The Fatty Acid Composition of Cottonseed Oil at Various Stages of Solvent Extraction

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The variation in the fatty acid composition of the glyceride portion of cottonseed oil at various stages of solvent extraction has been investigated. Prime cottonseed meats were flaked and extracted in glassware rate extraction apparatus, using commercial hexane up to different degrees of extractions. The fatty acid composition of cottonseed oil obtained after extracting the flakes to different residual oil contents was determined by gas-liquid partition chromatography. No difference was found.

SOME EVIDENCE indicates that the oil produced by solvent extraction of oil seeds to residual oil levels as low as 1% or less is lower in quality than that produced by extraction to higher residual levels. Bull and Hopper (1) have reported a high phosphatide content in the last 1.1% of soybean oil extracted. Karnofsky (6) has reported a high refining loss in a similar fraction. Eaves *et al.* (3) have studied the composition of the difficultly-extractable lipids of cottonseed meats. They also studied the effect of these lipids on the refining characteristics of crude oils. They found that the composition of successive lipids fractions varied with the degree of exhaustiveness of extraction. The fractions obtained by more exhaustive extraction contained greater amounts of undesirable nonneutral oil material and lesser amounts of desirable neutral oil. Crude oils equivalent to varying degrees of total lipids extraction were reconstituted from the crude lipids fraction and evaluated for refining characteristics. The impurities content of the reconstituted oils varied as the degree of total lipids extraction and increases in the impurities content of the oils were generally reflected in disproportionate increases in refining losses and/or refined oil color.

Eaves *et al.* (4) also studied the comparative absolute yields of crude and neutral oils from variously-prepared cottonseed meats. The results of the studies showed that the method used in preparing cottonseed meats for extraction had a significant effect on the yields of crude oil obtained but that the yields of neutral oil were virtually unaffected. Analyses of the crude oils showed that the differences in crude oil yields were caused by the relative amounts of nonneutral oil materials in the crudes from the differently-prepared meats.

The purpose of the present investigation is to see whether the fatty acid composition of the glyceride portion of cottonseed oil varies at various stages of solvent extraction.

Chang (2) found an increase in refining loss of about 2% as the residual oil content in the meal was reduced from 1.1 to 0.7%. The iodine values, saponification values, and refractive indices showed no significant changes, indicating no probable change in neutral oil composition. Changes in the composition of the glyceride portion of the oil have been studied.

Prime cottonseed meats were flaked and extracted in glassware rate-extraction apparatus similar to that used in previous studies (7) except somewhat larger. The extraction chamber was 12 in. high by 2 in. in diameter and designed to use 100-g. samples. The extraction chamber and the incoming solvent were heated to 150°F. The solvent, commercial hexane,[1] was passed through the sample continuously.

TABLE I

Composition of Cottonseed Oil Resulting from Extraction to Different Levels

Sample No.	Total oil extracted, %	Fatty Acid Composition, %					
		Myristic	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic
1	75.4	0.9	25.7	0.1	0.2	22.6	50.5
2	88.0	0.9	26.8	0.1	0.3	22.9	49.0
3	95.0	0.7	26.8	0.1	0.2	22.8	49.4
4	97.5	0.8	27.2	0.1	0.2	22.8	48.8

In the first series of experiments (Table I) different samples of flakes were extracted to different residual oil contents. This resulted in one sample of oil from each sample of flakes. In the second series (Table II) a sample was extracted for 50 min. to give the first fraction of oil. The extraction was continued, and fractions of miscella were taken every 10 min.; each fraction contained all of the oil extracted in that 10-min. interval. The solvent from each miscella fraction was evaporated under vacuum.

TABLE II

Fatty Acid Composition of Different Fractions of Cottonseed Oil

Fraction No.	Total oil extracted, %	Fatty Acid Composition, %					
		Myristic	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic
1	0–75.4	0.9	25.8	0.1	0.0	22.5	50.6
2	75.4–82.3	0.7	26.7	0.1	0.0	22.8	49.7
3	82.3–88.0	0.9	26.8	0.1	0.2	22.9	48.9
4	88.0–95.0	0.7	27.2	0.2	0.1	22.3	49.3
5	95.0–97.5	0.9	27.3	0.2	0.2	22.2	49.2
6	97.5–98.2	1.0	26.5	0.1	0.2	23.8	48.3
7	98.2–98.8	0.7	27.7	0.2	0.3	22.6	48.5
8	98.8–99.0	0.8	27.9	0.3	0.4	22.7	47.9

Each oil sample was saponified with a 4% solution of alcoholic sodium hydroxide, and the unsaponifiables were extracted with petroleum ether.[2] Fatty acids were liberated by 1:3 sulfuric acid. The fatty acids were then extracted with ethyl ether and dried over anhydrous sodium sulfate; the ether was evaporated off under vacuum. The fatty acids were next esterified with methanol containing 3% by weight of hydrogen chloride. The esters were removed from the mixture with ethyl ether and analyzed by gas-liquid chromatography (5) (Tables I and II).

