Fractionation of Soybean Phosphatides With Isopropyl Alcohol[1]

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ONLY a minor part of the "lecithin" potentially available in soybean oil has found industrial application. Interest is now increasing in extending the use of soybean phosphatides, in part because of recent research work that has resulted in new information concerning the specific chemical, physical, and biological properties of the particular phosphatidic components.

The crude phosphatides are a complex mixture including oil, lecithin, cephalin, phosphoinositides, and sugars in free and bound form, together with minor amounts of sterols, sterol glucosides, pigments, and antioxidants. In earlier work at this laboratory a sample of oil-free phosphatides from hexane-extracted soybean oil was fractionated and calculated to contain 24% lecithin, 25% cephalin, and 33% phosphoinositides (7). The phosphoinositide fraction is known to consist of at least two major components which differ in composition and solubility properties. Carbohydrates comprise about 6 to 8% of crude phosphatides. Sucrose, raffinose, and stachyose have been identified as free sugar components while galactose, mannose, and arabinose have been found only after hydrolysis (9).

Laboratory fractionations have variously employed acetone, ethyl alcohol, methyl alcohol, and acetic acid. For reasons of costs, use restrictions, heat of vaporization, flash point, and similar considerations, investigation of other solvents appeared to be pertinent prior to setting up larger scale separations. Since isopropyl alcohol appeared to fill many of the requirements of a commercial solvent, the present study of its efficiency as a fractionating solvent was performed.

In the current work, commercial unbleached crude phosphatides from hexane-extracted soybean oil were extracted with successive portions of isopropyl alcohol. Data were obtained on composition of fractions and volume of solvent required.

Analytical Methods

The acetone-insoluble material in the fractions resulting from isopropyl alcohol extraction was determined by mixing the sample at about 40°C. with acetone and then cooling at 0°C. until the acetone-insoluble material had settled out. Acetone extractions were continued until little more material was dissolved, and the acetone-insoluble residue was dried in vacuum. A small amount of phosphorous-containing material, together with triglycerides, was dissolved by the acetone, the amount being partly dependent on the free fatty acid content of the extracted material (6). However no more effective fractionating solvent is known for removing the triglycerides.

Phosphorous (12), total nitrogen, choline (3), amino nitrogen (2, 13), inositol (1), and sugar (10), were determined on the fractions as described previously (7, 8).

Lecithin was calculated as 56.8x choline nitrogen. This factor is based on a molecular weight for the fatty acids of 278.3 (11). In a similar manner cephalin in the isopropyl alcohol-soluble fractions was calculated as 52.3x Burmaster (periodate) nitrogen. Since the nitrogen measured by the Burmaster procedure is less than the non-choline nitrogen (total N-choline N), part of the nitrogen is unaccounted for. Use of Burmaster nitrogen therefore gives a minimum value. Since ethanolamine occurs as a constituent of the phosphoinositides, an estimate of cephalin in the isopropyl alcohol-insoluble fractions cannot be based on Burmaster values. Sugar was calculated as galactose in order to compare with previous work. The accurate estimate of phosphoinositides is not possible since their composition is not known, but, as an approximation, values have been calculated on the basis of 12.5% inositol in pure phosphoinositides, which was used in a previous publication (7).

TABLE I

Extraction of Crude Phosphatides With Isopropyl Alcohol

No. of extract	Extract (% of crude phosphatides)	Acetone-insoluble (% of extract)	Acetone-insoluble (% of crude phosphatides)	Solvent used (ml. per 100 g. crude phosphatides)
I	28.8	31.2	9.0	100
II	12.0	42.4	5.1	150
III	4.0	49.1	1.9	150
IV	4.5	55.2	2.5	150
V	2.3	65.2	1.5	100
Total	51.6	.....	20.0	650

Extractions of Crude Phosphatides with 99.4% Isopropyl Alcohol

The crude phosphatides were mixed with 99.4% isopropyl alcohol in a Waring blendor at room temperature. Although the temperature rose during mixing, it was not allowed to exceed 40°C. during the extraction. After each of the first three extractions the mixture was allowed to stand one hour or more in order to settle out most of the solid material. The supernatant was subsequently drawn off and centrifuged. Then this residue was combined with that remaining in the mixer for further extraction. After the third extraction sufficient oil had been removed to allow filtration of the extracts through a Buchner funnel. This filtration procedure was used to remove the fourth and fifth extracts. The increased amount of extract obtained in the fourth extraction was undoubtedly due to the more complete removal of extracts by filtration.

