[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF PITTSBURGH]

THE PHYTIN CONTENT OF FOODSTUFFS

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The quantity of phytin in vegetable foodstuffs is important because of the nutritive value of this substance. It supplies phosphorus and calcium in a readily assimilable form, and also exerts a mild laxative action. The naturally occurring product is mainly a mixed calcium, magnesium and potassium salt of inosite hexaphosphate, $C_6H_6O_6(PO(OH)_2)_6$.

Anderson[2] and others have, however, isolated esters of inosite having fewer phosphoric acid radicals. The data reported in the literature are based upon many different methods of analysis and are not consistent.

The present investigation was undertaken (1) to recheck some of the reported estimations of phytin; (2) to estimate the quantity of phytin in a number of important foodstuffs for which no data were available; (3) to estimate the loss of phytin during its preparation in a pure form; and (4) to find the extent of decomposition of phytin under certain conditions.

Method of Analysis

The best method for the estimation of phytin is that proposed by Heubner and Stadler.[3] It is based upon the fact that the iron salt of inosite phosphoric acid is very sparingly soluble in dilute acid. It is possible, therefore, to determine the amount of inosite phosphoric acid in the presence of phosphoric acid or other phosphoric acid esters whose salts are soluble in dil. hydrochloric acid by titrating with ferric chloride, using ammonium thiocyanate as indicator. The details of the method as modified are as follows.

Eight g. of the finely ground foodstuff was extracted with 200 cc. of 2% hydrochloric acid for three hours. The extract was filtered and aliquot portions of 50 cc. were taken for titration. Each portion was diluted so that the final concentration before titration was 0.6% hydrochloric acid. Ten cc. of 0.3% ammonium thiocyanate solution was used as indicator and the phytic acid was titrated with a standard solution of ferric chloride. The solution contained 0.00195 g. of iron per cc., which was close to the upper limit suggested by Heubner and Stadler. This solution was more satisfactory than a weaker one, because it gave a sharper end-point. The end-point is difficult to judge on account of the whitish precipitate of heptaferric inosite phosphate which forms. From the number of cubic centimeters of the ferric chloride solution used, the iron equivalent was obtained, and from this the equivalent of phytin phosphorus, using the factor 1.19. The weight of phytin phosphorus was calculated to the weight of phytin, by using the factor 3.55.

[1] This publication is based upon a thesis submitted by H. P. Averill in partial fulfilment of the requirements for the degree of Master of Science at the University of Pittsburgh.

[2] Anderson, *J. Biol. Chem.*, **20**, 4, 463 (1915); **18**, 3, 441 (1914).

[3] Heubner and Stadler, *Biochem. Z.*, **64**, 422 (1914).

Rather[4] has recommended 1.2% acid for extraction, but the results in Table I show that 2.0% hydrochloric acid is preferable. The former strength apparently does not inhibit completely the activity of the phytase.

TABLE I

EFFECT OF ACID CONCENTRATION

Sample	Percentage of phytin obtained by extraction with 1.2% HCl	2.0% HCl	Difference
Wheat No. 1	1.24	1.36	0.12
Wheat No. 2	1.04	1.19	.15
Barley	0.93	1.07	.14
Rye	.98	1.04	.06

A modification was necessary in the case of soy beans and nuts on account of the color and turbidity of the extract. This turbidity made it almost impossible to determine the end-point satisfactorily. It was

