Discussion and Summary.

Previous work done in this laboratory has shown that a comparatively large amount of nitrogen in amino form, as determined by the method of precipitation with phosphotungstic acid, is an indication of certain undesirable qualities in flour. These qualities in sound flour are of the same kind as those denoted by ash and acidity. Nitrogen in amino form, as measured by formol titration, is valuable together with the determination of ash and acidity in measuring quality in flour. This difference, however, should be noted: Titrable nitrogen is more uniformly distributed in the wheat kernel than are the materials which determine the amount of ash and acidity. Therefore, in clear and low grade flours, as compared with patent and straight flours made from the same sound wheat, the increase in titrable nitrogen is not proportionate to the increase in ash or acidity.

The relations of chemical constituents to the factors which determine quality in flour are not well known. The titrable nitrogen measures a certain degree of protein hydrolysis, or the presence of nitrogenous substances, similar in nature to those produced by protein hydrolysis. These substances are the ones which are indicated by the titrable nitrogen in flour from sound wheat. If wheat has been subjected to unfavorable conditions to such an extent that the proteolytic enzymes have caused splitting of the protein, the amount of titrable nitrogen would be increased. This is a field worthy of investigation. The chemical factors which determine quality in gluten are little known. The data in the present paper are offered as a contribution to the general problem. More extensive work must be done before definite conclusions can be drawn. The data show that the lower grades of flour such as the clear and low grade, made from sound wheat, do not contain nitrogenous substances such as are measured by the formol titration, in as large a proportion as ash and acidity.

[Contribution from the Laboratory of Agricultural Chemistry of the University of Wisconsin, Madison.]

THE PHYTIC ACID OF THE WHEAT KERNEL AND SOME OF ITS SALTS.

By P. W. Boutwell.[1]

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Part I.—Attention has often been called to the marked lack of uniformity which exists in the analysis of phytic acid and of its salts which have been published from time to time. An explanation of these divergent results ob-

[1] Abstract of a thesis submitted to the Graduate Faculty of the University of Wisconsin in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

tained by workers with wheat bran and of the difficulty encountered due to contamination of the product with inorganic phosphoric acid has been found by Anderson[1] to be due to the action of the enzyme phytase present in the bran, which in dilute acid solution rapidly hydrolyzes the organic phosphorus compound into inosite and phosphoric acid or lower phosphoric acid esters of inosite. Prior to this work, Anderson,[2] in an earlier paper on the organic phosphorus-containing compound in wheat bran, came to the conclusion that wheat bran does not contain phytin. As he was unable to isolate from the barium salts which he prepared any substance corresponding to a salt of inosite hexaphosphate he believed that his amorphous barium precipitates were mixtures probably of various organic phosphoric acids, and that wheat bran contains several different organic phosphoric acids. Of these he succeeded in isolating one—inosite monophosphate.[3] His method for the separation of the phytin from the bran was a modification of the method of Patten and Hart,[4] in which the substance was precipitated repeatedly from dilute hydrochloric acid solution with an equal volume of alcohol to insure the complete removal of inorganic phosphorus, which remained in solution in the dilute acid alcohol. We have made several attempts to duplicate the isolation of this compound, but without success. This failure to reproduce the results of Anderson is not strange in view of the fact that no two preparations of the crude phytin by the acid-alcohol method employed are exactly identical. We have made a large number of preparations of phytin by this method, and have found that the nature of the product varied according to the conditions of the extraction. This fact finds ready explanation in the recent work of Anderson on the hydrolysis of phytin by the enzyme phytase in dilute hydrochloric acid extracts of wheat bran.