Solvent was removed from aliquots under vacuum while carbon dioxide was bubbled through the solution. Traces of solvent remaining were taken off subsequently in a vacuum desiccator. The first extract with isopropyl alcohol was a brown fluid; the following extracts were yellow and had greasy to waxy consistency. The isopropyl alcohol-insoluble residue was a light brown powder.

In some cases proportional parts of the smaller fractions, e.g., III, IV, and V were combined for analyses. Amounts of extracts and analyses are shown

[1] Presented at the Fall Meeting of American Oil Chemists' Society, Oct. 8-10, 1951, in Chicago, Ill.

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TABLE II

Nitrogen, Phosphorous, Sugar, and Inositol of Fractions (% of Extract)

Fraction	Fraction (% of crude phosphatides)	Total N	Choline N	Van Slyke N	Burmaster N	Total P	Mol. ratio P/N	Inositol	Sugar
		%	%	%	%	%		%	%
Extract I	28.8	0.63	0.37	0.16	0.15	1.21	0.87	0.05	2.30
Extract II	12.0	0.98	0.43	0.25	0.24	1.91	0.88	0.06	2.74
Extract III, IV, and V	10.7	0.97	0.49	0.35	0.31	2.13	0.99	0.09	3.72
Isopropyl alcohol-insoluble	48.5	0.81	0.09	0.55	0.43	3.01	1.68	6.2	9.75
Ethyl alcohol extract I	6.8	1.22	0.47	0.57	0.52	2.74	1.02	.....	.....
Ethyl alcohol extract II	3.9	1.19	0.30	0.62	0.61	2.83	1.08	.....	.....

TABLE III

Composition of Fractions Obtained by Isopropyl Alcohol and Ethyl Alcohol

Fraction	Inositides		Cephalin		Lecithin		Sugar
	(% of extract)	(% of crude phosphatides)	(% of extract)	(% of crude phosphatides)	(% of fraction	(% of crude phosphatides)	(% of crude phosphatides)
Extract I	0.4	0.1	7.8	2.2	21.0	6.0	0.65
Extract II	0.4	0.1	12.5	1.5	24.4	2.9	0.33
Extract III, IV, and V	0.7	0.1	16.2	1.7	27.8	3.0	0.40
Total isopropyl-alcohol soluble	.....	0.3	.....	5.4	.....	11.9	1.38
Isopropyl alcohol-insoluble	50.0	24.2	.....	.....	5.1	2.5	4.72
Total	.....	24.5	.....	.....	.....	14.4	6.10
Ethyl alcohol extract I	.....	.....	27.2	1.9	26.6	1.8	.....
Ethyl alcohol extract II	.....	.....	31.9	1.2	17.0	0.7	.....

TABLE IV

Extension of Crude Phosphatides With Acetone Followed by Isopropyl Alcohol

Acetone extract no.	Acetone extract (% of crude phosphatides)	Acetone used (ml./100 g. crude phosphatide)	Isopropyl alcohol extract no.	Isopropyl alcohol extract (% of crude phosphatides)	Isopropyl alcohol used (ml./100 g. crude phosphatides)	Isopropyl alcohol extract (% of acetone-insoluble)
I	19.6	300	A	5.4	200	7.6
II	3.7	200	B	6.3	200	8.9
III	3.9	200	C	4.3	200	6.1
IV	1.1	150	D	3.0	200	4.3
V	0.7	150	E	2.2	200	3.2
VI	0.5	150	F	1.5	200	2.1
Totals	29.5	1150	Total	22.7	1200	32.2

in Tables I, II, and III. Of the crude phosphatides, 51.6% was soluble in isopropyl alcohol. The extract consisted of 20.0% acetone-insoluble phosphatides together with 31.6% oil based on the crude phosphatides. The phosphatides in the extract consisted of about 68% lecithin and 31% cephalin. Almost all of the phosphoinositides remained in the insoluble portion. In order to learn the completeness of isopropyl alcohol extraction, isopropyl alcohol-insoluble material was further extracted two times with absolute ethyl alcohol, using 7 ml. solvent per gram of material for each extraction. The ethyl alcohol extracted an additional 10.7% of the crude phosphatides. These contained practically all the residual lecithin together with additional cephalin.

