition of different corn oil specimens have been accomplished by three independent investigators; a table correlating the three sets of data is available (14). The results of our spectrophotometric analyses for trienoic and dienoic materials and our difference calculations for monenoic and saturated materials (calculated as linolenic, linoleic, oleic, and saturated acids, respectively) are presented in Table II.

## Experimental

A hexane-extracted corn oil prepared especially for this study through the courtesy of A. Richard Baldwin of the Corn Products Refining Company was used. Residual hexane was removed from the oil under reduced pressure at room temperature with a slow stream of dry nitrogen. Constants for the oil are given in Table II.

### A. Acetone-Insoluble Fraction.

It was found that a ratio of 1 to 6 between weight of oil in g. and volume of acetone in ml. insured nearly complete precipitation of the acetone-insoluble material in the oil; solution of the acetone-insoluble material in ethyl ether and filtration yielded a small quantity of ether-insoluble material. This material was insoluble in methanol, ethanol, and low-boiling petroleum ether; it was not investigated further. Removal of the ethyl ether from the filtrate under vacuum at 0° C. gave a light orange-brown solid identified as phosphatides. Several determinations gave a phosphatide concentration of 0.50 ± 0.02%; later developments indicated that this figure was slightly low since the first steps in the low-temperature fractionation procedure yielded small amounts of phosphatidic material (P-1 and P-2), Table II. Removal of the acetone from the oil under vacuum with a slow current of dry nitrogen at about 25° C. yielded a product of iodine value 126.5.

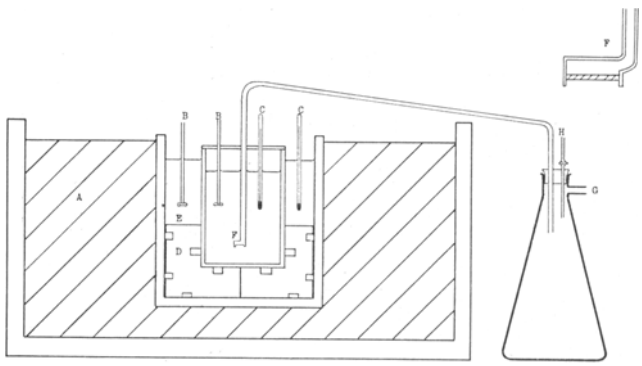


Fig. 1. Small crystallizer.

A. Asbestos insulation
B. Stirrer
C. Thermometer
D. Perforated tin support
E. External acetone bath
F. Filtering device
G. To vacuum
H. To atmosphere

### B. Low-Temperature Fractionation of the Oil.

The experimental determination of the glyceride structure of the oil involves the operation of a process capable of separating the glyceride components to a degree such that fractions of composition markedly simpler than that of corn oil itself are obtained. Low-temperature solvent precipitation was selected as the separation process; acetone was chosen as the solvent because of the marked change in its solvent properties for vegetable oils that occurs between room tem-

TABLE II

Characteristics of the Fractions

| Number of Frac. | (I) Expt. Wt. of Frac. | (II) Wt. of Frac. to 1,000 g. Corn Oil | (III) % Unsap. of Frac. | (IV) Wt. Unsap. of Frac. | (V) Glyceride Wt. of Frac. | (VI) I. V. of Frac. | (VII) I. V. of Unsap. | (VIII) I. V. of Glycerides |
|---|---|---|---|---|---|---|---|---|
| P-1 | 0.8g. | 0.8g. | 4.72 | 0.04g. | 0.0g. | 72.6 | 68.1 | ..... |
| P-2 | 0.5 | 0.5 | 4.61 | 0.02 | 0.0 | 77.5 | 77.2 | ..... |
| 1 | 5.4 | 5.5 | 2.80 | 0.15 | 5.4 | 56.5 | 66.6 | 56.2 |
| 2 | 13.8 | 14.1 | 1.98 | 0.28 | 13.8 | 59.3 | 85.5 | 58.9 |
| 3 | 4.5 | 4.6 | 3.44 | 0.16 | 4.4 | 70.3 | 57.0 | 71.4 |
| 4 | 9.6 | 9.8 | 5.48 | 0.54 | 9.3 | 90.3 | 62.4 | 91.5 |
| 5 | 24.6 | 25.1 | 1.35 | 0.34 | 24.8 | 107.7 | 100.6 | 107.7 |
| 6 | 62.6 | 63.8 | 1.78 | 1.14 | 62.7 | 103.8 | 89.6 | 104.0 |
| 7 | 101.4 | 103.2 | 1.17 | 1.21 | 102.0 | 114.5 | 102.1 | 114.6 |
| 8 | 19.9 | 20.3 | 1.60 | 0.32 | 20.0 | 121.5 | 103.5 | 121.7 |
| 9 | 114.9 | 117.1 | 0.92 | 1.08 | 116.0 | 116.9 | 41.7 | 117.6 |
| 10 | 84.1 | 85.6 | 1.14 | 0.98 | 84.6 | 132.4 | 73.1 | 133.1 |
| 11 | 101.5 | 103.3 | 1.96 | 2.02 | 101.3 | 138.8 | 91.0 | 139.7 |
| 12 | 171.9 | 175.1 | 1.80 | 3.15 | 171.9 | 144.2 | 80.0 | 145.4 |
| 13 | 101.7 | 103.6 | 1.08 | 1.12 | 102.5 | 138.7 | 129.8 | 138.8 |
| 14 | 3.7 | 3.8 | 17.8 | 0.68 | 3.1 | 118.7 | 105.1 | 122.5 |
| 15 | 14.0 | 14.3 | 1.90 | 0.27 | 14.0 | 123.5 | 104.0 | 124.1 |
| 16 | 21.2 | 21.6 | 1.14 | 0.25 | 21.3 | 108.1 | 99.8 | 108.5 |
| 17 | 4.7 | 4.8 | 1.32 | 0.06 | 4.7 | 130.9 | 126.3 | 132.1 |
| 18 | 17.5 | 17.8 | 1.58 | 0.28 | 17.5 | 116.5 | 81.7 | 117.2 |
| 19 | 103.4 | 105.3 | 4.66 | 4.91 | 100.4 | 133.2 | 70.0 | 136.3 |
| Total | 981.7 | 1,000 | ..... | 19.00 | 979.6 | ..... | ..... | ..... |
| Corn Oil | 1,000 | ..... | 1.95 | 19.5 | 980.5 | 126.5 | 97.1 | 127.1 |