From this earlier work it was apparent that the acid-alcohol method as previously employed for the preparation of phytin was unsatisfactory in many ways. The work of Clark[5] on the organic phosphorus-containing compound in the seeds of the Indian field mustard suggested a new method for the preparation of phytin. Schulze and Winterstein[6] called attention to the fact that phytin is less soluble in hot than in cold acetic acid. Clark in working with the seeds of the Indian field mustard made use of this principle, and from it perfected a method for the preparation of phytin. With a few modifications we have adapted this method to the preparation of phytin from wheat bran and the wheat embryo. We have found this method superior to the old acid-alcohol method,

[1] R. J. Anderson, *J. Biol. Chem.*, 20, 483 (1915).
[2] R. J. Anderson, *Ibid.*, 18, 425 (1914).
[3] R. J. Anderson, *Ibid.*, 18, 441 (1914).
[4] Patten and Hart, *Am. Chem. J.*, 31, 564 (1901).
[5] George Clark, *J. Chem. Soc.*, 105, 535 (1914).
[6] Schulze and Winterstein, *Z. physiol. Chem.*, 22, 90 (1896).

being more rapid, and giving with a better yield a homogeneous, crystalline product from which crystalline barium salts have been prepared.

Preparation of Phytin from Wheat Bran by the Method of Clark.—Two and one-half pounds of bran were extracted for five hours with 2.0% hydrochloric acid at a room temperature of about 20°. The extract was filtered through cheese cloth and allowed to settle overnight. The clear, supernatant liquid was syphoned off, heated to boiling to coagulate the protein, and allowed to cool. After settling the clear amber-colored liquid was again syphoned off from the residue, and heated to boiling. Ammonia was added until the solution was just alkaline and the boiling continued for a short time. A large quantity of a flocculent precipitate separated out. This was filtered while the solution was still hot, and was washed with boiling water. This brown, sticky precipitate was extracted with two liters of 8.0% acetic acid, and filtered from an insoluble residue. The amber-colored solution thus obtained was heated to boiling, and a large amount of phytin separated out as a fine, white precipitate which redissolved on cooling except for a slight insoluble residue which was filtered off. The clear filtrate was diluted with an equal volume of water and heated to boiling, and again made alkaline with ammonia. A large, white precipitate settled out, which was again filtered while hot, and washed thoroughly with boiling water. This white precipitate was extracted with the smallest possible amount of 0.8% acetic acid, and filtered from a slight insoluble residue. This acetic acid solution was heated to boiling, and the phytin separated out as a heavy, white, powdery precipitate, which was filtered off while hot, leaving in the filtrate all inorganic phosphates which were soluble in the acetic acid solution. This precipitate was thoroughly washed with hot water, alcohol, and ether, and dried in the air at room temperature. The yield was about 25 g. of a pure white powder, free from inorganic phosphates. It was insoluble in hot and cold water, but readily soluble in dilute mineral acids. It was soluble in cold, dilute, acetic acid, but was reprecipitated from the boiling solution. Under the microscope the substance appeared as spheroidal crystals similar in appearance to the crystalline barium salts described later.

Examination of the Crude Phytin.—From one of the air-dried samples prepared as described above a portion was dried in vacuum over phosphorus pentoxide at room temperature. After a period of six weeks constant weight was obtained. The material was extremely hygroscopic and absorbed moisture with great rapidity.

In the analysis of the sample the methods employed for the determination of calcium, magnesium and phosphorus were those suggested and used by Plimmer and Page[1] and also by Clark[2] in the analysis of similar

[1] Plimmer and Page, *Biochem. J.*, 7, 157 (1913).

[2] George Clark, *Loc. cit.*

compounds. In the estimation of carbon and hydrogen, in order to avoid the usual difficulty experienced in burning organic phosphorus-containing compounds and to prevent the formation of carbonates with the bases present, the material was mixed with a very finely ground recently fused mixture of lead chromate and potassium dichromate. In the case of the free acid and the barium salts, in order to secure complete combustion of the carbon, it was found necessary to reburn the sample, after grinding the residue with lead chromate and potassium dichromate as suggested by Anderson.[1]

On analysis the following results were obtained:

Found: C, 7.99; H, 2.49; P, 20.42; Ca, 3.65; Mg, 10.81.
Water: 22.53.

This compound does not agree with any of the simple calcium magnesium salts of inosite hexaphosphoric acid. However, the carbon and phosphorus are present, as in phytic acid, in the ratio of one atom of carbon to one atom of phosphorus.

