The Composition and Yield of Crude Lipids Obtained From Soybeans by Successive Solvent Extractions[1]

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Crude lipids extracted from soybeans by petroleum solvents consist roughly of three main fractions, triglycerides, unsaponifiable matter, and phosphatides. The present communication reports on the composition and yield of crude lipids obtained from soybeans by successive extractions with both Skellysolve F (boiling range, 35° to 58° C.) and Skellysolve B (boiling range, 63° to 70° C.).

To obtain these data, approximately one bushel lots of flaked 1938 Illini soybeans were extracted in a stainless steel batch-extraction apparatus of the Soxhlet type, operated intermittently in such a manner that the miscella obtained by each flooding of the flakes with solvent could be recovered separately.[3] The solvent was partially removed from each miscella fraction while still in the extraction apparatus, using external steam coils, and later the last portion was removed on a steam bath under an air jet. Error at this point, due to oxidation of the sample, was checked by removing the solvent from one part of a sample in an atmosphere of nitrogen and comparing the iodine numbers and refractive indices of the two samples. The two pairs of values were essentially the same, showing that the effect of the air jet during the time required to remove the solvent is negligible. The extraction temperature using Skellysolve F was 28° C., and using Skellysolve B, 40° C.

This work was originally carried out using Skellysolve F, the solvent that ordinarily is used for quantitative oil determination in the laboratory, and later when comparable oil samples became available from the same lot of beans which had been extracted with a commercial solvent, Skellysolve B, they were included in the investigation.

Six fractions were obtained using Skellysolve F and five fractions using Skellysolve B. Table I shows the weight of each fraction and the number of siphonings composing each fraction, together with the weights and percentages of the total of the unsaponifiable matter and phosphatides occurring in each crude extract.

Iodine and thiocyanogen numbers, refractive index, unsaponifiable matter, and phosphorus were determined on each fraction. These values are listed in Table II. For all data reported in this paper the iodine numbers were determined using Wijs solution with a 30-minute reaction time (2); the thiocyanogen and unsaponifiable values were determined by the methods of the A.O.C.S. (2); the refractive index was evaluated at 25° C. on a Zeiss dipping-type refractometer using water-jacketed double prisms; and the phosphorus was determined colorimetrically using the method of Truog and Meyer (1).

TABLE II
Analysis of Crude Lipid Extracts

Fraction	Iodine No. (Wijs)	Refractive index (25°C.)	Thio-cyanogen No.	Unsaponi-fiable matter	Phospha-tides (P x 25)
Skellysolve F extraction				*per cent*	*per cent*
1	132.4	1.47333	82.7	.65	.53
2	132.8	354	83.1	.82	1.40
3	132.5	405	83.7	1.12	2.80
4	128.9	572	86.5	1.59	7.25
5	123.6	657	86.3	1.56	12.82
6	114.9	932	86.9	1.91	23.95
Skellysolve B extraction					
1	129.7	1.47374	87.5	.64	1.29
2	129.2	442	89.0	.89	4.35
3	126.6	558	91.9	1.14	9.78
4	122.8	673	92.0	.97	14.08
5	119.6	764	92.1	1.18	18.62

TABLE I
Yield of Crude Lipid Extracts

Fraction	Siphonings included in fraction	Total crude lipids		Unsaponifiable matter		Phosphatides (P x 25)	
		Weight	Per cent of total	Weight	Per cent of total	Weight	Per cent of total
Skellysolve F extraction		*gm.*		*gm.*		*gm.*	
1	1	3,155	74.2	20.50	66.6	16.80	38.6
2	2	792	18.6	6.50	21.1	11.09	25.5
3	3	234	5.5	2.62	8.5	6.55	15.1
4	4-5	36	0.8	.57	1.8	2.60	6.0
5	6-9	15	0.3	.23	0.7	1.88	4.3
6	10-19	19	0.4	.36	1.2	4.55	10.5
Total	19	4,251	99.8	30.78	99.9	43.47	100.0
Skellysolve B extraction		*gm.*		*gm.*		*gm.*	
1	1	4,013	86.1	25.68	80.7	51.75	53.5
2	2	464	10.0	4.13	13.0	20.25	20.9
3	3	80	1.7	.91	2.8	7.75	8.0
4	4-5	52	1.1	.50	1.6	7.25	7.5
5	6-9	52	1.1	.61	1.9	9.75	10.1
Total	9	4,661	100.0	31.83	100.0	96.75	100.0

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[2] A cooperative organization participated in by the Bureaus of Agricultural Chemistry and Engineering and Plant Industry of the U. S. Department of Agriculture, and the Agricultural Experiment Stations of the North Central States of Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin.

