Two distinct 2,4-dinitrophenylhydrazones have been isolated from the carbonyl portions of both the hardened and unhardened soybean oils. The structure of the lower melting derivative, in each case, has been shown to correspond closely to the structure of α-heptenal.

Conjugated triene is suggested as a precursor to the formation of α-heptenal in reverted soybean oil.

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Adsorption Analysis of Lipids. II. The Fractionation of Soybean Oil and Derived Ethyl Esters[1]

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THE desire to fractionate glycerides of soybean oil arises from the need for a stable edible oil of low iodine value and a rapid-drying paint oil of high iodine value. Among the various methods of fractionation that have been employed are molecular distillation, low-temperature crystallization, and countercurrent extraction.

The molecular distillation process has been of little value in fractionating soybean glycerides with regard to unsaturation. A spread of 13.5 units in iodine value (126.6-139.1) is the maximum reported (1).

Fractional crystallization is more effective. By repeated recrystallization from acetone Bull and Wheeler (2) were able to obtain fractions as high in value as 166.1 and as low as 84.7, or a spread of 81.3 units. Golumbic, Martin, and Daubert (3) report a fractionation in which 16.7% of the total oil had an iodine value of 160.3; another fraction containing 25% of unsaponifiable matter comprising 0.9% of the total oil had an iodine value of 196.0. In a study of the component glycerides of soybean oil, Hilditch, Meara, and Holmberg (4) describe fractions ranging in iodine value from 114.3 to 163.7 and in linolenic acid content from 6.4 to 17.7%.

Extraction processes have given fractionations comparable or superior to those of fractional crystallization. The Solexol process (5), which uses propane as the solvent, is reported to fractionate oil yielding 30% of oil with an iodine value of 162 and 67.5% with an iodine value of 125. Using a two-solvent system of furfural and naphtha in a 50-foot countercurrent extraction column, Goss (6) reports iodine values of 116 to 173. Employing the same solvents in an 87-foot column, Gloyer (7) obtains a raffinate fraction comprising 30.1% with an iodine value of 95.5 and an extract fraction comprising 69.9% with an iodine value of 153.2.

No attempts to fractionate the glycerides of soybean oil by means of (chromatographic) adsorption analysis have been reported to date. However, Walker and Mills (8) applied this method to linseed oil and, using a single column, were able to separate fractions which had iodine values ranging from 116.2 to 202.8. Repeated adsorptions yielded fractions having iodine values of 117.7 to 246.5.

In the present study soybean glycerides and the mixed ethyl esters of soybean oil have been fractionated by adsorption on columns of aluminum oxide. These fractions were analyzed for fat acid composition by use of spectrophotometric and iodine value data. Total pigments, fraction weights, and the position of pigment bands were also measured. The utility of the adsorption column for the fractionation of glycerides of soybean oil and its ethyl esters is illustrated by the data presented.

Materials

The soybean oil used in these studies was a sample of commercially-produced, crude, degummed oil, extracted and stripped of solvent under mild temperature conditions. The ethyl esters used were prepared from this oil by trans-esterification with ethanol with use of sodium hydroxide as catalyst. The esters were extracted with petroleum ether, washed, dried, and stripped of solvent at 100°C. and then purified further by distillation at a pressure of 1 mm. and a temperature of 158°C., no attempt being made to fractionate them at this point. The iodine value of the crude oil was 134.2, that of the esters 125.8. Spectrophotometric analysis of the oil showed 8.1% linolenic acid, 52.7% linoleic acid, 26.2% oleic, and 13.0% saturated acids present as triglycerides.

The adsorbent used for this work was aluminum oxide, Harshaw's Al-2 powder.* This grade was selected after testing several brands for filtration

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* The mention of this product does not imply that it is endorsed or recommended by the Department of Agriculture over others of a similar nature not mentioned.

rate, fractionation, and oil recovery. Its strength of adsorption, measured on the Brockman scale (9), was class V.

Experimental Procedure

Alumina (approximately 700 gm.) was packed into a large glass tube under partial vacuum, forming a column 5 cm. in diameter and 44 cm. in length. Samples and solvents were introduced from a glass reservoir of a type which also permits the continuous application of gas pressure to the solvents at the top of the column (10). Purified nitrogen gas (0.002% oxygen) was first passed through the column to deaerate it, then 100 ml. of redistilled petroleum ether was introduced, followed by 15 gm. of the crude oil dissolved in 100 ml. of petroleum ether. After the solution of oil had entered the column, the column was developed with 3.9 liters of 35% diethyl ether in petroleum ether. A nitrogen pressure of 2 pounds per square inch was maintained on the column from the addition of the oil sample until the chromatogram was finished. Under these conditions the rate of flow of solvent through the column was approximately 2 liters per hour.