Separation of the Free Acid from the Calcium Magnesium Salt.—Ten grams of the crude phytin were dissolved in 200 cc. of 2.0% hydrochloric acid and the solution was precipitated with barium chloride and barium hydroxide. The barium salt was filtered off, washed with boiling water, and dissolved in 2.0% hydrochloric acid, and the barium salt again precipitated as before. This process was repeated until the barium salt had been precipitated for the fifth time. The final barium salt was dissolved in 2.0% hydrochloric acid, the barium was removed with a slight excess of dilute sulfuric acid and the filtrate precipitated with copper acetate. The copper salt was filtered off, washed thoroughly with boiling water, suspended in water, and the copper removed with hydrogen sulfide, and the filtrate concentrated on the water bath to a volume of 100 cc. The solution was perfectly clear and colorless at this point, and gave no test for inorganic phosphoric acid. It was then treated with five times its volume of alcohol, and a white flocculent precipitate formed, which was allowed to settle, and was filtered off. The filtrate was again evaporated to a small volume, and was filtered from a slight residue. The further addition of alcohol gave no precipitate. The solution was evaporated on the water bath to a volume of 250 cc., and from then on under diminished pressure at a temperature of from 25 to 30° until a volume of 25 cc. was reached. At this point the solution was quite turbid, but on filtering yielded a clear and almost colorless liquid still free from inorganic phosphoric acid. The solution was set aside to dry at room temperature in vacuum over phosphorus pentoxide. As the evaporation continued the liquid remained perfectly clear and almost colorless. While it assumed the usual sirupy consistency it was not accompanied by the

[1] R. J. Anderson, *Loc. cit.*

usual darkening which phytic acid invariably shows on drying. As the drying continued it was noticed that the sirup became much thicker than does phytic acid. When evacuating the desiccator the sample foamed up, and unless the vacuum was relieved the acid would slowly bubble up and overflow the container. The interior of the desiccator was filled with a strong, acrid gas, and gave evidence of a decomposition of the acid. To prevent this decomposition it was necessary to dry the acid for some time at room temperature under ordinary pressure over phosphorus pentoxide. As the drying continued the sample began to take on the consistency of a wax, and the tendency to foam up lessened. After several weeks instead of being the usual dark-colored sirupy liquid the sample was an amber-colored solid which could be powdered when dry but which was extremely hygroscopic and took on moisture immediately on exposure to the air. The acid was completely soluble in water and in alcohol. The aqueous solution was strongly acid to litmus, and gave no immediate precipitate in the cold with ammonium molybdate in nitric acid solution. In concentrated solutions ammonium molybdate gave a heavy, white flocculent precipitate, which dissolved upon diluting with water. Silver nitrate gave a heavy, white precipitate. There was no precipitate on the addition of ammonia. On analysis the following results were obtained:

Found: C, 10.27; H, 3.47; P, 24.83.
Calc. for $C_6H_{24}O_{27}P_6$: C, 10.08; H, 3.39; P, 26.07.
Calc. for inosite hexaphosphate, $C_6H_{18}O_{24}P_6$: C, 10.90; H, 2.72; P, 28.18.

This compound does not agree in its physical properties with those of phytic acid as previously reported. Its phosphorus content is also considerably too low. What effect the decomposition which took place during the course of the drying may have had on its composition has not yet been determined.

Preparation of the Crystalline Barium Salts.—Twenty-five grams of the crude phytin were dissolved in a small volume of 2.0% hydrochloric acid. A concentrated solution of barium chloride was added and the solution made alkaline with barium hydroxide. The barium salt was filtered off and after thorough washing was dissolved in 2.0% hydrochloric acid and the precipitation repeated three more times. The final product yielded 40 g. of a fine, white amorphous powder.

Crystallization of the Barium Salt from the Cold Solution.—Ten grams of the above amorphous barium salt were dissolved in 150 cc. of 2.0% hydrochloric acid and a dilute solution of barium hydroxide added until the precipitate which first formed just failed to redissolve. The solution was filtered from the slight turbidity and the clear filtrate was allowed to stand. At the end of a few hours a heavy granular precipitate began to settle out, which clung to the bottom and sides of the beaker. After

48 hours the precipitate had settled out completely and was filtered off and washed with boiling water till free from chlorides. It was then washed and dried with alcohol and ether. A little over 5 g. of a white, hard, granular substance was obtained. Under the microscope the material appeared to be made up of fine globoids of apparently crystalline structure. The above material was recrystallized as before, yielding 2.3 g. of a fine, white, crystalline powder free from chlorides and inorganic phosphorus, identical in appearance with the first product. After drying in vacuum over phosphorus pentoxide at 105°, the sample gave an immediate test for inorganic phosphates showing that a partial decomposition had taken place. To determine the extent of this hydrolysis an inorganic phosphorus determination was made. There was found 1.28% of phosphorus as inorganic or 8.33% of the total phosphorus as inorganic.

