Separation of Phytin from Oil Seed Protein Flours

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THERE has been a great deal of interest in the production of commercial protein products from de-fatted seed meals. Zein from corn and chemically purified protein from soybeans are probably the best known and most widely used oil seed protein products while a considerable amount of work has been carried out on the preparation of chemically purified proteins from cottonseed, peanuts, etc., by the Regional Laboratories of the Department of Agriculture and others. Comparatively little attention has been given however to the preparation of other products from the de-fatted seed meals although it has been recognized by all of the investigators in this field that certain by-products were inevitable. One of the most valuable of these by-products is phytin. This material and the inositol which may be prepared from it have considerable value and are of interest in the field of nutrition. Inositol also has possibilities for use as a polyhydric alcohol in the drying oil field.

The authors' work on the utilization of oil seeds led to the isolation of a fraction containing 13 to 17% organic phosphorus. The organic phosphorus in oil seed flour is present predominantly as phytin (2). This fraction was isolated in the preparation of chemically purified protein from Chinese tallow tree seeds, from the seeds of wild perennial gourds, and from peanut and flaxseed protein flour. Neither Painter and Nesbitt (8) nor Smith (10) isolated such a fraction in the course of extensive studies on flaxseed protein. Holland and Meinke (6) prepared stillingia protein but did not separate a phosphorus-containing fraction in the course of their investigation. Fontaine (3, 5) discusses the effect of phytic acid in cottonseed and peanut meals on the preparation of cottonseed and peanut proteins but does not report the separation of a phytic acid fraction from these meals. Of particular interest are their solubility curves of protein and phytin plotted against pH. McKinney (7) discussed a method for preparing proteins by the use of salts and dialysis that contained a minimum amount of phosphorus compounds.

The flours of flaxseed, stillingia, *Curcurbita digitata, C. foetidissima, C. palmata,* peanut, soybean, and castor bean were used in this study. The flaxseed was regular North American seed; the stillingia seeds were obtained in the Houston area; and the three Curcurbitae (wild gourds) were obtained through L. C. Curtis. The peanuts and soybeans were of normal quality, and the castor bean was a domestic variety. These seeds were ground and the oil removed with hexane. The coat of the various seeds was separated by a combination of screening and air separation to give the high protein flour.

The products were analyzed for protein by the regular Kjaldahl nitrogen method, using a factor of 6.25 to obtain the protein. Total phosphorus was determined by the method described by Fontaine (3). Inorganic phosphorus was obtained by the Pons method (9). The difference between the two gave organic phosphorus. Phytin phosphorus was obtained by a method described by Young (11). The amount of phytin was obtained by multiplying the phytin phosphorus by the factor 4.55.

During a study on protein preparation from the various flours it was noted that when the soluble material at a pH of 6.6 was boiled, a precipitation took place. This was first believed to be albumin but, on further examination, was found to be principally phytin. This fact permits an extra step to be placed in one of the methods for preparing protein to obtain a phytin concentrate. The advantage of this procedure is that it permits the isolation of a purer protein as well as a potentially valuable phytin concentrate.

The procedure used for the preparation of the various products from the flour is given in Figure 1. In this procedure the seed flour is slurried with about 15 parts of water and sufficient acid to reach a pH of 4.7. Hydrochloric or sulfurous are preferred. After agitating at room temperature for a short while, the slurry is filtered or centrifuged. The filtrate is brought to a pH of 6.6 with alkali and boiled. The resulting precipitate is removed by filtration. This dried precipitate is the phytin concentrate. The filtrate is added back as indicated in the figure. This procedure may be repeated to increase the yields to some extent. After extraction at pH of 4.7, the insoluble is reslurried in water and brought up to a pH of 11 with alkali. After agitation the dispersed protein is separated as a filtrate, leaving the residue. The dispersed protein is again brought to a pH of 4.7 with sulfurous or hydrochloric acids and the coagulated globulin removed by filtration or centrifugation. On drying, this coagulum is the chemically purified protein. The resulting solution, which is combined with that from the phytin filtration, is evaporated to form the solubles.