The adsorption and fractionation of ethyl esters of crude soybean oil was conducted in the same manner except that a 20-gm. sample of esters was adsorbed, and the chromatogram was developed with a solvent consisting of 1.75% diethyl ether in petroleum ether.

With both samples the percolate was collected in 100-ml. fractions in tared flasks. In the case of the crude soybean oil approximate measures of the pigment content were obtained by determining the optical density of the fractions on the Beckman spectrophotometer at 4500 Å. After evaporation of the solvent the weight of residual oil or esters in each fraction was determined.

The per cent composition of fat acids in each fraction was calculated from spectrophotometric determinations and iodine value. The "background correction" method of Brice, Swain, Schaeffer, and Ault (11) was used for the calculation of linolenic acid when present in amounts of less than 3%; when present in amounts greater than 3% the method of Mitchell, Zscheile, and Kraybill (12) was employed.

While current chromatographic studies of the unsaponifiable fraction indicate the presence of at least 7 or 8 carotenoid pigments in crude soybean oil, under the conditions of adsorption described here the pigments are separated into only three bands. The positions of the leading edge of each band were measured at intervals corresponding to the entry of each 100 ml. of solvent into the column. In order to remove the third pigment band from the column it was necessary to elute the crude oil chromatogram with 100% diethyl ether.

Results

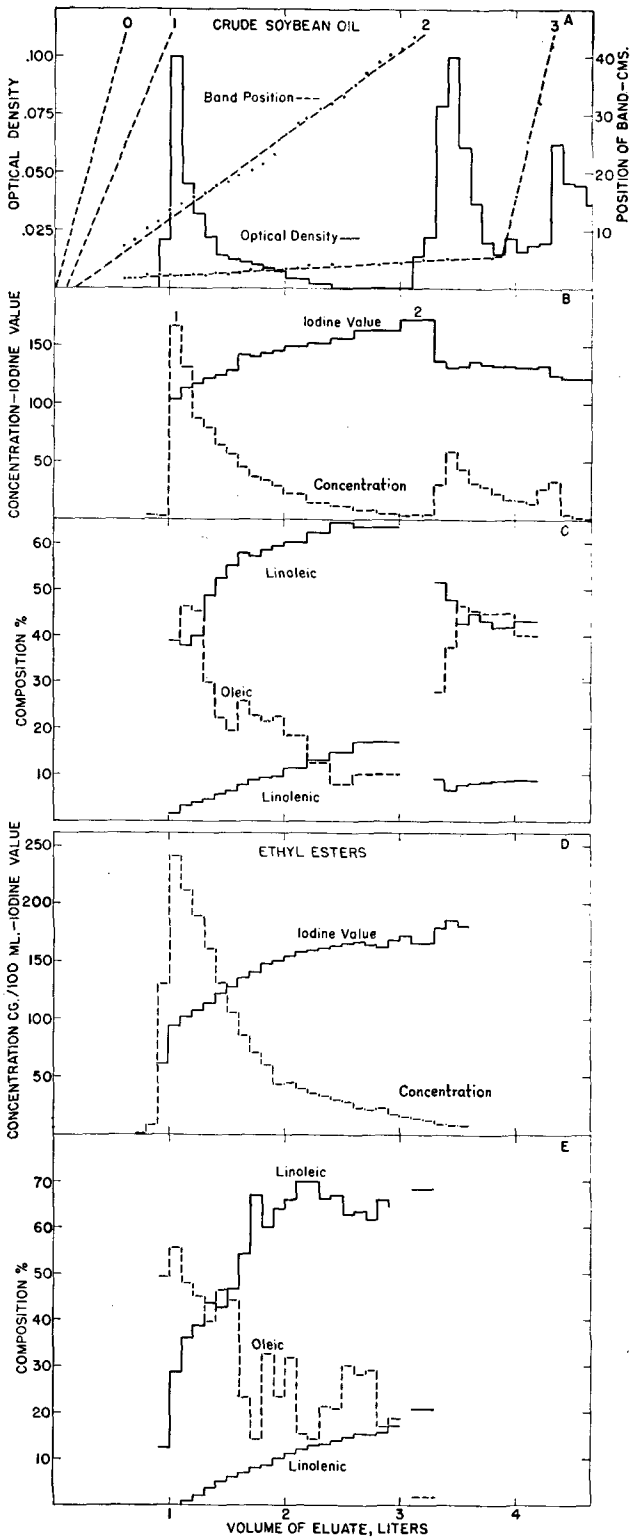
Adsorption Analysis of Crude Soybean Oil. The amount of fractionation obtained on a given column depends on conditions such as the amount of oil adsorbed, the composition of the developing solvent, the length of the column, and the activity of the adsorbent. Over 15 adsorptions of both crude and refined soybean glycerides have been performed with variations of these conditions. All desirable features for an adsorption analysis cannot be obtained with any one set of conditions. For example, those conditions giving the highest recovery for a given oil result in the lowest fractionation of that oil. Since this is true, data from only one typical adsorption analysis will be given here to illustrate the type of results obtained.