| Number of Frac. | (IX) Sap. Equiv. of Frac. | (X) Sap. Equiv. Corrected to Glycerides | (XI) % Linolenic Acid of Glycerides | (XII) % Linoleic Acid of Glycerides | (XIII) % Oleic Acid of Glycerides | (XIV) % Saturated Acid of Glycerides | (XV) Melting Point of Fraction (°C.) | (XVI) $[n]_D^{40°C.}$ of Frac. |
|---|---|---|---|---|---|---|---|---|
| P-1 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| P-2 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 1 | 305.5 | 296.9 | ..... | 23.5 | 15.1 | 57.0 | +31 to +32 | 1.46133 |
| 2 | 303.5 | 296.5 | ..... | 26.1 | 12.9 | 56.6 | +15 to +16 | 1.45965 (Min.) |
| 3 | 305.9 | 295.2 | ..... | 31.6 | 15.8 | 48.2 | +10 to +11 | 1.46178 |
| 4 | 308.0 | 291.4 | ..... | 37.6 | 25.8 | 32.2 | − 1.5 to − 1.0 | 1.46423 |
| 5 | 299.8 | 295.6 | ..... | 49.5 | 20.1 | 26.0 | −18 to −17 | 1.46583 |
| 6 | 300.5 | 295.2 | ..... | 49.0 | 17.0 | 29.6 | −17 to −16 | 1.46520 |
| 7 | 297.1 | 293.7 | ..... | 60.5 | 5.6 | 29.5 | −17 to −16 | 1.46578 |
| 8 | 297.1 | 292.2 | 0.17 | 63.2 | 7.5 | 24.7 | −25 to −23 | 1.46650 |
| 9 | 297.0 | 294.0 | 0.16 | 59.2 | 11.1 | 25.1 | −27 to −26 | 1.46643 |
| 10 | 295.5 | 292.1 | 0.23 | 61.0 | 24.5 | 9.9 | −25 to −24 | 1.46853 |
| 11 | 297.7 | 292.0 | 0.41 | 62.0 | 29.3 | 3.9 | −31 to −30 | 1.46923 |
| 12 | 297.7 | 292.3 | 0.52 | 64.7 | 29.8 | 0.6 | −28 to −26 | 1.46935 |
| 13 | 296.4 | 293.1 | 0.56 | 61.8 | 28.3 | 4.9 | −10 to − 8.5 | 1.46945 |
| 14 | 281.0 | 231.0 | 1.06 | 41.6 | 49.3 | 3.6 | +38.5 to +39.5 | 1.47483 |
| 15 | 298.8 | 293.1 | 8.18 | 40.9 | 30.7 | 15.8 | +15 to +16 | 1.47563 |
| 16 | 295.4 | 292.0 | 6.06 | 33.0 | 35.7 | 20.8 | −10 to − 9 | 1.47735 (Max.) |
| 17 | 299.0 | 295.0 | 0.92 | 50.2 | 43.0 | 1.5 | − 5 to − 4 | 1.47583 |
| 18 | 301.0 | 296.2 | 0.89 | 44.6 | 37.8 | 12.3 | + 3 to + 4 | 1.47423 |
| 19 | 306.4 | 292.1 | 0.91 | 61.3 | 25.4 | 8.0 | −24 to −22 | 1.47305 |
| Total | ..... | ..... | 5.88 g. | 569.4 g. | 219.6g. | 140.8 g. | ..... | ..... |
| Corn Oil | 297.6 | 292.0 | 0.56 | 59.0 | 20.9 | 15.1 | −19 to −18 | 1.46725 |

perature and temperatures attainable with dry ice.

a. *Preliminary Studies.* For preliminary work in determining the behavior of corn oil towards this type of fractionation, a small crystallizer, capable of handling up to four liters of solution, was constructed (Fig. 1). Pulverized dry ice was added to the external acetone bath until it attained the desired temperature; generally a period of 1½ to 2 hours was required for the internal container to attain the temperature. No dry ice was added directly to the solution being studied in the internal container, nor was the temperature of the external container ever taken below the desired final temperature. Approaching the heterogeneous equilibrium always from the warm side avoided the necessity for re-solution of material deposited from parts of solution locally cooled below the desired temperature. The stirrer in the internal container was set for a high speed to minimize supercooling; the device allowed temperature regulation to ± 0.1° C. in the internal container.

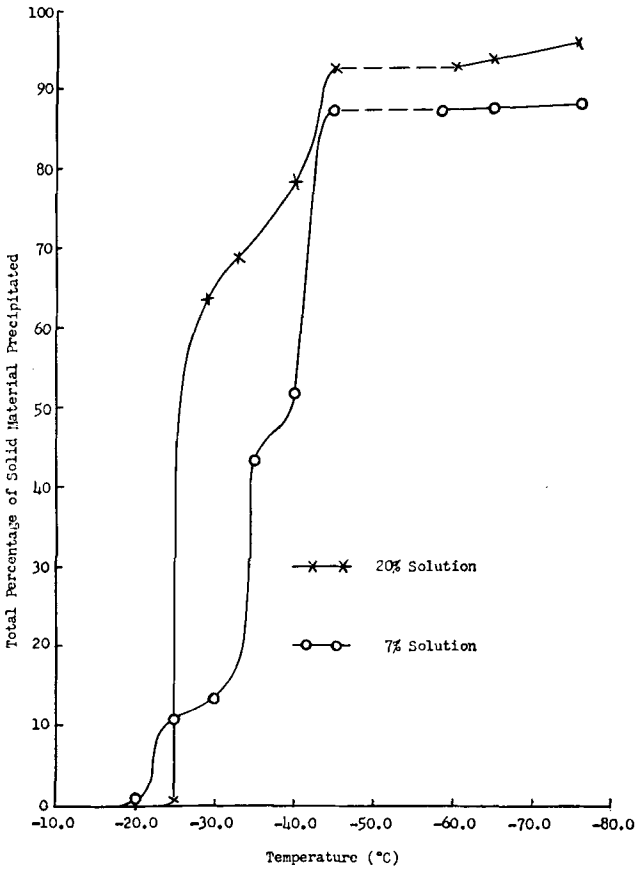
Hilditch and Maddison's work on cottonseed oil of iodine value 105.0 suggested an initial concentration of 30% (11); several runs, involving different filtering techniques, were made, but quantitative filtering and washing of the type of precipitate deposited from this strength solution of corn oil was not found possible. The solution strength was then changed to 20%, and a number of fractionations were made to determine the best method of filtering. It was desired to filter directly from the controlled-temperature confines of the crystallizer under conditions affording a minimum disturbance of the equilibrium attained and to avoid the technique sometimes used wherein the contents of the crystallizing vessel are emptied into a Büchner funnel and suction-filtered in the usual way. After several trials a coarse-grade, sintered-glass funnel 1½ cm. in diameter, covered with a piece of medium fine filter paper cut to fit snugly against the glass, was selected. After a two-hour equilibration period, the internal stirrer was stopped and half an hour allowed for settling; the filtering device, precooled in a separate bath, was immersed a short distance beneath the liquid's surface and gradually lowered as the filtration proceeded. The precipitate was filtered dry, packed around the filter, and washed twice with acetone precooled 10 C°. below the equilibration temperature. With the 20% solution a turbidity was first noted at —13.5° C.; but filtering at —20.0° C. yielded no precipitate, and further observations showed that a separation of the liquid corn oil from solution to form a two-liquid system had occurred rather than a deposition of solid. The first solid precipitate was obtained at —25.0° C.