This development of inorganic phosphorus on drying at 105° led to the investigation of other preparations, and in each case after drying at 105° the substance which had previously been free from inorganic phosphorus gave a decided test on the addition of a nitric acid solution of ammonium molybdate in the cold. Other workers have called attention to the decomposition of phytic acid and its salts. Starkenstein[1] is of the opinion that commercial phytin undergoes spontaneous decomposition. Moreover, he found that on drying his preparation at 100°, the percentage of inorganic phosphorus present was very great. Anderson[2] reports spontaneous decomposition both of phytic acid and its salts, and also states that a very perceptible increase in inorganic phosphorus occurs on drying at 105° in vacuum. In order to avoid this decomposition as far as possible it was decided to dry the material at room temperature in vacuum over phosphorus pentoxide. Accordingly from a fresh sample of the crude phytin the crystalline barium salt was again prepared according to the previous method, and after recrystallization was dried at room temperature in vacuum over phosphorus pentoxide until constant weight was obtained.

Found: C, 6.20; H, 1.37; P, 15.65; Ba, 42.19.
Water: 10.11.

Calculated for an equi-molecular mixture of the tri- and tetra-barium inosite hexaphosphates:

$C_6H_{12}P_6O_{24}Ba_3.C_6H_{10}P_6O_{24}Ba_4$ = 2267: C, 6.35; H, 0.97; P, 16.40; Ba, 42.39.
Calc. for 14 H_2O: 10.00 per cent.

Crystallization of the Barium Salt from the Boiling Solution.—Twelve grams of the amorphous barium salt previously described were dissolved in 200 cc. of 2.0% hydrochloric acid, and a dilute solution of barium hydroxide added till the precipitate which first formed just failed to re-

[1] E. Starkenstein, *Biochem. Z.*, 30, 65 (1910).
[2] R. J. Anderson, *J. Biol. Chem.*, 17, 171 (1914).

dissolve. The solution was filtered from a slight turbidity and heated to boiling, when a heavy, crystalline precipitate separated out which was washed with boiling water, alcohol and ether. The air-dried substance was a finely divided, white crystalline powder weighing 4.1 g., free from chlorides and inorganic phosphates. Viewed under the microscope the substance showed the same spheroidal structure as the preceding preparation. For analysis it was dried at room temperature in vacuum over phosphorus pentoxide, till constant weight was obtained.

Found: C, 6.83; H, 1.86; P, 16.34; Ba, 40.65.
Water: 8.76.

For two molecules of tri-barium inosite hexaphosphate, and one molecule of tri-barium inosite tetraphosphate

$$2(C_6H_{12}P_6O_{24}Ba_3).1(C_6H_{10}P_4O_{18}Ba_3) = 3{,}038.$$

Calc.: C, 7.11; H, 1.19; P, 16.33; Ba, 40.69.
For 15 H_2O: 8.88 water.

The composition of the above salt is quite close to that of tri-barium inosite hexaphosphate isolated from wheat bran by Anderson,[1] which shows the following percentage composition:

$C_6H_{12}P_6O_{24}Ba_3$ = 1066: C, 6.75; H, 1.12; P, 17.44; Ba, 38.65.