Extraction of Crude Phosphatides with Acetone Followed by Extractions with Isopropyl Alcohol

In order to evaluate the merits of various possible fractionation procedures, the direct isopropyl alcohol extraction of crude phosphatides was compared with the isopropyl alcohol extraction of oil-free phosphatides. The crude phosphatides (100 g.) were extracted with acetone in the Waring blendor to remove the oil. It was necessary to use more solvent in the mixer to get sufficient suspension of the material than was the case with isopropyl alcohol; moreover six extractions were judged necessary to remove the oil. A part of the acetone-insoluble material was dried in vacuum. Of this residue, which represented one-half of the insoluble material, 35.3 g. were extracted 6 times with 100-ml. portions of isopropyl alcohol, which volume corresponds to 200 ml. per 100 g. of crude phosphatides. The extracts after filtration and evaporation were analyzed in the same manner described for the direct isopropyl alcohol extraction.

As shown in Table IV, acetone removed 29.5% oil and isopropyl alcohol dissolved 22.7% phosphatides. The sum of extracted material is 52.5% compared with 51.6% extracted by isopropyl alcohol directly. The amount of solvent used was 11.5 ml. acetone and 12 ml. isopropyl alcohol per gram crude phosphatide compared with only 6.5 ml. isopropyl alcohol per gram by the direct extraction.

Discussion

Selection of a procedure for commercially fractionating "soybean lecithin" depends on the products and purity of products desired and on the cost of the necessary procedures. Based upon the information of this paper and previous work, several alternative schemes may be proposed to obtain various products.

One proposal is actually on the verge of commercial implementation. The first step consists in separation of the oil from the phosphatides by acetone extraction, a procedure which is a commercial operation both in this country and in Germany (4, 5). The second step consists in fractionation of oil-free phosphatides with ethyl alcohol to give a fraction containing lecithin and cephalin, and an insoluble fraction containing primarily the two phosphoinositide fractions (7).

A simpler fractionation can be performed, as shown here, by extracting crude phosphatides with isopropyl alcohol if there is no objection to oil occurring in the soluble fraction. In such a fractionation lecithin and

phosphoinositides are quite well separated, but cephalin is distributed between the two fractions. Thus in present work when commercial crude phosphatides were extracted five times with a total of 6.5 ml. of isopropyl alcohol per gram, the combined extracts contained 61% acetone-soluble material, 23.2% lecithin, and 10.6% cephalin. Practically all the inositol-containing material and 77% of the sugar remained in the insoluble fraction.

It is obvious that the separation between oil and phosphatide components achieved by acetone extraction followed by isopropyl alcohol or ethyl alcohol cannot be obtained by the use of isopropyl alcohol alone; but the direct extraction with isopropyl alcohol gives a good separation of the inositol containing fraction from the other components and, in addition, uses a much smaller volume of solvents. If it is desirable, the oil may later be removed from the soluble portion by acetone in the usual way.

While five extractions and a total of 6.5 ml. of isopropyl alcohol per gram of phosphatides were used in this work, for some purposes a less complete extraction may be satisfactory. For example, by extracting only two times with a total of 2.5 ml. isopropyl alcohol per gram 40.8% of the crude phosphatides would be extracted as indicated by the data in Table I. This extract would contain 34% acetone-insoluble material and the remainder would be mainly oil. The fraction remaining after the isopropyl alcohol extraction would contain 91.8% acetone-insoluble material.