The fractionation process was followed by decreasing the temperature through successive 5 C°. decrements and filtering to separate the precipitate formed

during each temperature interval. After each filtration the solution was returned to room temperature and the acetone added during the course of washing removed under vacuum with a current of dry nitrogen at 30° C. Adjustments were made so that the total volume of solution at room temperature was maintained constant at one liter, leaving temperature as the only independent variable changed. The temperature variation exerted its effect through a decrease in solvent properties of the liquid phase, through a concentration decrease resulting from glyceride precipitation into the solid phase, and through a concentration increase resulting from solution shrinkage. The net effect was a complex interplay among these three factors, but the readjustment of the solution to one-liter volume at room temperature after each precipitation and filtration lent continuity to the entire series of observations in that the cooling process for each run was begun always from the same room-temperature volume. Hence in the —35.0° C. run the solution, when it passed through the —30.0° C. temperature point, was in about the same state as was the supernatant liquid in the —30.0° C. run; and the material which precipitated from —30.0° C. to —35.0° C. in the —35.0° C. run was the same material which would have precipitated if the run at —30.0° C. had been extended to —35.0° C. without the filtration and washing. This lent a systematic character to the investigation which would not have been present had the solute-solvent ratio been varied more indiscriminately and which permitted the construction of graphs of properties of the fractions against temperature only. These graphs were of use in establishing the fact that fractionation was being accomplished and in selecting the most advantageous temperatures in the later, larger scale crystallizations.

The results of the fractionation of one liter of the 20% solution are presented in Table I and Graphs I and II. Several comparisons made between properties of the oil itself and the sums of properties of the fractions are presented in Table VI-A. As the iodine values and some further studies of the solution at —20° C. in a transparent container indicated, the important phenomenon occurring in the 20% solution was not the relatively small degree of fractionation but rather the separation of three-fifths of the oil in the liquid form and the solidification, rather than the fractionation, of the separated oil in the interval between —25.0° C. and —29.0° C. The situation, with liquid separating rather than solid depositing out of a 20% solution, is not common; Hilditch and Maddison (12), working with a 20% solution of olive oil in acetone, reported a solid fraction at —10° C. with no mention of liquid separation, and a brief experiment carried out here with a 40% solution of cottonseed oil in acetone gave a solid fraction at —15°C. with no previous liquid separation. The close approximation of the iodine value of the separated liquid to the iodine value of corn oil itself suggested that liquid separation could be avoided by subtracting the weight of the separated portion from the amount of oil used in preparing the next solution; consequently the next run was made on a liter of a 7% solution.

The results of the fractionation of the 7% solution, which showed turbidity first at —10.0° C., are presented in Table I and Graphs I and II. Comparisons similar to those made on the 20% solution are presented in Table VI-A. A comparison of both the weight *vs.* temperature and iodine value *vs.* temperature graphs for the 20% and the 7% solutions show that the fractionation is very much greater in the case of the 7% solution. The interval of no precipitation which occurred in the lower temperature regions of both the 20% and the 7% solutions is of interest; it might represent an interval of supercooling, despite the rapid stirring and two-hour equilibration period, or it might represent a separation of




GRAPH I

TABLE I

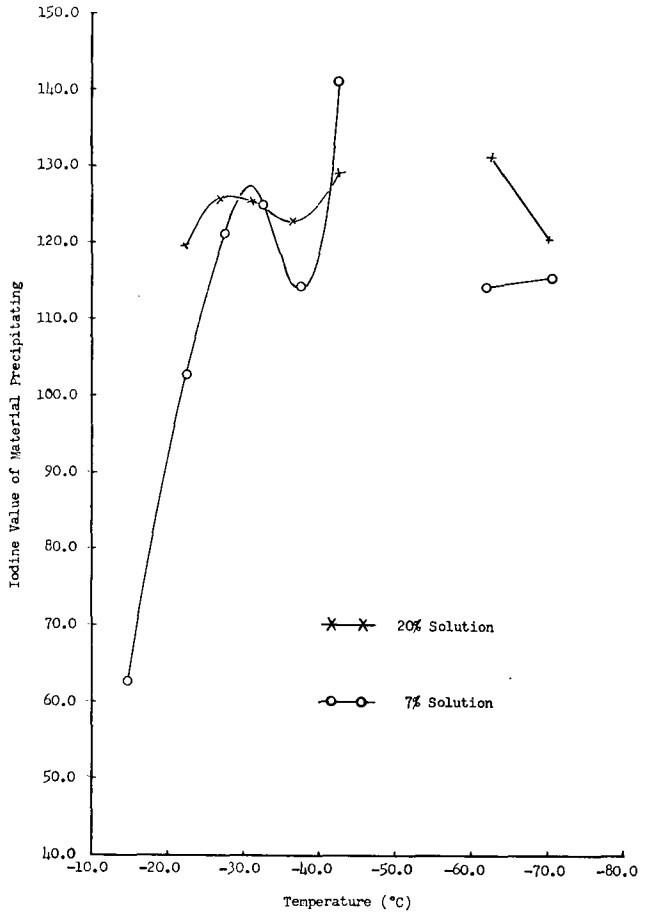
Preliminary Studies of Solvent Fractionation

20% Solution of Corn Oil (1 Liter)

| T(°C.) | I. V. | Weight(g.) |
|---|---|---|
| —20.0 to —25.0 | 119.3 | 1.3 |
| —25.0 to —29.0 | 125.6 | 126.1 |
| —29.0 to —33.0 | 125.3 | 10.4 |
| —33.0 to —40.0 | 122.8 | 18.8 |
| —40.0 to —45.0 | 129.1 | 28.7 |
| —45.0 to —60.2 | No precipitate | ..... |
| —60.2 to —65.0 | 131.4 | 2.5 |
| —65.0 to —75.5 | 120.7 | 4.0 |
| Residue | 145.5 | 7.5 |
| | | 199.3 |

7% Solution of Corn Oil (1 Liter)

| T(°C.) | I. V. | Weight(g.) |
|---|---|---|
| —10.0 to —20.0 | 62.6 | 0.3 |
| —20.0 to —25.0 | 102.6 | 7.3 |
| —25.0 to —30.0 | 121.0 | 1.8 |
| —30.0 to —35.0 | 125.0 | 21.0 |
| —35.0 to —40.0 | 114.2 | 5.9 |
| —40.0 to —45.0 | 141.1 | 24.9 |
| —45.0 to —58.5 | No precipitate | ..... |
| —58.5 to —65.0 | 114.4 | 0.2 |
| —65.0 to —76.0 | 115.6 | 0.3 |
| Residue | 132.2 | 7.5 |
| | | 69.2 |




GRAPH II

the oil into two sets of components on the hypothesis that any glyceride precipitating into both the higher temperature division and the lower temperature division would have been observed precipitating into the precipitate-free region in between.

b. *Full-scale Crystallization Studies.* The final study on corn oil, using 1,000 g. of oil in 7% acetone solution (14.29 l.), was made using a larger crystallizer (Fig. 2), capable of handling this quantity at one time and of maintaining a constant temperature for 48 hours. Using a thermostatically controlled fan to force the circulation of air used as the heat transport medium between two insulated chambers, one containing dry ice and one containing the jar with the solution, it offered a temperature regulation of ± 0.10° C. at temperatures of —25° C. or lower. Periods of from 5 to 10 hours were required to cool the 14.29 l. to the various temperatures. A 24-hour equilibration period, during which time the solution was stirred at the given temperature, was followed by a 2-hour settling period with no stirring. The filtering device, a modified Büchner funnel (Fig. 2), was clamped in place above the solution during the cooling process for temperature equilibration; a fine grade of filter paper was used. The fractions were refractionated in the small crystallizer.

