The Component Fatty Acids of Soybean Lecithin[1]

M. H. THORNTON, C. S. JOHNSON and M. A. EWAN
Department of Agricultural Chemistry, Purdue University Agricultural Experiment Station
Lafayette, Indiana

Although many preparations of lecithin from animal tissues have been made, its isolation from plant sources is considerably more difficult. For many years it has been known that soybeans contain both lecithin and cephalin. The crude phosphatides are separated from soybean oil and find extensive use in industry. However, there is relatively little information as to the fatty acids present in soybean lecithin. Some preparations of lecithin have been made and analyzed but in many cases there is considerable doubt as to the purity of material. Until recent years it was considered that the carbohydrates present in the crude phosphatides might be chemically combined with the lecithin and cephalin. In 1936 Rewald (1) showed that the phosphatides could be freed from carbohydrates by purely physical means.

Levene (2) prepared a sample of lecithin from soybeans by the use of the cadmium chloride procedure and found that the fatty acids consisted of a mixture of palmitic, stearic, oleic, linoleic and linolenic acids. The cadmium chloride procedure developed by Levene was the principal method used in attempts to prepare pure lecithin until it was modified by the work of Pangborn (3) in 1941.

Hilditch and Pedelty (4) reported the component fatty acids of soybean phosphatides. However, in this work no pure compounds were separated, the crude phosphatides being merely divided into two parts on the basis of solubility in ethanol.

In the present investigation an attempt was made to isolate a pure sample of lecithin from soybean oil for the purpose of determining its fatty acid content. The sample prepared was apparently pure lecithin except for the presence of about three per cent of cephalin. The fatty acids present were determined by the usual ester distillation method.

EXPERIMENTAL

Isolation of Lecithin

The lecithin was prepared from crude commercial soybean phosphatides which contained about 64% of acetone-insoluble material. Determination of choline and amino nitrogen indicated that these crude phosphatides contained approximately 20% lecithin and 20% cephalin.

Approximately 4 kg. of the crude phosphatides were dissolved in ethyl ether and the phosphatides precipitated by pouring the solution into two volumes of acetone. This operation was repeated twice in order to free the phosphatides from oil. The resulting precipitate of waxy material was thoroughly extracted with absolute ethanol by stirring at room temperature. The ethanol was separated from the solid by filtration. This extraction was repeated 8-10 times until the alcoholic extract was no longer highly colored.

To the alcoholic solution of phosphatides solid anhydrous cadmium chloride was added with shaking until further additions no longer caused a precipitate. The precipitated cadmium chloride salt was then separated from the alcohol by centrifugation. This precipitate was treated by the method developed by Pangborn (3). It was suspended in an amount of petroleum ether equivalent to 150 ml. for each 10 gms. of salt and this suspension was repeatedly extracted with one-fourth its volume of 80 per cent ethanol. About fifteen extractions were necessary to transfer the greater part of the salt to the ethanol layer. The dissolved petroleum ether was removed from the 80 per cent ethanol solution of the cadmium chloride salt by evaporation under reduced pressure. The alcoholic solution was then allowed to stand over night at about —10° C. The precipitate which formed was collected by centrifugation, suspended in petroleum ether, and the extraction and crystallization procedure was repeated twice. At this time the precipitate was entirely free from color. It was dissolved in chloroform and the lecithin freed from cadmium chloride by the addition of a saturated solution of ammonia in methanol. After removal of the precipitated cadmium chloride by centrifugation, the chloroform solution was evaporated to dryness under reduced pressure at 40° C. in an atmosphere of nitrogen. The excess ammonia was removed by this operation. The washing procedure suggested by Pangborn caused formation of emulsions which could be broken only with great difficulty. After evaporation of the chloroform, the lecithin was dissolved in absolute ethanol in which it was extremely soluble. The solution was practically colorless. The yield was 160 gm.

Analysis of the Lecithin

To determine the purity of the isolated material it was analyzed for nitrogen, phosphorus, fatty acids, sugar, choline and amino nitrogen. The choline was determined by the photometric modification of Beattie's method described by Thornton and Broome (5). The results of these analyses are given below.