[3] The authors are indebted to Mr. V. F. Pfeifer of the U. S. Regional Soybean Industrial Products Laboratory for his aid in obtaining the oil samples.

Analysis of the flakes showed an original oil content of 19.00 per cent by laboratory quantitative extraction with Skellysolve F,[4] and a moisture content of 8.3 per cent. The residue from the intermittent extraction with the same solvent gave an oil and moisture content of 0.18 and 7.5 per cent, respectively. Calculated from the total amount of extract obtained by intermittent extraction, 4,251 gm., and the weight of the charge used in the extractor, 23.06 kg., the oil content of the beans before extraction was 18.43 per cent. The flakes showed an original oil content of 19.50 per cent when Skellysolve B was used as the solvent for the laboratory quantitative analysis. The residue from the intermittent extraction with this solvent contained 0.43 per cent oil and 7.4 per cent moisture. Calculated from the amount of extract obtained, 4,661 gm., and the weight of the charge used in the extractor, 24.60 kg., the oil content before extraction was 18.95 per cent. In both cases the intermittent extraction removed approximately 97 per cent as much oil as the quantitative extraction.

The iodine numbers and refractive indices were determined on the oil obtained in the analytical quantitative extractions and found to be as follows: For oil extracted with Skellysolve F, 132.0 and 1.47367, respectively; for oil extracted with Skellysolve B, 131.0 and 1.47385.

The mixed fatty acids were prepared from each sample using the A.O.C.S. method, and unsaturated acids in the mixtures were then determined by the thiocyanogen method of Kaufmann as adopted by the A.O.C.S. (2). The determination of saturated acids in the mixed fatty acids was made using the low temperature crystallization method of Earle and Milner (3). Because of the small sample weights of the Skellysolve F fractions 4, 5, and 6, the unsaponifiable matter was determined on the same sample from which the mixed fatty acids were prepared.

The results of the iodine and thiocyanogen determinations on the mixed fatty acids together with the percentages of saturated acids and the calculated percentages of oleic, linoleic, and linolenic acids are given in Table III. For the calculation of the unsaturated acids the empirical thiocyanogen values for linoleic and linolenic acids, as recently determined by Kass and coworkers (4), were substituted for the theoretical values ordinarily used in the equations for these calculations.

TABLE III

Analysis of the Mixed Fatty Acids

Fraction	Iodine No. of mixed fatty acids	T. C. No. of mixed fatty acids	Saturated acids	Oleic	Linoleic	Linolenic
Skellysolve F extraction			*per cent*	*per cent*	*per cent*	*per cent*
1	133.3	83.0	14.4	26.6	54.2	4.2
2	133.8	83.0	14.4	25.8	54.5	4.4
3	133.7	82.5	14.5	25.0	55.4	4.0
4[1]	131.5	83.0	15.0	28.8	51.9	4.2
5[1]	128.8	81.1	15.6	28.7	53.4	2.4
6[1]	125.2	79.4	16.9	29.5	51.8	1.8
Skellysolve B extraction						
1	136.9	85.3	14.2	26.1	52.2	6.9
2	137.3	85.6	14.3	25.8	51.1	7.9
3	136.6	84.3	14.7	24.2	53.3	6.7
4	134.8	84.0	15.1	25.7	51.4	6.8
5	133.5	81.5	15.8	22.7	56.0	4.3

[1] Unsaponifiable removed from sample before preparation of the mixed fatty acids.

The fatty acid components do not show the great differences from fraction to fraction that are found in the phosphatide and unsaponifiable percentages. However, the preparation of the mixed fatty acids for the later fractions showed a recovery of only approximately 70 per cent of the original starting material and when tested for solubility in acetone, 25 per cent of fraction 6 was insoluble. The figures for the percentages of the fatty acids in these fractions would be lower if they were revised to read as percentages of the original extract instead of percentages of the mixed fatty acids.

The nature of the unaccounted-for compounds in these later fractions has not been determined as yet, but they are possibly of the same general type as the phytosterols. In the preparation of the mixed fatty acids from the later fractions there was an increasing amount of solid material separating at the interface of the two liquids during the washing of the acid-ethyl ether solution with water. This material was organic and crystalline, but its actual composition has not been determined. Since the main purpose of this investigation was to determine what effect the number of extractions had on the percentage of the various fatty acids in the extract, no work was done on these other materials except rough determination of their amount.