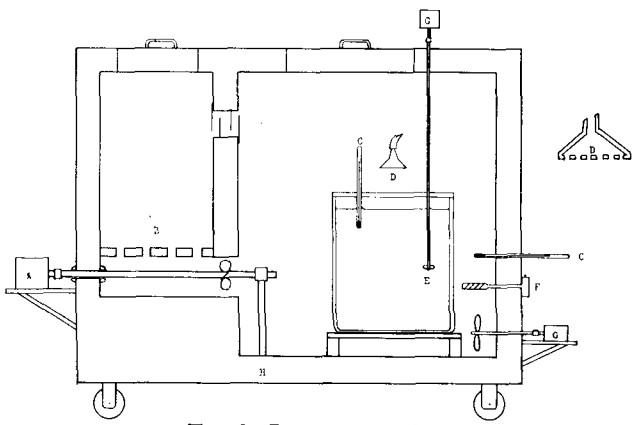


FIG. 2. Large crystallizer.

A. Thermostatically controlled motor
B. Dry ice
C. Thermometer
D. Modified Büchner
E. Stirrer
F. Thermoregulator and relay
G. Motor
H. Glass wool insulation

A diagrammatic representation of the total fractionation is given in Figure 3. The major fractions (a, b, c, etc.) were all precipitated from a constant volume of solution of 14.29 l. in order to make the process consistent with the preliminary studies on the 7% corn oil solution, allowing the use of that data in determining the temperatures most advantageous from the standpoint of separation. For Fraction a (Fig. 3), —26.0° C. was chosen because it allowed the precipitation of most of the more saturated material and because the temperature regulation of the large crystallizer was markedly better below —25.0° C. For Fractions b and c, —34.0°C. and —42.0°C. were chosen because they represent maximum rate of change in iodine value of material precipitating with respect to temperature. For Fraction d, —45.0° C. was chosen and no carry-over was made from Fraction d to Fraction e in order to maintain separately the glycerides precipitating on opposite sides of the no-precipitate interval. Precipitation of Fraction e was accomplished at —78.5° C., the lower limit of the device since the entire interval from Fraction d to —78.5° C. represented only a very small quantity of material.

The combinations of concentration and temperature used for the refractionations in the small crystallizer of the major fractions were more arbitrary, although not completely so. The scheme actually presented on the crystallization diagram is the result of a much larger number of attempts to find procedures yielding greater degrees of separation between successive fractions. In the case of Fraction a the final fractions were made small since the iodine value was changing very rapidly with temperature; in the later stages, larger final fractions were possible. Ease of filtration also influenced the recrystallization schemes; certain combinations of concentration and temperature gave rise to precipitates which could not be filtered.

It was found in the precipitation of Fraction d-1 and d-1-a that a filterable precipitate could not be obtained above —45.0° C., at which temperature a very large percentage of Fraction d had precipitated. Hence the extra step and the eventual change from 4 to 8 liters were made in order that Fraction 12 would not constitute more than half of Fraction d. In general, conditions were controlled so that one-third to one-fifth of a given major fraction would be carried over into the next fraction. It was noted that as a given fraction progressed further through the recrystallization process the precipitations became sharper; that is, the bulk of the total material coming out over a given temperature range precipitated through increasingly smaller fractions of the total temperature interval, leaving the remainder of

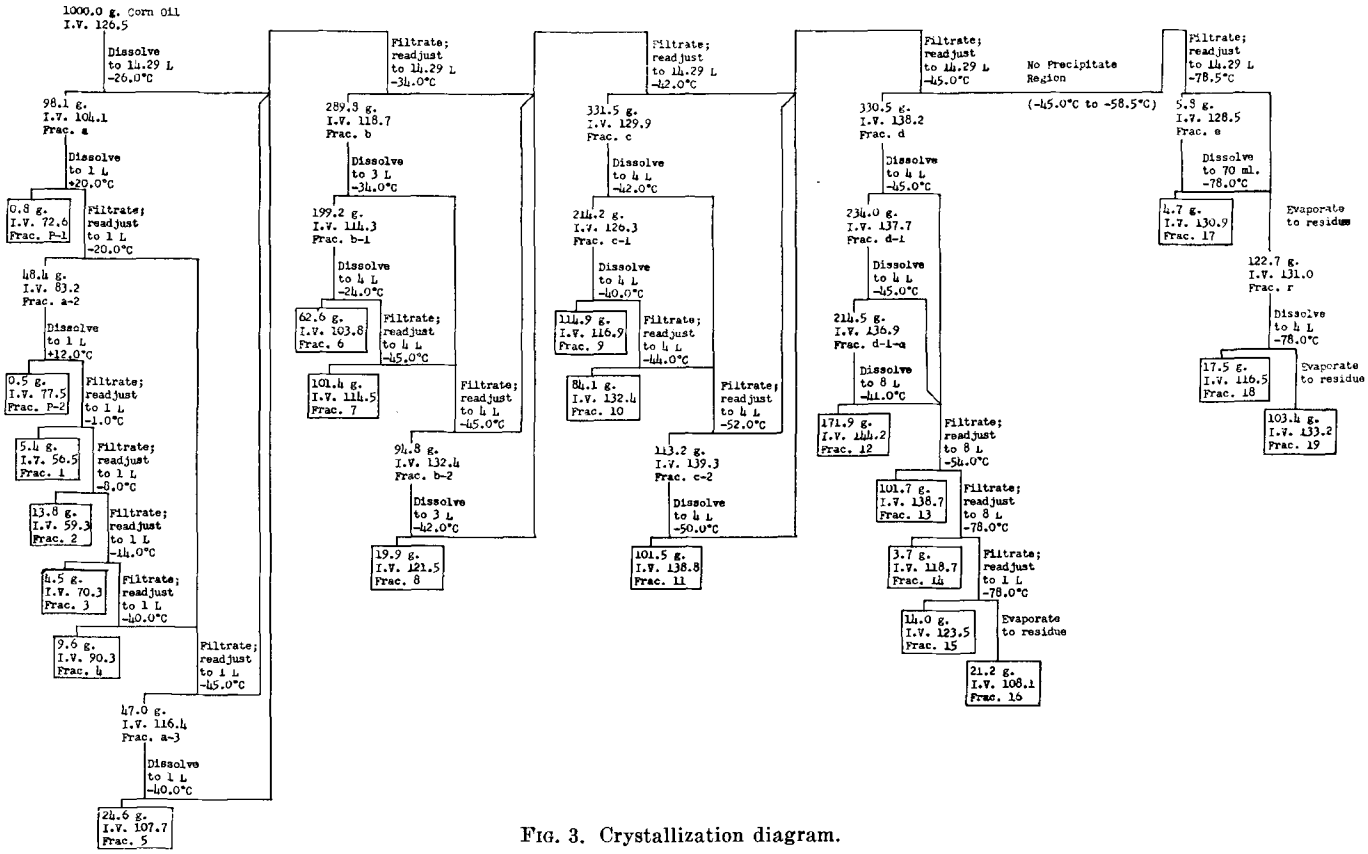



FIG. 3. Crystallization diagram.