Data showing the fractionation of crude soybean oil are given in the accompanying Figure. Plotted on the abscissa common to the Figure, parts A, B, and C, is the volume of solvent which has entered the column since the introduction of the sample. Part A, curve 0 describes the rate of progress of the solvent through the column. The pore volume of the adsorbent, or the volume which entered the column before the leading boundary of solvent reached the bottom of the tube, is seen to be 600 ml. After the passage of 400 additional ml. of solvent, the first pigment band emerged from the column (part A, curve 1). Since 100 ml. of petroleum ether preceded the sample, the retention volume (13) for the first carotenoid band (curve 1) is therefore 300 ml. The second carotenoid pigment band progressed down the column at less than one-tenth the rate of the first pigment (curve 2). When this band reached the bottom of the column, the leading edge of the third pigment band (curve 3) had moved less than 6 cm. with the percolation of 3,800 ml. of solvent. Subsequently, the column was developed with diethyl ether, and with this eluant the rate of movement of the pigment down the column approximately equaled that of the solvent.

Part B, curve 1, gives the concentration of glycerides eluted in each fraction in centigrams per 100 ml. of eluate. This curve is composed of three peaks. The shape of the first peak is that predicted by theory—a sharp leading boundary and a trailing rear boundary (14). The retention volume of the glycerides is nearly identical with that for the first carotenoid band, or 300 ml.

The occurrence of a second peak in the concentration curve at 3,500 ml. is at present unexplained. Possibly some difference in glyceride structure comprising approximately 23.7% of the total and possessing markedly different adsorption characteristics from the rest of the oil causes this recurrent adsorption pattern. No humps are found in either peak corresponding to the elution of specific glyceride isomers, probably because the glyceride mixture is too complex to be completely resolved. The third peak is merely the result of the emergence of the diethyl ether boundary. The over-all percentage recovery of glycerides placed upon the column was 82.4%. No combination of solvents yet tried has increased the recovery appreciably.

A comparison of the concentration curve with the optical density curve (parts A and B) suggests that the first two carotenoid bands may serve as valuable indicators. The position of the first carotenoid band gives the position of the leading edges of the first glyceride band on the column. The second pigment band likewise coincides with the second band of glycerides. Since the fractions having the highest iodine value immediately precede the second carotenoid band (part B) and the first carotenoid indicates the position of low-iodine-value fractions, these pigments have the practical value of locating on the column the high- and low-iodine-value glycerides in crude oils.



Adsorption analysis of crude soybean oil and derived theyl esters.

While the fraction size gradually falls off as observed in the concentration curve, the iodine values (part B, curve 2) rise from 104.2 to 173.2. This iodine value and concentration behavior is typical for both crude and refined oils. However, under different adsorption conditions, fractions with iodine values as high as 200 have been obtained. Based on experience with other columns, the more general behavior of the iodine value curve over the fractions of the second peak is first to decrease and later to increase, thus tending to repeat the configuration of the iodine value curve over the first peak.

While the iodine values of the fractions give a rough measure of the fractionation obtained, a more comprehensive evaluation is given by spectrophotometric analyses for the polyunsaturated fat acids (part C). The linolenic acid content of the fractions rises uniformly from 1.6% in the eleventh fraction eluted (fractions 9 and 10 were too small to analyze) to a value of 17.2% in the twenty-second fraction. The iodine value of the next fraction indicates a still higher percentage of linolenic acid, but this fraction was too small for spectrophotometric analysis. The linoleic acid content also increases from 38.7% to 64.5%. Meanwhile, oleic acid, which was calculated from both spectrophotometric data and iodine value, reaches a maximum of 46.5% and then decreases to 10.4%. Errors in spectrophotometric and iodine value determinations combine in the calculation of oleic and saturated acids to such an extent as to make conclusions uncertain. However, it appears that saturated acids are present in large amounts in early fractions and occur to some extent in all other fractions.