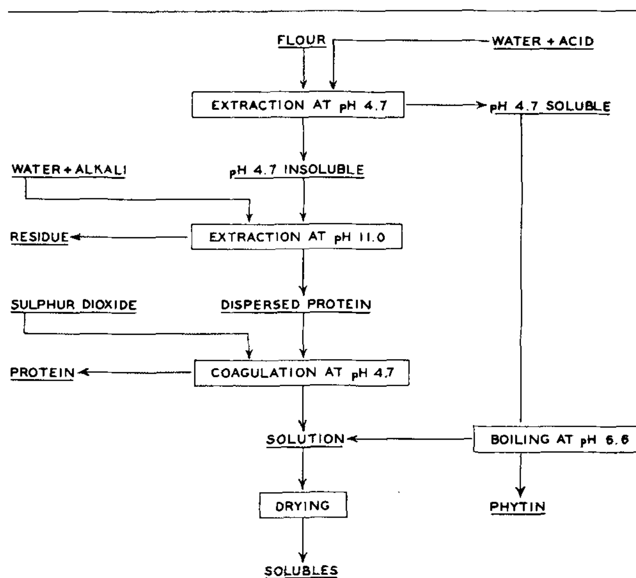

FIG. 1. Preparation of Seed Products.

The various flours used, along with the analysis for protein and phytin, are given in Table I. Flaxseed 1

represents the flour which was obtained from hexane extracted meal while Flaxseed 2 was similarly treated with acetone. It was of interest to note that stillingia flour has the highest protein content while the various wild gourds were high in phytin phosphorus.

The amount of product in each fraction that was obtained from the various flours in the procedure described is also given in Table I. It may be noted that a small but appreciable amount of crude phytin was obtained for the various seed flours with the exception of soybean, which was very small. Castor bean shows a large phytin-rich fraction, but this was due to an abnormal amount of albumin, resulting in a phytin-rich fraction of relatively low phytin content. As might be expected from the protein analysis, the highest yield of protein-rich fraction was obtained from stillingia.

The various fractions were analyzed for nitrogen, which was multiplied by 6.25 to give protein (See Table I). This factor would perhaps vary somewhat with the various proteins, but its use at least permits a comparative picture. The 100% protein figure for stillingia and *C. foetidissima,* for example, is undoubtedly not actual but is a result of the application of this arbitrary figure. With the exception of castor bean the protein-rich fraction is fairly pure. Low nitrogen was obtained on the phytin-rich fraction, indicating only minor amounts of protein present. Castor bean shows up as an exception due to the presence of albumin previously mentioned.

In Table I an indication is also given of the phytin content of the various fractions. This was obtained from the analysis of organic phosphorus and the use of a factor 4.55. Since phytin is not a definite compound, the factor is arbitrary. It was obtained as an average of various reported phosphorous contents of phytin and as the result of analysis of commercial phytins. The results on the phytin-rich fraction analysis indicates a high concentration of this material. The analytical results obtained on the various fractions from castor flour were inconsistent and have not been included in the tabulation.

The total amount of phytin present in the flour is distributed as shown in Table I. With the exception of peanut and particularly soybean, the largest amount of phytin is concentrated in the phytin-rich fraction. Considering that the phytin-rich fraction is very much less in weight than the others, this indicates a fair degree of separation. The protein-rich fraction still contains a considerable amount of organic phosphorus, but this has been cut to about half the value it would have been without the phytin coagulation step. The best results were obtained on the stillingia flour. It may be noted that the percentage of phytin recovered does not add up to 100. Since this was based on analysis of the original flour, it shows the errors one obtains in this type of analysis and distribution of products.

In the previous tables the amount of phytin was obtained by multiplying the organic phosphorus by a factor. The organic phosphorus was obtained as a difference between the total phosphorus and the inorganic phosphorus. This implies that all the organic phosphorus is present as phytin. It is known this is not strictly true. Phytin phosphorus was run on the various phytin fractions and is compared to the organic phosphorus in Table II. As one might expect, the organic phosphorus is slightly higher than the phytin phosphorus. However the phytin phosphorus analysis is difficult and is subjected to numerous errors. Therefore the organic phosphorus determinations were used in the tabulations.

TABLE II

Comparison of Phytin Analysis

Phytin from	Organic P	Phytin P
	%	%
Flaxseed 1	13.1	13.1
Flaxseed 2	13.4	10.3
Stillingia	17.6	15.4
C. digitata	16.9	16.5
C. foetidissima	17.3	15.2
C. palmata	16.1	15.7
Peanut	16.6	15.0
Soybean	15.9	12.9

In order to be sure that the material considered as phytin was truly a salt of phytic acid and not some other organic phosphorous compound, it was hydrolyzed and inositol prepared from it by the method described by Bartow and Walker (1). The oil seed inositols prepared by this procedure were tested with the results given in Table III. Inositol was also prepared from a sample of commercial phytin and all of the inositol compared with a sample of commercial i-inositol from the Corn Products Refining Company. These results confirm the actual separation of phytin.