the interval with little material coming out in it, suggesting that the complex oil was being separated into significantly simpler mixtures of very similar materials. The characterization of the fractions tends to corroborate this view, adjacent fractions possessing markedly different properties.

e. *Characterization of the Fractions.* All fractions were characterized by determination of weight, unsaponifiable, iodine value, saponification equivalent, melting point (15), and refractive index. Dienoic (linoleic) and trienoic (linolenic)[4] acids were determined spectrophotometrically by ultraviolet absorption measurements; monenoic (oleic) and saturated acids by difference (Table II) (16, 17).

## Discussion of the Fraction Characteristics of Table II

Column I (experimental weights of fraction) lists the weights as experimentally obtained; a recovery of 981.7 g. of total fractions from the original 1,000 g. of oil was achieved. Column II (weight of fraction to 1,000 g. of corn oil) corrects the weights of Column I to total 1,000 g. on the assumption that the loss for a particular fraction is proportional to the weight of that fraction rather than on the assumption that the 18.3 g. loss should be divided equally among the fractions. The corrected fraction weights of Column II are taken as the actual fraction weights and are used in all future percentage calculations, etc. Column IV (weight unsaponifiable of fraction) is the product of Column II and 0.01 times Column III (% unsaponifiable of fraction). Column V (glyceride weight of fraction) is the difference between Column II and Column IV on the assumption that all the material in the fraction except unsaponifiable is glyceride, except in the cases of Fractions P-1 and P-2. They are composed of high-melting, acetone-insoluble material and are considered phosphatide with no glyceride content. Column VIII (I. V. of glycerides) is derived using the equation:

$$(\text{I.V. Glyceride})(\text{Wt. Glyceride}) = (\text{I.V. Frac.})(\text{Wt. Frac.}) - (\text{I.V. Unsap.})(\text{Wt. Unsap.})$$

Columns V, VI, II, VII, and IV supply all unknowns except the I. V. of the glycerides. Column IX (saponification equivalent of fraction) was derived by use of:

$$\text{Sap. Equiv.} = \frac{(\text{Constant})(\text{Wt. Sample})}{(\text{ml. Blank}) - (\text{ml. Sample})}$$

while Column X (saponification equivalent corrected to glyceride) used:

$$\text{Sap. Equiv. Corrected to Glycerides} = \frac{(\text{Constant})[(\text{Wt. Sample}) - (0.01)(\%\,\text{Unsap.})(\text{Wt. Sample})]}{(\text{ml. Blank}) - (\text{ml. Sample})}$$

on the assumption that the unsaponifiable material absorbed no alkali. Table VI-B compares several properties of the oil with the sum of corresponding properties of the fractions.

[4] The presence of linolenic acid in corn oil is in doubt; bromination in ethyl ether of the fatty acids of Fraction 15, freed from unsaponifiable material, did not result in hexabromostearic acid separation. Considerable doubt exists, though, regarding the precision of the hexabromide precipitation method in the determination of linolenic acid in the presence of large quantities of other unsaturated acids (1); bromination in ethyl ether of a synthetic fatty acid mixture, composed of pure acids prepared in this laboratory and possessing a composition identical to that determined spectrophotometrically for Fraction 15, did not result in hexabromostearic acid separation. Bromination, by a procedure identical to that of the two previous cases, of an ethyl ether solution, possessing the same concentration of linolenic acid as the solution used in the case of the synthetic mixture but containing no other solute, yielded a hexabromide precipitate, confirming the marked interfering action exerted by the unsaturated acids on the hexabromide determination of linolenic acid in concentrations as high as 8%. The trienoic material as determined spectrophotometrically was calculated as linolenic acid.

### Interpretation of the Experimental Data

The solvent-fractionation process yields fractions with glyceride compositions determined solely on the basis of solubilities, the more soluble molecules being retained longer in solution until the lower temperatures near the end of the process are attained. It is desired to obtain a correlation between solubility and structural features of the glyceride molecules in a form restricting the glyceride composition of the individual fractions to mixtures simple enough to allow a direct glyceride calculation from fatty acid composition without the assumption of any scheme of fatty acid distribution. Since all the glycerides are tri-esters of glycerol with straight-chain, unsubstituted fatty acids, only two types of structural differences among the molecules are possible to account for the observed solubility differences.

1. *Molecular Weight Differences.* Glycerides containing fatty acids of different molecular weights would exhibit different solubility properties. In the case of corn oil, however, as will be demonstrated later, saponification equivalents determined on all the fractions show only negligible differences from 18-carbon chain length fatty acids; hence the observed solubility differences are not due primarily to molecular weight differences.

2. *Double Bond Differences.* Two factors must be considered: first, the total number of double bonds in the glyceride molecule and, second, given a particular number of double bonds, the effect of different arrangements of those bonds within the molecule. If the solubility is predominantly a function of the total number of double bonds within the molecule and is not grossly affected by the arrangement of those bonds, a separation process yielding fractions in order of increasing solubility would be expected to yield fractions also in order of increasing unsaturation. If, however, the arrangement of a given number of double bonds within the molecule exerts an effect equal to or predominant over the effect of the total number of double bonds present, such a simple, continuous increase in iodine value of fractions obtained would not appear. A molecule with two double bonds fortuitously placed for increased solubility would precipitate after a molecule with three double bonds less advantageously arranged in the molecule and the progression of iodine values for fractions arranged in order of increasing solubility would be an irregular one.

The results for corn oil show an overall increase in iodine value with solubility; the few decreases that are noted are of relatively small magnitude in comparison with the overall increase, suggesting that a classification scheme, for use with solvent fractionation data, be devised for the glyceride molecules on such a basis that all molecules of a given degree of unsaturation occur in one class and that the restrictions imposed upon the composition of the fractions be in terms of such classes. For corn oil, where the acids are primarily 18-carbon fatty acids, the degree of unsaturation of the various glyceride classes, given in terms of iodine value, is expressed by:

$$\text{Iodine Value} = \frac{(100)(\text{Mol. Wt. } I_2)(N)}{(\text{Mol. Wt. Tristearin}) - (\text{Mol. Wt. } H_2)(N)} = \frac{(100)(253.84)(N)}{(891.46) - (2.016)(N)} \quad N = 0,1,2 \ldots .$$

where $N$ is the number of double bonds present in the members of the class being considered. In corn oil, the four acids—linolenic (Ln), linoleic (Lo), oleic (Ol), and saturated (S)—allow only a relatively few glyceride members to exist in each glyceride class; application of these ideas to corn oil results in the scheme of classification presented in Table III. The Arabic numerals separated from the molecular formulas by commas indicate the number of positional isomers represented by the particular formula; diacid triglycerides, for example, are written in symmetrical form but the numeral following indicates that both positional isomers are represented. The procedures used here are not capable of distinguishing positional isomers from each other. Iodine values, molecular weights, saponification equivalents, and fatty acid composition of the glycerides are tabulated. Comparison of the experimental saponification equivalents of the glycerides with the tabulated ones based on the assumption of 18-carbon fatty acids substantiates the assumption of primarily 18-carbon fatty acids in corn oil.