When calculated as percentage of the mixed fatty acids, the amounts and trends of each acid in corresponding fractions show good agreement between the two solvents (Fig. 1), and there is remarkable agreement in the percentage of the total acids extracted in each fraction by each solvent (Table IV).

TABLE IV

Percentage of the Total Acids Extracted in Each Fraction

Fraction	Per cent of total oleic	Per cent of total linoleic	Per cent of total linolenic	Per cent of total saturated
Skellysolve F extraction				
1	75.1	74.7	74.3	74.5
2	18.4	18.5	19.2	18.7
3	5.3	5.6	5.5	5.6
4	0.7	0.6	0.6	0.6
5	0.3	0.3	0.2	0.3
6	0.3	0.3	0.2	0.3
Skellysolve B extraction				
1	86.4	86.2	85.3	85.8
2	9.9	9.8	11.3	10.0
3	1.6	1.8	1.7	1.8
4	1.1	1.1	1.1	1.2
5	1.0	1.2	.7	1.2

The greater solvent power of the Skellysolve B seems to be mainly for other constituents than the triglycerides and this may be due principally to the higher extraction temperature. Although the amounts of the acids removed by the Skellysolve B are slightly greater than those removed by the Skellysolve F, the difference in iodine and thiocyanogen numbers between the original extract and the mixed fatty acids prepared from the extract is much greater in the case of the Skellysolve B (Fig. 2). This seems to show the presence of some material in the Skellysolve B extract which affects the iodine and thiocyanogen numbers either because of the presence of components of lesser absorption capacity or by deleterious effect on the actual determinations. The latter would seem to be the case inasmuch as the recovery

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[4] A.O.C.S. Skellysolve F extraction method using regrind.

of the mixed fatty acids for corresponding fractions of the two solvents did not show a great deal of variation, and the odd behavior of the thiocyanogen values seems to indicate the presence of an interfering substance in the original extract which is removed during the preparation of the mixed fatty acids.

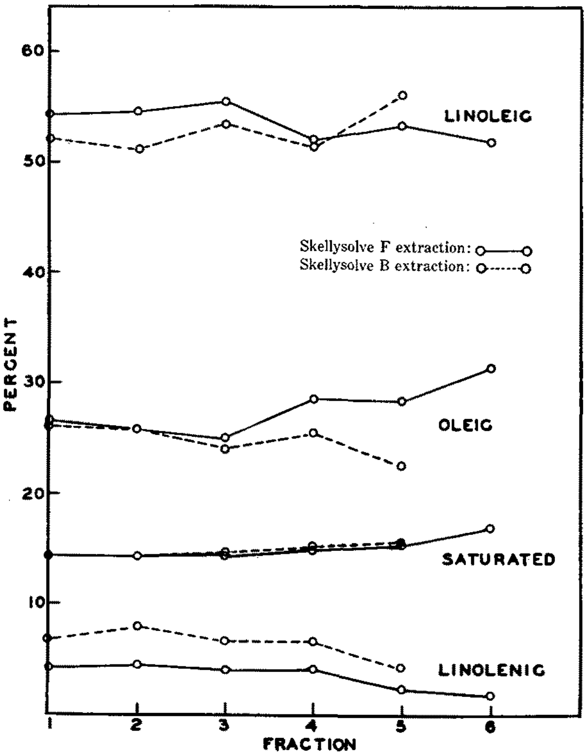

FIG. 1. Variation in percentages of fatty acids.