Adsorption Analysis of Ethyl Esters. One of the limitations of the glyceride fractionations just described is the expected random or even distribution of unsaturated and saturated fat acids in the same glyceride molecule. For this reason, better fractionation was anticipated for the esters of soybean oil than for the glycerides. This prediction was verified experimentally, as shown in the data of the Figure, parts D and E. Iodine values rise from a low of 60.8 to a high of 184.6. Linolenic acid could not be detected in the tenth and eleventh 100-ml. fractions but rose to 20.8% in the thirty-second and thirty-third fractions. Again the highest iodine value fraction was too small for spectrophotometric analysis. Linoleic acid increased from 12.5% to a high of 70% while oleic acid decreased from a high of 55%. Here, as with glyceride fractionations, conclusions are drawn with considerable reserve. However, there is a general trend for both oleic and saturated ester content to decrease with volume of eluate, the initial content of saturated esters being relatively high (38.3%) and decreasing to low values. Probably it is always present in small amounts.

It will be noted also in these data that with the conversion of the glycerides to ethyl esters, the second peak in the weight curve does not occur. This tends to support the hypothesis that the second peak of the glyceride curve results from glyceride structure rather than the fat acids themselves. The recovery of the esters originally adsorbed was 90.0%.

Discussion

Adsorption analysis compares favorably with other methods of fractionation of glycerides reported to date. The spread of iodine values reported herein for soybean oil and soybean oil esters for a 1½-foot adsorption column is equivalent to that reported for a 50-foot countercurrent extraction column. The passage of soybean glycerides through a single adsorption column also gives a fractionation equal or superior to that of repeated low-temperature crystallization.

Other considerations, however, limit the usefulness of adsorption analysis. These include the compara-

tively small amounts of material separated. This limitation results from the high solvent to solute and the high adsorbent to solute ratios. Also, as currently applied, adsorption analysis has the disadvantages inherent in a batch process.

In the fractionation of soybean oil by adsorption analysis reported here, no evidence has been obtained for the occurrence of glycerides more unsaturated than dilinoleo-monolinolenin or for the occurrence of di- or trisaturated glycerides. These observations are in accordance with the theory of even distribution of fat acids.

The immediate application anticipated in these fundamental studies is the preparation of soybean oil fractions of both high and low linolenic acid content and a study of these fractions in regard to the "linolenic acid" theory of flavor instability (15). Consequently, the next step is the preparation of high- and low-iodine-value fractions in quantity using the naturally occurring carotenoid pigments as internal indicators for the location of glyceride fractions.

Acknowledgment

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Summary

A rapid method of fractionating soybean glycerides and their ethyl esters by adsorption analysis on an aluminum oxide column is described. It compared favorably with other methods of fractionation. In the adsorption analysis of crude soybean oil, the iodine values of fractions ranged from 104 to 173, a spread of 69 units. However, under other conditions of adsorption, iodine values as high as 200 were obtained. In the analysis of ethyl esters of soybean oil, iodine values ranged from 61 to 185, a spread of 124 units. The fat acid composition of each fraction was calculated from spectrophotometric and iodine value determinations. Recoveries from the columns were 82.4% in the case of the glycerides, and 90.0% in the case of the esters.

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Adsorption Analysis of Lipids. III. Synthetic Mixtures of Ethyl Stearate, Oleate, Linoleate, and Linolenate*

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THE separation of esters derived from naturally occurring fats is commonly achieved by distillation. Esters of saturated fat acids which differ in length by two carbon atoms may be separated satisfactorily for analytical purposes (1, 2) by the use of modern, efficient columns or by the amplified distillation technique. Mixtures of fat acid esters of the same carbon chain length but with varying degrees of unsaturation, however, can be only partially fractionated, if at all. Norris and Terry (3), using a Podbielniak column, recovered fractions containing 95% methyl oleate from a mixture of equal parts of methyl stearate and methyl oleate; however, with a mixture of equal parts of methyl oleate and methyl linoleate, no appreciable fractionation resulted. Little success was anticipated for the separation of other members of the homologous series of unsaturated fat acids, presumably because of "association of the acids."

Fractional crystallization and countercurrent extraction methods appear more promising for the separation of unsaturated esters of the same carbon chain length than do distillation procedures. However, since no data comparable to that presented herein have been published, comparison is limited in this paper between adsorption analysis and the less-efficient distillation procedure. In the previous paper of this series comparisons were made of the fractionation of soybean glycerides accomplished by adsorption analysis, molecular distillation, countercurrent extraction, and fractional crystallization (4).

The feasibility of applying chromatographic adsorption analysis to the separation and preparation of individual fat acids has been studied qualitatively by several workers (5, 6, 7, 8), but few systematic investigations which give quantitative information as to column performance have been reported. Cassidy (9) has studied quantitatively the completeness of separation in the homologous series of saturated fat acids on carbon columns by the use of conventional chromatographic development and elution techniques. In connection with the "frontal analysis" technique Claesson (10) has made a remarkable contribution by developing the theory and obtaining data which permit the analytical determination of mixtures of saturated fatty acids and a few unsaturated fat acids and

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