The calculation of the glyceride composition of the fractions was based on the assumption that each fraction contains only two, adjacent glyceride classes. The validity of such an assumption is dependent upon the efficiency of the fractionation process; indeed, the entire purpose of the fractionation process is to elaborate, from the complex oil, fractions simple enough to allow an assumption of this sort to be valid. The efficiency of the fractionation process can be evaluated qualitatively from the characteristics of the fractions. Differences in iodine value and melting point between adjacent fractions suggest that the degree of fractionation has been considerable; the refractive index data permits a somewhat more than qualitative evaluation of the efficiency of the fractionation process. Synthesis and characterization studies of pure glycerides have shown that the refractive index of the molecules increases with increasing degree of unsaturation, the positions of the double bonds within the molecule exerting a secondary although measureable effect. In corn oil the most unsaturated glyceride expected, under any system of fatty-acid distribution, to exist to an important extent is trilinolein; the most saturated, di-saturated-mono-olein. The refractive indices of trilinolein and *sym*-oleyl-distearyl-glycerol at 40.0° C. are respectively 1.47205 (18) and 1.45607 (19), yielding, to a first approximation, a maximum expected spread of

$$1.47205 - 1.45607 = 0.01598.$$

The actual spread obtained was

$$1.47735 - 1.45965 = 0.01770.$$

The discrepancy observed may be assigned partly to the effect of the unsaponifiable material in the fractions and partly to the difference between naturally occurring unsaturated fatty acids and the debromination products used in the syntheses. The net indication is, however, that the fractionation process used does bring about a marked separation of the component glycerides and that the assumption of only two glyceride classes to a fraction is a justifiable one.

The determination of the two adjacent classes of glycerides that exist in a given fraction was made on the basis of iodine value; hence, a fraction of iodine value 133.1 would contain Classes 4 and 5. Inspec-

TABLE III

Glyceride Classification Based Upon Total Unsaturation

1. One Double Bond; I.V. 28.54; Mol. Wt. 889.44; Sap. Equiv. 296.48

| | | |
|---|---|---|
| Ol | 31.76% Ol | |
| S | 63.97% S | |
| S,2 | | |
| (A) | | |

2. Two Double Bonds; I.V. 57.21; Mol. Wt. 887.43; Sap. Equiv. 295.81

| | | | |
|---|---|---|---|
| Lo | 31.60% Lo | Ol | 63.66% Ol |
| S | 64.11% S | Ol | 32.06% S |
| S,2 | | S,2 | |
| (B) | | (C) | |

3. Three Double Bonds; I.V. 86.01; Mol. Wt. 885.41; Sap Equiv. 295.14

| | | | | | |
|---|---|---|---|---|---|
| Ln | 31.45% Ln | Lo | 31.67% Lo | Ol | 95.70% Ol |
| S | 64.26% S | Ol | 31.90% Ol | Ol | |
| S,2 | | S,3 | 32.13% S | Ol,1 | |
| (D) | | (E) | | (F) | |

4. Four Double Bonds; I.V. 114.9; Mol. Wt. 883.40; Sap. Equiv. 294.47

| | | | | | |
|---|---|---|---|---|---|
| Ln | 31.52% Ln | Lo | 31.75% Lo | Lo | 63.49% Lo |
| Ol | 31.97% Ol | Ol | 63.95% Ol | Lo | 32.20% S |
| S,3 | 32.20% S | Ol,2 | | S,2 | |
| (G) | | (H) | | (I) | |

5. Five Double Bonds; I.V. 144.0; Mol. Wt. 881.38; Sap. Equiv. 293.79

| | | | | | |
|---|---|---|---|---|---|
| Ln | 31.59% Ln | Ln | 31.59% Ln | Lo | 63.64% Lo |
| Lo | 31.82% Lo | Ol | 64.10% Ol | Lo | 32.05% Ol |
| S,3 | 32.28% S | Ol,2 | | Ol,2 | |
| (J) | | (K) | | (L) | |

6. Six Double Bonds; I.V. 173.2; Mol. Wt. 879.36; Sap. Equiv. 293.12

| | | | | | |
|---|---|---|---|---|---|
| Ln | 63.32% Ln | Ln | 31.66% Ln | Lo | 95.67% Lo |
| Ln | 32.35% S | Lo | 31.89% Lo | Lo | |
| S,2 | | Ol,3 | 32.12% Ol | Lo,1 | |
| (M) | | (N) | | (O) | |

tion of Table III shows that any two adjacent classes never contain a total of more than six glycerides; as shown below, the determination of weight, percentages of the four fatty acids, and iodine value of a fraction allows the writing of five independent simultaneous linear equations containing the glyceride components as the only unknowns. For fractions 1 through 7 (Ln = 0) the number of equations equals or exceeds the number of unknowns and ordinary determinant methods apply; for the remaining fractions the presence of traces of linolenic acid allows six unknowns and mandates the use of the approximation method discussed later under *The Pure Even Pattern.* The calculation of glyceride composition is illustrated by the case of Fraction 10; the glyceride iodine value of 133.1 indicates that Class 4 (I. V. 114.9) and Class 5 (I. V. 144.0) are present, permitting glycerides G, H, I, J, K, and L. The equations are:

% Saturated Acid of Glycerides,
$$32.20(G)+0(H)+32.20(I)+32.28(J)+0(K)+0(L)=(9.9)(84.6)=838$$

% Oleic Acid of Glycerides,
$$31.97(G)+63.95(H)+0(I)+0(J)+64.10(K)+32.05(L)=(24.5)(84.6)=2{,}073$$

% Linoleic Acid of Glycerides,
$$0(G)+31.75(H)+63.49(I)+31.82(J)+0(K)+63.64(L)=(61.0)(84.6)=5{,}160$$

% Linolenic Acid of Glycerides,
$$31.52(G)+0(H)+0(I)+31.59(J)+31.59(K)+0(L)=(0.23)(84.6)=19.5$$

Iodine Value of Glycerides,
$$114.9(G)+114.9(H)+114.9(I)+144.0(J)+144.0(K)+144.0(L)=(133.1)(84.6)=11{,}260$$

A set of numerics satisfying the above equations within the limits of experimental error is: G = 0.62 g.; H = 5.64 g.; I = 25.4 g.; J = 0 g.; K = 0 g.; L = 53.0 g. Substitution of these values in the original equations as a check yields: Sat. Acid, 838 = 838; Oleic Acid, 2078 = 2073; Linoleic Acid, 5162 = 5160; Linolenic Acid, 19.5 = 19.5; Iodine Value, 11,264 = 11,260. One such set of simultaneous equations was solved for each of the 19 fractions; the resulting composition of each fraction and the sum of the compositions of all the fractions is listed in Table IV. In all cases a set of values satisfying the equations and containing no negative values was found; this absence of negative values substantiates the assumptions, noted before, on which the equations are based, as it might be expected that nonsensical assumptions would yield nonsensical answers. Table VI-C compares values obtained by direct analysis of corn oil with the corresponding values deduced from the sum of the calculated structures of the fractions and provides a check on the accuracy of solution of the sets of equations.