TABLE I

The Analysis of Seed Flour Fractions

Flour	Flour Analysis		Recovered Fractions															
	% Protein	% Phytin	Phytin-Rich				Protein-Rich				Solubles				Residue			
	(% N_2 × 6.25)	% Org.P × 4.55)	% of Flour	% Protein	% Phytin	% of Flour Phytin	% of Flour	% Protein	% Phytin	% of Flour Phytin	% of Flour	% Protein	% Phytin	% of Flour Phytin	% of Flour	% Protein	% Phytin	% of Flour Phytin
Flaxseed 1	56	6.4	6	28	60	56	34	94	4	21	38	45	5	30	22	12	0	0
Flaxseed 2	51	5.9	5	24	61	52	36	98	5	31	36	42	1	6	22	16	1	4
Stillingia	71	6.4	6	6	80	75	57	100	1	9	23	32	3	11	14	17	1	2
C. digitata	67	8.4	5	8	77	46	42	96	5	25	35	24	5	21	18	18	0	0
C. foetidissima	67	7.7	4	6	79	41	38	100	5	25	40	38	2	10	18	19	1	2
C. palmata	66	9.1	6	8	73	48	37	96	5	20	32	36	2	7	25	25	1	3
Peanut	51	3.2	2	11	76	47	43	99	4	54	30	17	1	9	25	....	0	0
Soybean	56	3.6	1	16	72	20	47	94	4	52	32	12	1	9	20	22	1	6
Castor Bean	69	5.9	10	63	....	....	37	78	....	....	37	30	....	....	16	48	....	....

TABLE III
Inositol Analysis

Inositol from	Melting Point	Hydroxyl Content
	°C.	%
Flaxseed 1	226	55.4
Flaxseed 2	224	55.7
Stillingia	224	56.1
C. digitata	224	53.2
C. foetidissima	226	53.7
Peanut	223	53.8
Phytin	226	56.8
Inositol	225	56.6

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ABSTRACTS . . . . E. S. Lutton, Editor

• Oils and Fats

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Simplified procedures for determination of hypochlorous acid value of fatty oils. S. K. Basu (Calcutta Univ.). *Indian Soap J.* **17**, 216-18(1952). HOCl adds quantitatively to the ethylenic bonds of unsaturated fatty acids when these are present in the form of their Na salts. The following method was developed: Saponify 0.1-0.2 g. of the sample in a 250-ml. glass-stoppered conical flask. Neutralize the excess alkali with 0.5 *N* HCl, evaporate the alcohol on a water bath, and add 2 ml. water to dissolve the soap. Then add 5 ml. NaOCl reagent and 10 ml. glacial acetic acid. Stopper immediately, shake well, and keep in the dark for 15 minutes. Add 10 ml. 20% KI solution, allow to stand for 5 minutes in the dark, and titrate the liberated iodine with $Na_2S_2O_3$. Observed and calculated HOCl values were determined for the following fats, respectively: butter 6.7, 6.9; coconut 2.0, 2.0; castor 18.4, 18.4; peanut 18.7, 18.8; mustard 20.5, 21.1; and linseed 38.2, 38.3. (*Chem. Abs.* **46**, 8394)

Dissociation temperatures of urea complexes of long-chain fatty acids, esters and alcohols. H. B. Knight, L. P. Witnauer, J. E. Coleman, W. R. Noble, Jr., and D. Swern (East. Reg. Res. Lab., Philadelphia, Penna.). *Anal. Chem.* **24**, 1331-4 (1952). Dissociation temperatures of urea complexes with 42 long chain compounds (fatty acids, methyl esters, alcohols) have been determined. This temperature is characteristic for each compound and suggested as a method of identification.