Separations of lecithin from cephalin in the soluble portion and of the phosphoinositides of the insoluble portion have been accomplished as yet only by methods suitable for small-scale laboratory purposes. However, current reports indicate that commercial fractionations of soybean phosphatides are following closely upon knowledge of composition and laboratory separations and that further developments depend upon the growth of more fundamental information.

Summary

Isopropyl alcohol has many advantages as a commercial solvent and gives useful separations of soybean phosphatides. When commercial crude phosphatides were extracted five times with a total of 6.5 ml. isopropyl alcohol per gram, 31.5% oil, 12% lecithin, and 5.5% cephalin were dissolved. Practically all the inositol-containing material and about 77% of the sugars remained insoluble. Further extractions with ethyl alcohol removed the remaining lecithin together with additional cephalin.

After removal of the oil from the crude phosphatides with acetone, the extraction of the isopropyl alcohol-soluble material required twice the amount of isopropyl alcohol used for the direct extraction described above.

Acknowledgment

The authors are indebted to H. H. Hall and Margaret C. Shekleton for the inositol bioassays and to C. H. Van Etten, T. A. McGuire, and Mary B. Wiele for Kjeldahl nitrogen and total phosphorous determinations.

REFERENCES

1. Atkins, L., Schultz, A. S., Williams, W. L., and Frey, C. N., Ind. Eng. Chem., Anal. Ed., *15*, 141 (1943).
2. Bu-master, C. F., J. Biol. Chem., *165*, 1 (1946).
3. Glick, D., J. Biol. Chem., *156*, 643 (1944).
4. Goss, W. H., "The German Oilseed Industry," p. 29, Hobart Publishing Company, Washington, 1947.
5. Markley, K. S., "Soybeans and Soybean Products," Vol. II, p. 604, New York, Interscience Publishers Inc., 1951.
6. Linteris, L., and Handschumaker, E., J. Am. Oil Chem. Soc., *27*, 260 (1950).
7. Scholfield, C. R., Dutton, H. J., Tanner, F. W., Jr., and Cowan, J. C., J. Am. Oil Chem. Soc., *25*, 368 (1948)
8. Scholfield, C. R., McGuire, T. A., and Dutton, H. J., J. Am. Oil Chem. Soc., *27*, 352 (1950).
9. Scholfield, C. R., Dutton, H. J., and Dimler, R. J., J. Am. Oil Chem. Soc., *29*, 293-298 (1952).
10. Somogyi, M., J. Biol. Chem., *160*, 61 (1945).
11. Thornton, M. H., Johnson, C. S., and Ewan, M. A., Oil and Soap, *21*, 85 (1944).
12. Truog, E., and Meyer, A. H., Ind. Eng. Chem., Anal. Ed., *1*, *136* (1929).
13. Van Slyke, D. D., J. Biol. Chem., *83*, 425 (1929).

[Received December 3, 1951]

Variations in the Chemical, Physical, and Organoleptic Properties of Soybean Oil Hydrogenated Under Widely Varying Conditions

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IT is generally accepted that there is a relationship between the flavor stability of soybean oil and its oxidative stability. Consequently many workers in the field subscribe to the assumption that the flavor deterioration of fats is due to an autoxidative process of some type involving the polyunsaturated glycerides (1, 2).

In the work to be reported here soybean oil was hydrogenated to various degrees of hardness under a variety of conditions so as to produce fats of widely varying oxidative stability. No correlation was observed between flavor scores of fresh or aged products and their oxidative stabilities as measured by the active oxygen method (3, 4). Furthermore the content of polyunsaturated glycerides appeared to bear no relationship to flavor stability.

As reported by other workers (5), at moderate pressure chemical selectivity was found to vary directly with catalyst concentration and with temperature up to 200°C., and inversely with hydrogen pressure and rate of agitation. But at hydrogen pressures over 500 psi. the effect of changes in catalyst concentration and agitator speed was reversed. That is, selectivity then varied inversely with catalyst concentration and directly with agitator speed.

The percentage of high-melting trans isomers formed during hydrogenation (6) is also a function of pressure and varies inversely with it. Yet higher melting products were obtained, for a given drop in refractive index, when higher pressures were used. Apparently the amount of fully saturated glycerides