The gas-liquid partition chromatograph was assembled from the following units: the detector—a Gow Mac Model TR-11-B Thermal Conductivity Cell with a Model 9293 Power Supply Control Unit operating on current from two 12-volt storage batteries in series; potentiometer—Bristol Model IPH 570 Dynamaster,

[1] Phillips Petroleum Company, "high-purity normal hexane."
[2] Skellysolve F, Skelly Oil Company, Kansas City, Mo.

recording type; column—eight feet of ¼-in. copper tubing packed with polyester succinate on −40 + 65 Tyler mesh red Chromosorbe; carrier gas—helium.

The results indicate practically no difference in the fatty acid compositions of the various glyceride fractions. The slight down-trend of linoleic acid with a compensating up-trend of palmitic acid would have no significance if it were not repeated in both series. It is interesting also to note that the percentage of linoleic acid in Sample 4 in Table I is 48.8 as compared with a weighed cumulative value of Fractions 1 to 5, inclusive, of 49.2. These two results represent values from two similar samples from different flakes and extractions. It is apparent that there are no significant variations in the total fatty acid composition of the first 95% of the oil extracted under the conditions studied. This agrees with the general conclusion from the work of Chang (2) on soybeans.

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Partial Fractionation of Fatty Acid Triglycerides on a Silicic Acid Column

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A silicic acid column chromatographic method for the fractionation of glyceride types, employing an all-glass apparatus which permits gradient changes in solvent mixtures under pressure is described. Data on seven natural fats are discussed on the basis of the iodine values of individual fractions. The general trend in elution with the increasing concentration of ethyl ether in n-hexane is governed by a) chain length and b) unsaturation of the constituent fatty acids. Glycerides containing short-chain acids are more strongly adsorbed than those containing long-chain acids and glycerides containing unsaturated fatty acids are more strongly adsorbed in direct relation to unsaturation than those containing saturated acids of the same chain-length.

IN RECENT YEARS a number of techniques have been applied to the separation of natural triglycerides, prominent among which are the low-temperature crystallization (3,5,15) and countercurrent distribution (6,7). Separation of glycerides has also been attempted on adsorption columns of alumina with some success (17,23).

Silicic acid has been used for lipid separations by a number of workers (2,8,9,14,21,22,24). One of the more recent publications on the subject is that of Hirsch and Ahrens (12), who demonstrated the separation of complex lipid mixtures into chemical classes by elution from a single column.

The present work was undertaken in an attempt to standardize a method for the fractionation of the triglyceride types on a silicic acid column and to study the fatty acid distribution in natural fats. This report is a description of the method and its application to some representative fats and oils.

Experimental

Apparatus. The all-glass apparatus used in the study is shown diagramatically in Figure 1. It is essentially a modification of the one used by Sahasrabudhe and Tuckey (18) in their studies on organic acids. The apparatus is made up of 3 parts: a reservoir (A), a mixing flask (B), and the column (C). The different parts are provided with ball and socket joints and are held together with mechanical clamps.

The reservoir is a 3-liter flask with a standard ball joint on the upper end and a socket at the lower end. The lower end of the flask is sealed into a 1-mm. capillary (E) which extends through the socket into the mixing flask (B). An 8-mm. tube (F) opening at one end in the upper part of the reservoir, extends through the joint at the base into the mixing flask. The length of the tube extending into the mixing flask is such that the lower tip is about 5 mm. below the 500-ml. level of solvent in the mixing flask.

The mixing flask is a three-necked, 1-liter flask with ball joints on the necks and a socket joint at the lower end. It is also provided with a capillary tubing (E) and an 8-mm. tube (F) extending into the column through the adapter (D).

The stirring device in the mixing flask is a modification of the one used by Hirsch and Ahrens (11). The glass rod holding the Teflon collar and the stirring bar is fused into a socket to form a cap. Rapid mixing is achieved with the rotating magnetic bar held close to the externally-placed magnetic field.

The column is 60 mm. in diameter and 210 mm. long. At one end it is connected with a stopper and a Beketel adapter. On the top is a ball joint 65/40. The column is enclosed in a water jacket.

The adapter (D), with a 35/20 ball joint on top and 65/40 socket joint at the bottom, connects the column to the mixing flask.

The length of the capillary (E) and the solvent-levelling tube (F) are so adjusted that the capillary reaches to within 1 cm. of the solid packing, and the tube (F) gives the required solvent head of the column. The three air pockets in the apparatus are interconnected and thus help maintain constant levels in the mixing flask and the column head.

Adsorbent. Silicic acid [1] (100 mesh powder) is used (16). A 5-lb. lot of silicic acid is suspended in dis-

[1] Mallinckrodt Chemical Company, Montreal, Canada, labelled as "suitable for chromatography by the method of Ramsay and Patterson."