Rendering lard at low temperatures. C. E. Swift and O. G. Hankins (Bur. Animal Ind., Beltsville, Md.). *Food Tech.* **6**, 161-5(1952). The yield of lard produced by a modified open-kettle process operated on a pilot plant scale from skin-free fatty tissue averaged 96-98% in cutting and leaf fats, respectively. The highest yields were obtained by rendering tissue which had been cooled to —18°F. and ground through an 0.125-inch plate. Lard rendered at low temperatures (203°F.) had a very light color (2.0 γ, 0.2 R Lovibond) and a less pronounced odor and flavor than ordinary commercial lard. The average stability of the samples was 6 hours AOM.

Clarification of used sunflower oil. A. S. Ivanov. *Rybnoe Khoz.* **25**, No. 3, 16-18(1949); *Chem. Zentr.* **1949**, 1026. Used sunflower oil accumulating in the production of canned fish is subjected to a step-by-step alkali refining. (*Chem. Abs.* **46**, 7795)

Contents of oil and mineral substances in the seeds of yew and fir. A. Nemec (State Research Inst. Forestry Production, Prague). *Sbornik Ceskoslov. Akad. Zemedelske* **24**, 419-21 (1951). The undried seeds of the yew *Taxus baccata* contain 63.5% oil, which is about double the amount in fir seeds. The K content of the yew seed is the highest among the conifers. (*Chem. Abs.* **46**, 7795)

The modification of fish fat during the process of its extraction. R. R. Perepletshik. *Rybnoe Khozyaistvo* **24**, No. 5, 38-41(1948); *Chem. Zentr.* (Russian Zone Ed.) **1949**, I, 748. A study was made of the changes in fish diet during boiling, pressing, drying, extraction, and distillation. During drying the acid no. increased from the original of 1.58 to 25.4; after distillation it was 26.9. The iodine no. dropped from 119.5 to 102.4 and the n^{17} increased from 1.4775 to 1.4800 during drying, owing to hydrolysis and oxidation and polymerization. During the processing the color changed from light yellow to dark brown and it acquired a characteristic sharp odor and taste. The fat was scarcely changed by extraction with naphtha. It is recommended that drying of the expressed intermediate product be eliminated by using higher pressure when expressing the oil. (*Chem. Abs.* **46**, 7795)

An approach to continuous Twitchell fat splitting. C. B. Cox. *Trans. Inst. Chem. Engrs.* (London) **27**, 123-37(1949). The paper consists of a brief description of the Twitchell process, a discussion of the basic kinetic and equilibrium data, an examination, in the light of experimental data, of the case where fresh water is used for each stage of a 3-stage split, an examination in which the same water is used over and over again for different stages of successive batches, and a consideration of the possibility of using the Twitchell process continuously under moderate pressure. The analytical methods discussed in this paper should be applicable to the operation of a counter-current column. (*Chem. Abs.* **46**, 7794)

The precipitation of oxidized linseed fatty acids by urea. G. N. Catravas and G. Knafo (Lab. Chevruel, Paris). *Bull. mens. ITERG* (Inst. tech. etudes et recherches corps gras) **6**, 43-8 (1952). The new method of precipitating aliphatic straight-chain compounds with urea was tried in the quantitative separation of oxidized products of linseed oil fatty acids from those not affected by the oxidation. It was proved that the complex compounds contain oxidized and nonoxidized products and that the proposed method is not selective. Formulas of the possible oxidation products are discussed. (*Chem. Abs.* **46**, 7794)

Ternary mixtures of fatty acids. VII. Freezing points of mixtures of capric, lauric, and myristic acids. C. Paquot and Mme. J. Petit (Lab. C.N.R.S., Bellevue, France). *Bull. soc. chim. France* **1952**, 139-40. A ternary diagram of the freezing points of mixtures of capric, lauric, and myristic acids has the same general appearance as that reported previously for mixtures of myristic, palmitic, and stearic acids. The ternary eutectic occurs at 15.5° and contains 68, 21, and 11 mole % of C_{10}, C_{12}, and C_{14}, respectively.

VIII. Freezing points of mixtures of lauric, myristic, and palmitic acids. C. Paquot, J. Mercier, and Mme. J. Petite. *Ibid.* 140. The ternary eutectic for this system occurs at 30.1° and contains 67, 21, and 12 mole % of C_{12}, C_{14}, and C_{16}, respectively. (*Chem. Abs.* **46**, 7793)

Paper chromatography in the field of fats. X. Fluorescent dyes as indicators in the paper chromatographic analysis of fat acids and fats. H. P. Kaufmann and J. Budwig (Chem. Landesuntersuchungsamt Nordrhein-Westfalen, Munster/Westf.,