TABLE II

PHYTIN CONTENT OF FOODSTUFFS CALCULATED AS $C_6H_{18}O_{24}P_6$ ON AIR-DRIED SAMPLE

Foodstuff	Percentage of phytin	Foodstuff	Percentage of phytin
Barley, Success	1.07	Hempseed	2.75
Barley, Beldigrant	1.19	Millet seed	1.12
Buckwheat, Sample No. 1	1.25	Rapeseed	2.63
Buckwheat, Sample No. 2	2.39	Soy beans, Manchu	2.26
Buckwheat pure flour	1.86	Soy beans, Manchuria	1.79
Buckwheat grain minus hull	1.29	Soy beans, Ita San	2.55
Buckwheat hull	1.00	Soy beans, Elton	2.58
Oats	0.77	Soy Beans, Hamilton	1.87
Rye, Sample 1	1.04	Soy bean, Midwest	2.03
Rye, Sample 2	1.87	Soy bean, Ohio, 7496	2.44
Rye, Sample 3	1.12	Almond nuts, Sample 1	2.74
Rye flour, dark, No. 1	1.03	Almond nuts, Sample 2	2.41
Rye flour, dark, No. 2	1.46	Brazil nuts, Sample 1	2.62
Rye flour, light, No. 1	0.42	Brazil nuts, Sample 2	3.30
Rye flour, light, No. 2	.74	Filberts, Sample 1	1.72
Rye flour, straight	.96	Filberts, Sample 2	1.60
Rye middlings	3.33	Hickory nuts, Sample 1	1.67
Wheat, Baart	1.16	Hickory nuts, Sample 2	1.48
Wheat, Jenkins	1.19	Pecan nuts, Sample 1	1.40
Wheat, Marquis	1.36	Pecan nuts, Sample 2	1.52
Wheat bran	4.53	Peanuts, unroasted, No. 1	2.17
Wheat flour, Larabees Best	1.28	Peanuts, unroasted, No. 2	1.77
Wheat flour, Western Maid	1.07	Peanuts, roasted, No. 1	1.34
Wheat flour, Golden Loaf	0.66	Peanuts, roasted, No. 2	1.66
Wheat flour, Cornerstone	.68	Walnuts, English, No. 1	1.42
Wheat flour, Stewarts Special	.96	Walnuts, English, No. 2	1.45
Wheat flour, Liona	1.23	Walnuts, Black, No. 1	2.03
Wheat flour, Wabash	0.66	Walnuts, Black, No. 2	2.04
Wheat flour, Wabash, pastry	.85		

[4] Rather, THIS JOURNAL, 39, 2506 (1917).

thought that the difficulty might be due to oil in the seed, so an extraction with ether was carried out previous to the acid extraction. The difficulty was completely obviated in this way and results were obtained which are believed to be reliable.

Amount Present in Foodstuffs

Table II gives the results obtained for a variety of foodstuffs. All analyses were made in duplicate and the average result recorded. Unless otherwise stated in the table, the value given is for the whole seed or grain.

Very little work had been previously reported on the phytin content of nuts, soy beans, buckwheat or rye, so it is thought that these results will be of special interest in that respect. It will be noted that the different samples of the same kinds of nuts show fair uniformity, but that in the grains and flours there is a fairly wide variation. The hard winter wheat flours are in general higher in phytin content than the soft and spring wheat flours. The present investigation indicates that there is practically no difference between wheat and rye, either for the whole grain or for the flour, so far as the phytin content is concerned. The statement will be found in several books, however, that rye contains much more phytin than wheat. The results in general are somewhat higher than those previously recorded. This is thought to be due, in part, to improvements in the method of estimation, though other factors such as previous treatment, variety, location of growth, etc., may have been contributing factors.

Purification and Gravimetric Estimation

It was thought advisable to make a brief study of the course of purification of phytin, to find the approximate quantities lost at various stages of the procedure, and to find which treatment accomplished the greatest degree of purification. The procedure followed is that used by Anderson[5] in the preparation of a crystalline barium salt of the inosite hexaphosphoric acid. The details of the experiment are as follows.

Fifty g. of wheat bran was extracted with 2% hydrochloric acid for five hours. The extract was filtered, and to the filtrate saturated barium chloride solution was added until complete precipitation was obtained. This precipitate was filtered, dried and weighed, and a small portion (0.02 g.) was taken for analysis by the Heubner and Stadler method. The barium salt was decomposed by adding dil. sulfuric acid, and the barium sulfate was filtered off. To the filtrate, copper acetate solution was added until the copper salt of phytin was precipitated. The precipitate was filtered, washed and suspended in water. A stream of hydrogen sulfide was passed through until all of the copper had been precipitated as copper sulfide. The phytin was then separated by filtration and precipitated by barium chloride. The precipitate was filtered, washed and dried, and sampled. It was then dissolved in dil. hydrochloric acid, reprecipitated by barium hydroxide, filtered off, washed and redissolved. This re-solution and reprecipitation was repeated thrice. The precipitate was again dried, weighed and sampled.

[5] Anderson, *J. Biol. Chem.*, **44**, 2, 429 (1920).

It was then dissolved in dil. hydrochloric acid and precipitated with alcohol. This procedure was repeated once, after which the precipitate was dried, weighed and sampled. The results are shown in Table III.