	Calculated *	Found
	Pct.	*Pct.*
Phosphorus	3.89	4.04
Nitrogen	1.76	1.90
Choline	15.22	14.85
Fatty Acids	69.9	69.2
Amino Nitrogen	0.0	0.06
Sugar	0.0	0.0

* This calculation was based on the mean molecular weight of 278.3 found for the fatty acids of this material.

Glycerophosphoric acid was isolated from the aqueous portion of the hydrolyzate of the lecithin by the addition of barium hydroxide and subsequent precipitation of the barium salt with ethanol. It was identified by its barium and phosphorus content.

	P	Ba
Calculated for $C_3H_8O_6PBa$	10.06%	44.55%
Found	10.0 %	44.95%

[1] Journal Paper No. *124* Purdue Agricultural Experiment Station. This work was supported in part by the former U. S. Regional Soybean Industrial Products Laboratory of Urbana, Illinois, now a part of the Northern Regional Research Laboratory at Peoria, Illinois.

The Fatty Acids of Soybean Lecithin

A sample of 120 gm. of the lecithin was hydrolyzed by refluxing it with 520 ml. of 1.2 N hydrochloric acid in absolute methanol for three hours in an atmosphere of nitrogen. The methanol was evaporated under reduced pressure in a nitrogen atmosphere and the small amount of hydrochloric acid remaining was neutralized with potassium hydroxide. The hydrolysis was completed by refluxing the residue with 400 ml. of 1 N potassium hydroxide in ethanol for one hour. The ethanol was removed from the soaps by evaporation under reduced pressure in a nitrogen atmosphere. The soaps were taken up in water and the fatty acids were freed with 20% sulfuric acid. They were then extracted with ethyl ether and the ether was removed under reduced pressure in an atmosphere of nitrogen. These mixed acids had the following properties: iodine No.—134.5; thiocyanogen value—75.6; neutralization equivalent—278.3.

By the use of the crystallization method of Earle and Milner (6), the mixture was found to consist of 22.2 per cent saturated and 77.8 per cent unsaturated acids.

The saturated and unsaturated acids were esterified separately by refluxing with methanol containing 2 per cent sulfuric acid. Distillation was carried out in a fractionating column with a distilling tube 15 mm. in diameter and 33 inches in length equipped with Ewell-Lecky packing (7). The pressure during fractionation was approximately 0.5 mm.

TABLE I

Fractionation of Saturated Fatty Acid Esters

Fraction	Weight	Neutralization Equivalent	Palmitic Acid Ester	Stearic Acid Ester
	gm.		*Pct.*	*Pct.*
Original esters	.....	277.7	.....	.....
1	7.05	268.1	100.0	.....
2	2.81	268.9	100.0	.....
3	1.22	271.4	100.0	.....
4	2.56	293.5	16.0	84.0
Residue	2.45	299.6	.....	100.0
Total	16.09		71.4	28.6

On the basis of the neutralization equivalents of the fractions the saturated acids were made up of 71.4 per cent palmitic acid and 28.6 per cent stearic acid.

The free acid was recovered from Fraction 1. After recrystallization from acetone, it melted at 62.0-62.5° C. and this melting point was unchanged when the acid was mixed with palmitic acid. The free acid obtained from Fraction 4 was recrystallized four times from acetone. The crystals then melted at 69.0-69.5° C. and a mixed melting point determination confirmed the fact that it was stearic acid.

The fractionation data for the unsaturated acid esters are given in Table II.

Neutralization equivalents of the fractions indicated that only C_{18} unsaturated acids were present. The high neutralization equivalent of the residue, which was quite small, was assumed to be due to polymerization during distillation. The composition of Fractions 1 and 2 and of the residue was calculated from their iodine numbers since the thiocyanogen numbers showed that no linolenic acid was present in these fractions. The composition of the other fractions was calculated from the iodine and thiocyanogen numbers, using the empirical values developed by Kass (8).