## Corn Oil Glyceride Structure Expected from Different Schemes of Fatty-Acid Distribution

The structure expected for corn oil was calculated from the oil's fatty-acid composition and each of the four postulated schemes of distribution. From the data in Table II it was calculated that 1,000 g. of corn oil contains 979.6 g. of glycerides with 140.8 g. saturated acid, 219.6 g. oleic acid, 569.4 g. linoleic acid, and 5.88 g. linolenic acid (935.7 g. total acid); these figures represent a form of the analytical data more convenient for the calculations below.

1. *The Mono-Acid Triglyceride Pattern.* The procedure here involves only ratios between molecular weights. Thus,

$$\text{Weight trilinolein/1,000 g. corn oil} = \frac{(\text{Mol. Wt. Trilinolein})(\text{Wt. Linoleic Acid/1,000 g. Oil})}{(3)(\text{Mol. Wt. Linoleic Acid})}$$

$$= \frac{(879.36)(569.4)}{(3)(280.44)} = 595 \text{ g.}$$

2. *The Random Pattern* (20). It has been shown that the number of chemically distinguishable triglycerides possible from $n$ fatty acids is given by $(n^3 + n^2)/2$. In this study positionally different glyceride isomers could not be distinguished; hence, only the number of constitutionally different triglycerides possible was of interest. A line of reasoning somewhat parallel to the one yielding the above expression gives $(n^3 + 3n^2 + 2n)/6$ as the function denoting the number of constitutionally different triglycerides possible from $n$ fatty acids. For corn oil, the four acids yield 40 chemically distinguishable triglycerides and 20 constitutionally different triglycerides. If the assumptions of equal reactivity of all three glycerol hydroxyl groups and of equal reactivity of all acids are made, it follows that the probability of a given hydroxyl group being esterified by fatty acid A out of a mixture of fatty acids is proportional to the mole fraction of A. Extension of these ideas to the formation of the three types of triglycerides possible yields:

$$\text{Mole Fraction of } \begin{matrix} A \\ B \\ C \end{matrix} = (2)(F_A)(F_B)(F_C)$$

$$\text{Mole Fraction of } \begin{matrix} A \\ B \\ B \end{matrix} = (3/2)(F_A)(F_B)^2$$

$$\text{Mole Fraction of } \begin{matrix} A \\ A \\ A \end{matrix} = (F_A)^3$$

TABLE IV

Experimental Glyceride Structure

| Fraction | (A) Ol, S, S,2 | (B) Lo, S, S,2 | (C) Ol, Ol, S,2 | (D) Ln, S, S,2 | (E) Lo, Ol, S,3 | (G) Ln, Ol, S,3 | (H) Lo, Ol, Ol,2 | (I) Lo, Lo, S,2 | (J) Ln, Lo, S,3 | (L) Lo, Lo, Ol,2 | (O) Lo, Lo, Lo,1 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | .18g. | 4.02 | 1.20 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 2 | ..... | 10.6 | 2.40 | ..... | .82 | ..... | ..... | ..... | ..... | ..... | ..... |
| 3 | ..... | 2.22 | ..... | ..... | 2.18 | ..... | ..... | ..... | ..... | ..... | ..... |
| 4 | ..... | ..... | ..... | ..... | 7.53 | ..... | ..... | 1.77 | ..... | ..... | ..... |
| 5 | ..... | ..... | ..... | ..... | 6.08 | ..... | 4.74 | 14.0 | ..... | ..... | ..... |
| 6 | ..... | ..... | ..... | ..... | 23.8 | ..... | 4.88 | 34.1 | ..... | ..... | ..... |
| 7 | ..... | ..... | ..... | ..... | 1.10 | ..... | 8.50 | 92.4 | ..... | ..... | ..... |
| 8 | ..... | ..... | ..... | ..... | ..... | .11 | .13 | 15.1 | ..... | 4.66 | ..... |
| 9 | ..... | ..... | ..... | ..... | ..... | .59 | 14.6 | 90.1 | ..... | 10.7 | ..... |
| 10 | ..... | ..... | ..... | ..... | ..... | .62 | 5.64 | 25.4 | ..... | 53.0 | ..... |
| 11 | ..... | ..... | ..... | ..... | ..... | 1.32 | 2.50 | 11.0 | ..... | 86.6 | ..... |
| 12 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 3.19 | 159.8 | 8.9 |
| 13 | ..... | ..... | ..... | ..... | ..... | 1.82 | 2.70 | 14.3 | ..... | 83.8 | ..... |
| 14 | ..... | ..... | ..... | ..... | ..... | .10 | 1.93 | .24 | ..... | .83 | ..... |
| 15 | ..... | ..... | ..... | ..... | ..... | 3.65 | 2.68 | 3.24 | ..... | 4.43 | ..... |
| 16 | ..... | ..... | ..... | 4.10 | .29 | ..... | 11.8 | 5.04 | ..... | ..... | ..... |
| 17 | ..... | ..... | ..... | ..... | ..... | .14 | 1.70 | .09 | ..... | 2.77 | ..... |
| 18 | ..... | ..... | ..... | ..... | ..... | .50 | 9.41 | 6.21 | ..... | 1.38 | ..... |
| 19 | ..... | ..... | ..... | ..... | ..... | 2.90 | 1.50 | 22.1 | ..... | 73.9 | ..... |
| Total | .18 | 16.8 | 3.60 | 4.10 | 41.5 | 11.8 | 72.7 | 335.1 | 3.19 | 481.9 | 8.9 |

(F), (K), (M), and (N) = 0.

Since the various positional isomers of the above are all equally possible,

$$\text{Mole Fraction of } \begin{matrix}A\\B\\C,3\end{matrix} = (3)(2)(F_A)(F_B)(F_C)$$

$$\text{Mole Fraction of } \begin{matrix}A\\B\\B,2\end{matrix} = (2)(3/2)(F_A)(F_B)^2$$

$$\text{Mole Fraction of } \begin{matrix}A\\A\\A,1\end{matrix} = (F_A)^3$$

In corn oil, the 0.611 mole fraction of linoleic acid yields:

Weight trilinolein/1,000 g. corn oil = $(0.611)^3(979.6)$ = 223 g.