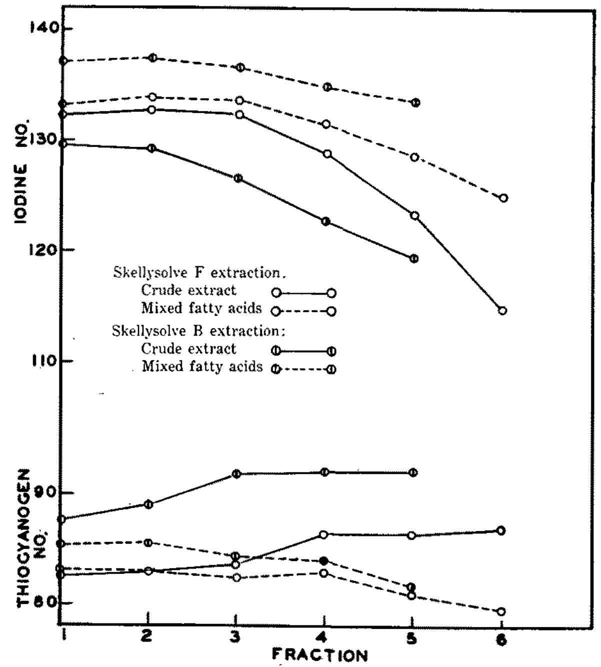

FIG. 2. Variation in iodine and thiocyanogen values on the crude extract and on the mixed fatty acids.

The extracts removed by the two solvents do not differ a great deal except as to phosphatide content which is much greater in the case of the Skellysolve B. With either solvent there is first a rapid extraction of the triglycerides, which are easily accessible because of ruptured cells, and following this a slower removal of the portions which are more inaccessible. This inaccessibility is perhaps due to unbroken cells from which removal of lipids can only be effected by diffusion through the cell walls. Simultaneously there are removed compounds which are less soluble in the solvent and which are only completely removed after more extensive extraction.

The extraction of phosphorus compounds starts more slowly than that of any other constituent measured. However, the essentially linear portion of the extraction-rate curve (Fig. 3) for the first four fractions seems to indicate that the extraction of the phosphatides is not primarily dependent upon the degree of accessibility to the solvent or to the concentration of material present, but is rather dependent upon the breaking of some bond which is holding them or some other factor not concerned with mere solubility. This is further corroborated by work now in progress in this laboratory. If quantities in individual fractions are plotted as per cent of the total extract removed in any one fraction, any of the fatty acids or the unsaponifiable matter will follow very closely the curve for the total extract. The extraction-rate curves for the total extract, unsaponifiable matter, and phosphorus are shown for the Skellysolve F extract in Fig. 3. With the solvents used, there seems to be no selective removal of triglycerides containing any one fatty acid at any point in the extraction, thus again emphasizing the fact that the soybean contains mixed, rather than simple, triglycerides.

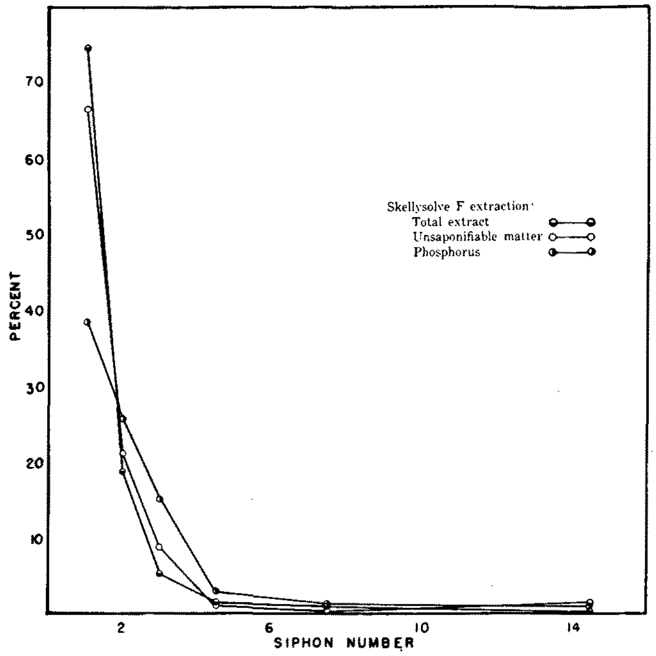

FIG. 3. Percentage of total extract per average siphon.

The six indicated points on the abscissa mark the position of the six fractions.

Summary

The composition and yield of the crude lipids obtained from soybeans by successive solvent extractions were determined for two solvents: Skellysolve F (boiling range, 35° to 58° C. and Skellysolve B (boiling range, 63° to 70° C.). The crude lipids from the first and sixth Skellysolve F extractions showed a variation of 17.5 units in iodine number and of 23.42 per cent in phosphatides. Those from the first and fifth Skellysolve B extractions showed a variation of 10.1 units in iodine number and of 17.33 per cent in phosphatides. With each solvent the series of intermittent extractions removed approximately 97 per cent as much oil as did an analytical quantitative extraction using that same solvent. The variation in percentages of the saturated and unsaturated acids was determined and found to be small. No preferential extraction of any glyceride was found. The Skellysolve B removed about the same amount of unsaponifiable matter, and slightly more than twice the weight of phosphatides, as was extracted by the Skellysolve F.