In our crystalline salt the barium is too high and the phosphorus is too low, for the simple tri-barium salt of inosite hexaphosphate. It is probable that our compound is largely tri-barium inosite hexaphosphate admixed with a small amount of the barium salts of certain lower phosphoric acid esters of inosite. Such a possible mixed salt, agreeing closely in composition with our compound, could be represented by the mixture of two molecules of the tri-barium salt of inosite hexaphosphate with one molecule of the tri-barium salt of inosite tetraphosphate. While the phytin probably exists in the bran in the form of a salt of inosite hexaphosphoric acid, during its process of separation from the bran it undoubtedly suffers considerable alteration. Anderson[2] has shown that the hydrolytic cleavage effected by the enzyme phytase results in the production of lower phosphoric esters of inosite. That a similar hydrolytic cleavage could take place in the boiling 8.0% acetic acid solution from which the phytin is prepared as the calcium magnesium salt is very probable. Again, this crystalline barium salt is prepared by precipitation from a boiling solution containing dilute hydrochloric acid where further opportunity for hydrolysis is afforded. Thus other barium salts of lower phosphoric acid esters of inosite may be precipitated along with the tri-barium salt of inosite hexaphosphoric acid.

Phytin from Wheat Embryo.—In studying the organic phosphorus-containing compound of wheat, it has been customary to look to the bran

[1] R. J. Anderson, *J. Biol. Chem.*, **20**, 493 (1915).
[2] R. J. Anderson, *Ibid.*, 20, 475 (1915).

as the most logical source of this material. In this outer, or bran layer, is concentrated a large proportion of the total phosphorus, of which only a small fraction exists in inorganic combination. There is always present in wheat bran small quantities of wheat embryo, and the question was raised as to the form in which the phosphorus exists in the embryo. The phosphorus content of the embryo is quite high, approaching that of wheat bran. In an especially good sample of wheat embryo which was used in this work the total phosphorus content was 1.21% as compared with the total phosphorus content of 1.31% in the wheat bran examined. Of this total phosphorus in the embryo a large percentage is soluble in water, and in dilute hydrochloric acid. In five hours 100 cc. of 1.0% hydrochloric acid will extract from 10 g. of embryo 0.72% phosphorus, or 57.43% of the total phosphorus. Of this total phosphorus only 0.13% exists in inorganic combination. Thus of the total soluble phosphorus in the wheat embryo about 70% exists in some form of organic combination. It was of interest to see if this organically combined phosphorus was present in the form of phytin, and if so, to see if the wheat embryo could serve as a source of this material.

Accordingly two and one-half pounds of wheat embryo were extracted for five hours with eight liters of 1.0% hydrochloric acid at a room temperature of about 20°. The extract, which was very sticky and difficult to handle, was filtered through cheese cloth and allowed to settle, and treated according to the method previously described for the preparation of phytin from wheat bran by the method of Clark. From the final acetic acid solution, on boiling, a heavy, white, crystalline precipitate of phytin settled out, which was similar in every way to the preparation obtained from wheat bran. From this preparation crystalline barium salts were prepared by the same method as employed in the case of the phytin from the wheat bran. These salts are identical in appearance and agree in composition with the corresponding salts prepared from the phytin obtained from wheat bran.

Summary.

The conflicting results obtained in the preparation of phytic acid and its salts from wheat bran by the earlier method led to the investigation of a new method for the study of this problem.

Phytin free from inorganic phosphates has been prepared both from wheat bran and from wheat embryo by precipitation from a boiling acetic acid solution. The product is a crystalline calcium-magnesium salt insoluble in water and represents a new compound from the wheat not hitherto described. It does not agree in composition with any simple calcium-magnesium salt of inosite hexaphosphoric acid.

The free acid has been separated from this preparation of phytin. It differs from the phytic acid previously described in that it is a solid sub-

stance readily undergoing spontaneous decomposition while drying in vacuum.

The same crystalline barium salts have been prepared from the phytin obtained both from wheat bran and from wheat embryo by this new method.

The barium salt crystallized from the cold dilute hydrochloric solution agrees in composition with an equi-molecular mixture of the tri- and tetra-barium salts of inosite hexaphosphoric acid.

The barium salts crystallized from the dilute hydrochloric acid solution on boiling do not agree in composition with any salts of inosite hexaphosphoric acid, but with a mixed salt consisting of two molecules of tri-barium inosite hexaphosphate, and one molecule of tri-barium inosite tetraphosphate.

The fact seems established that phytin exists in the wheat kernel as salts of inosite phosphoric acid and that phytic acid is an ester of inosite and phosphoric acid.

Part II.—Concerning the Phytase of Wheat Bran and Wheat Embryo.