3. *The Pure Even Pattern.* The first step in the calculation involves writing all the constitutionally different molecules consistent with the fatty-acid percentages. The percentages of saturated, oleic, and linolenic acids are all below 33.3%; hence, no glyceride molecule may possess two of any one of them. The percentage of linoleic acid is between 33.3% and 66.7%; hence, every glyceride molecule in the mixture must include one linoleic acid molecule, and some glyceride molecules will contain two. Only six constitutional possibilities compatible with the above requirements exist:

| Lo, Lo, S,2 | Lo, Lo, Ol,2 | Lo, Lo, Ln,2 | Ln, Lo, Ol,3 | Ln, Lo, S,3 | Lo, Ol, S,3 |
|---|---|---|---|---|---|

Here the number of unknowns exceeds the number of equations and the system is indeterminate. For such a case, an approximation method, involving the selection of an arbitrary ratio between the unknowns, comparison of the fatty-acid composition of that mixture with the fatty-acid composition of the oil, and readjustment of the ratio to closer correspondence with the oil's fatty-acid composition, has been proposed; such a procedure is, however, the exact equivalent of the simultaneous solution of the linear equations and hence does not yield a strictly unique solution under conditions rendering the equation system indeterminate. The indeterminism of the equation system is, however, partially removed by the fact that the nature of the problem allows the imposition of the so-called "sensible conditions," e.g., in this case, no negative answers. This condition reduces sharply the number of sets of answers satisfying the equations and renders the answer obtained from the approximation process very nearly unique; this is particularly true when several of the fatty acid components exist to small percentages and only a few exist to relatively large percentages as is the case with corn oil.

4. *The Partial Random Pattern.* The partial random scheme as applied to corn oil allows the formation of some trilinolein, introduces two oleic acid molecules or two saturated acid molecules in some of the triglycerides, and removes the restriction that every triglyceride must possess one molecule of linoleic acid. This allows the formation of 14 constitutional possibilities:

| Lo, Lo, Lo,1 | Lo, Lo, S,2 | Lo, Lo, Ol,2 | Lo, Lo, Ln,2 | Ol, Ol, S,2 | Ol, Ol, Lo,2 | Ol, Ol, Ln,2 |
|---|---|---|---|---|---|---|
| S, S, Ol,2 | S, S, Lo,2 | S, S, Ln,2 | S, Ol, Lo,3 | Ol, Lo, Ln,3 | S, Ol, Ln,3 | S, Lo, Ln,3 |

TABLE VI

Comparison of Properties of the Oil With Sums of Corresponding Properties of the Fractions

| | 20% Solution | 7% Solution |
|---|---|---|
| A. Preliminary Fractionation Studies: | | |
| Weight of Oil | 200.0 g. | 70.0 g. |
| Σ Weight of Fractions | 199.3 g. | 69.2 g. |
| (Weight of Oil) (I.V. of Oil) | 25,300 | 88,600 |
| Σ (Weight of Fractions) (I.V. of Fractions) | 25,200 | 88,400 |
| B. Full Scale Fractionation Studies: | | |
| Weight of Oil | 1,000 g. | |
| Σ Weight of Fractions | 981.7 g. | |
| (Weight of Oil) (I.V. of Oil) | 126,500 | |
| Σ (Weight of Fractions) (I.V. of Fractions) | 126,300 | |
| Weight Unsaponifiable of Oil | 19.5 g. | |
| Σ Weight Unsaponifiable of Fractions | 19.0 g. | |
| (Wt. Unsap. of Oil) (I.V. Unsap. of Oil) | 1,890 | |
| Σ (Wt. Unsap. of Frac.) (I.V. Unsap. of Frac.) | 1,570 | |
| (Weight of Oil) (Sap. Equivalent of Oil) | 297,600 | |
| Σ (Wt. of Frac.) (Sap. Equiv. of Frac.) | 298,200 | |

| | Linolenic Acid | Linoleic Acid | Oleic Acid | Saturated Acid |
|---|---|---|---|---|
| Wt. Acid in Oil | 5.5g. | 579 | 205 | 148 |
| Σ Wt. Acid in Fracs | 5.9 | 569 | 220 | 141 |

C. Comparison of Analysis of Calculated Glyceride Structure With Analysis of the Fractions:

| | Linolenic Acid | Linoleic Acid | Oleic Acid | Saturated Acid |
|---|---|---|---|---|
| Σ Wt. Acid in Fracs | 5.9g. | 569 | 220 | 141 |
| Σ Wt. Acid of Expt. Glyc. Struc | 6.0 | 570 | 220 | 141 |

TABLE V

Comparison of Experimental With Calculated Glyceride Structures

| | Ln Ln Ln,1 | Lo Lo Lo,1 | Ol Ol Ol,1 | S S S,1 | Ln Ln S,2 | Ln Ln Ol,2 | Ln Ln Lo,2 | Lo Lo S,2 | Lo Lo Ol,2 | Lo Lo Ln,2 |
|---|---|---|---|---|---|---|---|---|---|---|
| Mono-Acid Triglyc. Distribution | 6.15 g. | 596 | 230 | 147 | 0 | 0 | 0 | 0 | 0 | 0 |
| Random Distribution | 0.0003 | 223 | 12.5 | 3.24 | 0.02 | 0.03 | 0.07 | 164 | 257 | 6.96 |
| Even Distribution | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 291 | 524 | 0 |
| Partial Random Distribution | 0 | 8.0 | 0 | 0 | 0 | 0 | 0 | 332 | 485 | 0.50 |
| Experimental Distribution | 0 | 8.9 | 0 | 0 | 0 | 0 | 0 | 335 | 482 | 0 |
| | Ol Ol S,2 | Ol Ol Lo,2 | Ol Ol Ln,2 | S S Ol,2 | S S Lo,2 | S S Ln,2 | S Ol Lo,3 | Ol Lo Ln,3 | S Ol Ln,3 | S Lo Ln,3 |
| Mono-Acid Triglyc. Distribution | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Random Distribution | 23.9 | 98.0 | 1.02 | 15.3 | 39.9 | 0.41 | 125 | 5.34 | 1.30 | 3.40 |
| Even Distribution | 0 | 0 | 0 | 0 | 0 | 0 | 146 | 15.5 | 0 | 3.00 |
| Partial Random Distribution | 1.69 | 70.0 | 0.50 | 4.16 | 17.5 | 3.20 | 40.8 | 1.00 | 10.5 | 2.94 |
| Experimental Distribution | 3.60 | 72.7 | 0 | 0.18 | 16.8 | 4.10 | 41.5 | 0 | 11.8 | 3.19 |

Here the number of unknowns exceeds the number of equations and the approximation process, discussed above, was used. The expected weight of trilinolein is 8.0 g.

Complete results of the four distribution schemes are tabulated in Table V along with the experimentally obtained glyceride structure; the experimental structure agrees most nearly, with respect both to number and quantity of glycerides, with the partial random scheme.

## Summary

Corn oil was subjected to a low-temperature solvent-fractionation process and separated into 19 glyceride fractions; the glyceride structures of the fractions were calculated and added to obtain the glyceride structure of the oil. The experimentally obtained glyceride structure was compared to the structures calculated according to the mono-acid triglyceride, random, even, and partial random schemes and was found to agree most closely with the partial random system.

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