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Azo Dyes From Oleic Acid[1]

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Previous publications on the chemical conversion of oleic acid to useful products have described the arylstearic and sulfoarylstearic compounds (1, 2). The present report concerns the mononitration of the arylstearic compounds and the azo dyes which may then be prepared by the further application of typically aromatic reactions.

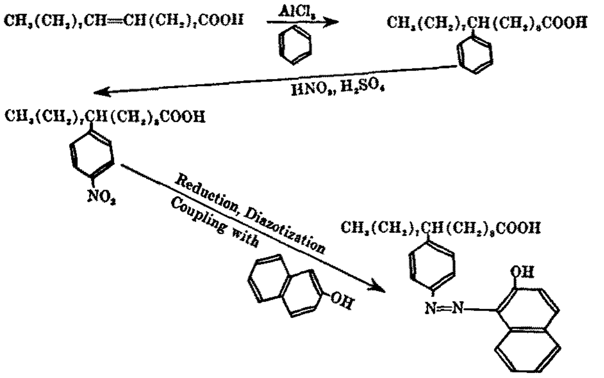
Phenylstearic acid, prepared by the Friedel and Crafts reaction between oleic acid and benzene in the presence of anhydrous aluminum chloride, is a viscous uncrystallizable oil. Nicolet and deMilt found that dinitrophenylstearic acid, which they prepared in an attempt to form a crystalline derivative, was likewise a viscous oil (3). The mononitroarylstearic compounds which we have now prepared also fail to be crystalline, presumably because they are a mixture of the 9- and 10-isomers (4).

The arylstearic compounds may be smoothly mononitrated in yields of about 90 per cent by means of a mixture of concentrated nitric and sulfuric acids. A molar ratio of nitric acid to sulfuric acid to compound to be nitrated of 2.2:2.7:1 gave satisfactory results. The following compounds were nitrated: phenylstearic acid, tolylstearic acid, ethoxyphenylstearic acid, phenoxyphenylstearic acid, phenylundecylic acid and ethyl phenylstearate. After isolation, the purity of the nitro compound was determined by titration with standard titanium trichloride solution (5) using a slightly modified method which was found to give accurate results for nitro compounds and for oil soluble azo dyes of known purity. The nitroarylstearic compounds could not be purified by distillation under reduced pressure because of extensive decomposition occurring at about 150° C.

The calcium, monoethanolamine, and triethanolamine soaps of nitrophenylstearic acid are insoluble in lubricating oil. This behavior is unexpected since calcium phenylstearate is readily dispersable and may be used to improve the properties of a Diesel lubricant (6). The insolubility of the soaps as well as the instability of the nitro compounds at higher temperatures indicate that these compounds cannot be used as addition agents to lubricants.

In the case of mononitrophenylstearic acid, the presence of the nitro group in the para position of the benzene nucleus was established by oxidative degradation with sodium dichromate and sulfuric acid. p-Nitrobenzoic acid was isolated in a yield of 60 per cent and identified by the method of mixed melting points.

From selected nitroarylstearic compounds, dyes were made by the reduction of the nitro compound to the amine, diazotization of the amine, and coupling of the diazonium compound with β-naphthol or β-naphthylamine. Using phenylstearic acid as a typical example, the equations representing the preparation of azo dyes from oleic acid are as follows:



β-Naphthol-azo-phenylstearic Acid

The dyes prepared in this manner were β-naphthol-azo-phenylstearic acid, β-naphthylamine-azo-phenylstearic acid, β-naphthol-azo-tolylstearic acid, β-naphthol-azo-ethoxyphenylstearic acid and ethyl β-naphthol-azo-phenylstearate. All of the dyes were viscous red oils ranging in purity from 45 per cent to 65 per cent as determined by titration with standard titanium trichloride solution. The colors of the dyes in alcoholic solution were quite similar and varied from a light yellow to a deep red depending upon the concentration of the dye. The dyes are not very soluble in cottonseed oil, but are readily soluble in alcohol, benzene and other organic solvents and completely insoluble in water.

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[1] Presented at the 14th Fall Meeting of the American Oil Chemists Society, Chicago, Illinois, October 2-4, 1940.