TABLE III

PURIFICATION OF PHYTIN

Precipitation	Loss from one weighing to next, % I	Loss from one weighing to next, % II	Titratable phytin, % I	Titratable phytin, % II
1. First $BaCl_2$ pptn.	..	..	77.75	77.75
2. $CuAc_2$ + 1 $BaCl_2$	58.2	59.7	84.90	88.50
3. Three more Ba pptns.	22.7	24.9	92.00	92.00
4. Two alcohol pptns.	33.1	42.9	99.00	99.00

Effect of Heating, Steaming and Moisture upon Titratable Phytin

The effect of various factors upon the decomposition of phytin was studied in the following manner.

Five 8-g. samples were weighed out from each of two different varieties of wheat. The first sample was given no treatment preliminary to the extraction. The second sample was placed in the electric oven at 105° for two hours and then extracted in the usual way. The third sample was steamed for one-half hour, care being taken that there was no loss of phytin by extraction with condensed water. The fourth sample was allowed to stand for five hours with 190 cc. of water, and enough concd. hydrochloric acid was added to bring the extracting liquid to 200 cc. of 2% hydrochloric acid. The fifth sample was slightly moistened and allowed to stand for ten hours before extraction with 2% hydrochloric acid.

Table IV shows the effect of these factors upon the percentage of titratable phytin.

TABLE IV

EFFECT OF HEATING, STEAMING AND MOISTURE UPON PHYTIN CONTENT

Treatment	I		II	
	Phytin, %	Diff. due to treatment, %	Phytin, %	Diff. due to treatment, %
None	1.70	..	1.42	..
Heated at 105° for 2 hrs.	1.50	0.2	1.32	0.1
Steam for 1/2 hr.	1.62	.08	1.12	.3
Excess of water for 5 hrs.	0.94	.76	0.86	.56
Moistened for 10 hrs.	1.26	.44	.98	.44

It is evident from Table IV that the determination of phytin in foodstuffs gives results varying greatly with the previous treatment of the material under investigation. Foods cooked in either a dry or moist condition, or milled products that have been tempered before milling, will show a phptin content below that originally present. In view of the ease with which the hydrolysis takes place, it is not surprising that few investigators have succeeded in isolating significant amounts of the hexaphosphoric acid compound, even though it were the main constituent during active growth of the seed. Wide variations in the amounts of phytin found present by different investigators may be due to the same factors. Since the purified compound is comparatively stable towards

heat and acids, it seems likely that the decompositions noted above are all closely related to enzyme hydrolysis.

Summary

The phytin contents of 57 samples of foodstuffs have been estimated by the method of Heubner and Stadler. It was found preferable to use 2% hydrochloric acid for extraction. Heating, soaking and steaming were found to bring about a very perceptible decrease in the amount of phytin as estimated by titration. The course of purification of phytin when separated by Anderson's procedure has been studied to show the effect of the various steps upon the loss and purity of the different fractions.

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LEAF CYTOPLASMIC PROTEINS[1]

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The nutrition of any organism, whether animal or vegetable, is essentially a problem of cell biochemistry. Each organism utilizes its own foodstuffs, which may be presented to it in various ways, yet the nutrition of the single cells making up such an organism depends on the selective absorption of certain fairly simple substances, inorganic salts, sugars, amino acids, etc., from some medium external to the cell into the cell itself.

We are, as yet, far from having a clear idea of the mechanism of this selective absorption or, in other words, of cell permeability; it is this want of knowledge which has prevented the plant physiologist from gleaning very much beyond an empirical knowledge of plant nutrition.

As Stiles[2] has recently pointed out in his useful summary of the literature on permeability,

> "Research on the problems involved has proceeded along two rather distinct lines. In one the whole living organism has been the unit of experimentation, while in the other isolated cells and tissues have been employed..... The methods as employed today have provided a quantity of empirical information on the relation between the amount of growth of plants and the constitution of the medium external to their roots; as far as permeability problems are concerned they have not led us much further than the experiments of Sachs, Knop and other workers of their time....."

While it is probable that the cell membrane is impermeable to certain substances, there is no doubt that the protoplasmic membrane is the seat

[1] Read at the Chemistry and Plant Life Symposium, Los Angeles, August 3, 1925.

[2] Stiles, "Permeability," New Phytologist Reprint No. 13, Wheldon and Wesley, London, 1924.