The presence of a phytin-splitting enzyme in bran has been shown by Suzuki, Yoshimura and Takaishi,[1] and also by Plimmer.[2] Anderson[3] has further studied the action of this phytase, and has determined certain conditions under which it displays a maximum activity, and other conditions under which its activity is inhibited or destroyed. It was of interest in connection with the work on the phytin of wheat bran and wheat embryo, to determine, if possible, other factors affecting the activity of the phytase in the bran, and to see if its presence could be detected in the embryo.

The effect of dry heat on the activity of the phytase was first tried. Samples of wheat bran were heated at different temperatures for varying lengths of time. These samples were then extracted with water or dilute hydrochloric acid. The total phosphorus extracted as well as the inorganic phosphorus in the extract was determined. The ratio of the inorganic phosphorus to the total phosphorus extracted was used as a measure of the activity of the enzyme showing the extent to which the hydrolysis of the organic phosphorus-containing compound in the bran had been carried. As a means of comparing the results obtained from the heated bran a number of similar determinations were made on the unheated bran. In the main these results on the unheated bran are in accord with those obtained by Anderson.[4]

[1] U. Suzuki, K. Yoshimura and M. Takaishi, *Bull. Coll. of Agr. Tokyo*, **7**, 503 (1907).

[2] Plimmer, *Biochem. J.*, **7**, 43 (1913).

[3] Anderson, *J. Biol. Chem.*, **20**, 483 (1915).

[4] Anderson, *Loc. cit.*

As shown by Table I the per cent. of inorganic phosphorus extracted from the unheated bran is relatively greater during the first twenty-four hours.

In Table I when the bran was extracted for 48 hours with 0.1% hydrochloric acid, 91.21% of the total phosphorus extracted appeared as inorganic phosphorus as compared with 77.94% after 24 hours. The enzyme apparently exhibits its maximum activity with this concentration of acid.

Referring to Table I relatively little cleavage into inorganic phosphorus takes place in the first two hours of extraction with 0.2% hydrochloric acid. Contrary to the results obtained by Anderson[1] the hydrolysis in water is much greater than in the case of 0.2% hydrochloric acid. The total amount of phosphorus extracted is greater in the case of the 0.2% hydrochloric acid than with the water, but the per cent. of the total phosphorus as inorganic is much less. In the case of extracting with water for 24 hours about 80% of the total phosphorus appears as inorganic, while about 25% appears as such when 0.2% hydrochloric acid is used as the extractive. Thus it appears that acid of this concentration exerts an inhibitory action on the phytase. It is possible that this divergence from the results obtained by Anderson can be explained by differences in the bran, or even in the temperature and conditions of extraction. The fact that the activity of the enzyme may be checked by acid of this concentration should not be overlooked in interpreting the results obtained by earlier workers. Patten and Hart[2] came to the conclusion that the organic phosphorus-containing acid obtained from wheat bran was identical with phytic acid. In extracting the bran, 0.2% hydrochloric acid was used. Anderson, on the other hand, extracting with acid of the same concentration did not obtain phytic acid from bran, or salts of inosite hexaphosphate, but obtained largely inosite monophosphate[3] and inosite triphosphate.[4] We have been unable to isolate inosite monophosphate from bran after extracting with 0.2% hydrochloric acid following the method of Anderson.

With 2% hydrochloric acid the enzyme is completely destroyed. Reference to Table I will show that from 0.08 to 0.09% of inorganic phosphorus is present in the bran, or about 7% of the total phosphorus extracted. This figure is in close agreement with the work of Hart and Andrews[5] who obtained 0.088% of inorganic phosphorus or about 7% of the total phosphorus extracted, after extracting bran for 40 hours with 1% hydrochloric acid.

[1] Anderson, *Loc. cit.*

[2] A. J. Patten and E. B. Hart, *Am. Chem. J.*, 31, 566 (1904).

[3] R. J. Anderson, *J. Biol. Chem.*, 18, 441 (1914).

[4] R. J. Anderson, *Ibid.*, 20, 463 (1915).

[5] E. B. Hart and W. H. Andrews, *Am. Chem. J.*, 30, 470 (1903).