Since methyl linoleate is more volatile than methyl oleate, and shows a tendency to concentrate in the first fractions of the distillate, it was expected that methyl linolenate would also be more concentrated in these first fractions than in succeeding fractions. However, the results in Table II show that the reverse is true. This concentration of methyl linolenate in the last fractions of the distillate is also apparent in the results obtained by other investigators (9), (10), (11).

For qualitative proof of the presence of the individual acids, Fraction 6 was used because of its relatively high linolenic acid content. A sample of 3.9 gm. of the free acids from this fraction was brominated according to the method of Eibner and Muggenthaler (12). Crystals were separated from the ethereal solution by filtration. After three recrystallizations from xylene, a yield of 0.172 gm. was obtained. These crystals melted at 179.8° C. A mixed melting-point showed that this material was hexabromostearic acid. The ether was removed from the filtrate and the residue recrystallized from petroleum ether. The melting-point of 114-114.6° C. showed that this substance was tetrabromostearic acid. This was further confirmed by the use of a mixed melting-point.

The petroleum ether solution was evaporated to dryness, debrominated, and the free acids recovered. These acids were oxidized with potassium permanganate according to the method used by Sullivan and Bailey (13). The dried hydroxystearic acids were extracted with dry ether in order to separate the dihydroxy acids. The ether was removed from the ether-soluble portion and the residue was recrystallized six times from ethanol. The crystals then melted at 130.5-130.8° C. This melting-point was unchanged when a portion of the sample was mixed with dihydroxystearic acid.

TABLE II

Fractionation of Unsaturated Fatty Acid Esters

Fraction	Weight	Neutralization Equivalent	I_2 No.	T. V.	Oleic Ester	Linoleic Ester	Linolenic Ester
	gm.				*Pct.*	*Pct.*	*Pct.*
Original esters		296.9	161.4	92.4	15.12	82.55	2.33
1	4.96	294.6	153.0	85.9	22.48	77.52	0.0
2	1.54	294.8	161.4	89.8	12.70	87.30	0.0
3	9.83	297.3	164.2	91.9	10.86	87.87	1.27
4	14.35	295.6	162.8	92.3	13.54	84.12	2.34
5	4.07	296.3	162.0	93.9	16.80	78.51	4.69
6	5.12	296.1	160.5	95.9	22.10	69.71	8.19
Residue	1.41	346.6	126.9	77.3	52.47	47.53	0.0
Total	41.28	.....	.....	.....	16.65	80.74	2.59

Table III shows a comparison between the fatty acids found in the lecithin and those of the soybean glycerides as reported in the literature (14).

TABLE III

Fatty Acids of Soybean Lecithin and Soybean Glycerides

Fatty Acid	Lecithin	Glycerides
Palmitic	15.77	6.8-14.3
Stearic	6.30	2.4- 5.5
Arachidic	0.0	0.3- 0.9
Oleic	12.98	25.9-33.7
Linoleic	62.92	50.7-58.8
Linolenic	2.02	2.1- 6.5

The glycerides were much lower in saturated acids than was the sample of lecithin analyzed. However, since the lecithin contains more linoleic and less oleic acid than do the glycerides, the iodine numbers of the acids from the two sources were approximately equal.

Summary

Lecithin was prepared from crude soybean phosphatides by the Pangborn modification of the cadmium chloride method. Results of analysis indicated that this preparation was 97 per cent lecithin and 3 per cent cephalin. Its fatty acid composition was as follows: palmitic—15.77 per cent; stearic—6.30 per cent; oleic—12.98 per cent; linoleic—62.92 per cent; linolenic—2.02 per cent.

BIBLIOGRAPHY

1. Rewald, B., Food *6*, 7 (1936).
2. Levene, P. A., and Rolf, I. P., J. Biol. Chem. *62*, 759 (1925).
3. Pangborn, M. C., J. Biol. Chem. *137*, 545 (1941).
4. Hilditch, T. P., and Pedelty, W. H., Biochem. J. *31*, 1964 (1937).
5. Thornton, M. H., and Broome, F. K., Ind. and Eng. Chem., Anal. Ed. *14*, 39 (1942).
6. Earle, F. R., and Milner, R. T., Oil and Soap *17*, 106 (1940).
7. Ewell, R. H., and Lecky, H. S., Ind. and Eng. Chem., Anal. Ed. *12*, 544 (1940).
8. Kass, J. P., Loeb, H. G., Norris, F. A., and Burr, G. O., Oil and Soap, *17*, 118 (1940).
9. Kaufmann, H. P., and Keller, M., Z. Angew. Chem. *42*, 73 (1929).
10. Gunde, B. G., and Hilditch, T. P., J. Chem. Soc. 1980 (1938).
11. Hilditch, T. P., and Jasperson, H., J. Soc. Chem. Ind. *58*, 187 (1939).
12. Eibner, A., and Muggenthaler, H., Farben. Z. *18*, 131 (1912).
13. Sullivan, B., and Bailey, C. A., J. Am. Chem. Soc. *58*, 388 (1936).
14. Hilditch, T. P., The Chemical Composition of Natural Fats. John Wiley and Sons, Inc., New York, p. 152, 1940.

Relation Between the Fatty Acid Composition and the Iodine Number of Soybean Oil

C. R. SCHOLFIELD[1] and W. C. BULL[1]
U. S. Regional Soybean Industrial Products Laboratory[2]

It has been shown by Dollear, Krauczunas, and Markley (1) that there is a high degree of constancy in the ratio of total saturated acids to total unsaturated acids in different soybean oils, but that the proportions of individual unsaturated acids vary, with corresponding variations in iodine number. This conclusion is based on the analysis of seven samples of soybean oil ranging in iodine number from 102.9 to 151.4. The present investigation was undertaken to determine the fatty acid composition of a large number of samples of soybean oil, in which the iodine numbers were distributed over a wide range of values, and to determine the relation between the iodine number and the percentage of each of the fatty acids by the use of statistical methods. This has been done, and from the relations determined it is possible to estimate the fatty acid composition of a soybean oil from a determination of its iodine number. Such an estimation may be of value in technical work on soybean oil and as a guide in breeding experiments with soybeans.

Ninety-five samples of soybeans with oils having a range in iodine values from 99.6 to 147.6 were chosen from those produced by the Bureau of Plant Industry, Soils, and Agricultural Engineering and previously analyzed in this laboratory (2). The beans were grown in 1936 to 1940 inclusive, and since harvesting had been stored at a temperature of 70° F. and a relative humidity of 18 percent.

Acid numbers were run on all samples as an indication of the preservation of the oil. The results, which were mostly low, indicated excellent preservation.

The samples were ground and, without drying the samples, the oil was extracted with petroleum ether in a percolator. The solvent was removed on a steam bath under an air jet, experience having shown that the use of an air jet in removal of the solvent has a negligible effect on the composition of crude soybean oil.

The acid number and unsaponifiable matter were determined on one portion of the oil (3-gm. sample), and the mixed fatty acids were prepared from another portion by the methods of the A.O.C.S. (3). The thiocyanogen numbers of the mixed acids were also determined by the methods of the A. O. C. S., using approximately 0.2 normal thiocyanogen solution (3). The iodine numbers of the oil and of the mixed acids were determined using Wijs solution with a 30-minute reaction time (3). The saturated acids were determined by the low-temperature crystallization method of Earle and Milner (4).

From these data the percentage of each of the unsaturated acids was calculated assuming that oleic, linoleic, and linolenic were the only unsaturated acids present. The empirical values for the thiocyanogen numbers of linoleic and linolenic acid as determined by Kass and coworkers (5) were used instead of the theoretical values formerly used. The analytical data are given in Table 1.

The linear equations best expressing the relations between the percentages of individual fatty acids in

[1] Northern Regional Research Laboratory, Peoria, Illinois.

[2] The Chemical and Engineering Sections of the Soybean Industrial Products Laboratory, Urbana, Illinois, were merged with the Northern Regional Research Laboratory, Peoria, Illinois, July 1, 1942.