The effect of dry heat on the bran was to increase the amount of inorganic phosphorus extracted without apparently destroying the enzyme. Comparing Tables I and II it will be seen that for corresponding periods the per cent. of inorganic phosphorus extracted by 0.2% hydrochloric acid from the heated wheat bran was greater than that extracted from the unheated bran by acid of the same concentration. In the case of the bran heated for 10 hours at 125 to 135°, 2% hydrochloric acid extracted over twice as much inorganic phosphorus as from the unheated bran. Due to this increase in the amount of inorganic phosphorus produced on heating the bran, it is impossible to decide whether the action of the enzyme has been inhibited. The results of Tables II, III and IV would seem to indicate that the enzyme has not been destroyed. Even after heating to 165° for a short time and extracting for 48 hours with 0.1% hydrochloric acid, the concentration in which the enzyme effects the maximum hydrolysis, 84% of the total phosphorus extracted appeared as inorganic as compared with 91% under similar conditions of extraction before heating.

Formaldehyde is without effect on the activity of the enzyme, as shown by Table V. The presence of formaldehyde inhibits the solubility of the phosphorus in the water.

In the case of the wheat embryo the phytase was also found to be present, and exhibited its activity in a manner similar to that shown by the bran. Reference to Table VI will show that again the maximum hydrolysis took place in 0.1% hydrochloric acid. That the rate of hydrolysis is quite rapid with 0.1% hydrochloric acid is shown by the fact that after extracting for six hours, 57.78% of the total phosphorus extracted is inorganic, while at the end of 24 hours 82% appears as such. As in the case with bran with 0.2% hydrochloric acid the amount of hydrolysis decreases, and with 0.4% hydrochloric acid the activity of the enzyme is practically destroyed.

Experimental.

In each case 10 g. of the sample were extracted with 100 cc. of the solvent, and the extraction continued at a room temperature of about 20° over varying periods of time. After filtering, 20 cc. of the extract were used in each case in determining total phosphorus and inorganic phosphorus. The method adopted for determining the inorganic phosphorus was a modification of that of Hart and Andrews[1] by precipitating with ammonium molybdate in the presence of dilute nitric acid at 65°. While the results obtained by this method are not absolute, yet it can be depended upon to give with a fair degree of accuracy the amount of inorganic phosphorus present. The bran used in these experiments contained 1.31% total phosphorus, and the wheat embryo 1.21% total phosphorus. The results of the determinations are tabulated below.

[1] E. B. Hart and W. H. Andrews, *Loc. cit.*

TABLE I.

Time of extraction. Hrs.	Inorganic phosphorus in extract. Per cent.	Total phosphorus in extract. Per cent.	Total phosphorus as inorganic. Per cent.	Time of extraction. Hrs.	Inorganic phosphorus in extract. Per cent.	Total phosphorus in extract. Per cent.	Total phosphorus as inorganic. Per cent.
Unheated bran. Solvent water.							
24	0.518	0.649	79.84	48	0.801	1.186	67.60
24	0.459	0.569	80.64				
Unheated wheat bran. Solvent 0.1% hydrochloric acid.							
24	0.771	1.003	76.94	48	1.097	1.203	91.21
Unheated wheat bran. Solvent 0.2% hydrochloric acid.							
2	0.097	0.958	10.17	24	0.256	0.936	27.38
24	0.240	0.965	24.94	44	0.365	1.055	34.57
Unheated wheat bran. Solvent 2.0% hydrochloric acid.							
2	0.089	1.178	7.36	24	0.081	1.173	6.92

Wheat bran was heated to 105–110° for different lengths of time, and extracted with 0.2% hydrochloric acid, as indicated below.

TABLE II.

Time of heating, 105–110°. Hrs.	Time of extraction. Hrs.	Inorganic phosphorus in extract. Per cent.	Total phosphorus in extract. Per cent.	Total phosphorus as inorganic. Per cent.
1	2	0.111	0.910	11.09
2	2	0.139	0.916	12.43
4	2	0.174	1.001	17.21
1	24	0.409	1.075	38.08
2	24	0.489	1.159	42.19
2	24	0.326	1.036	31.45
4	24	0.280	0.894	31.46

Wheat bran was heated to 125–135° and extracted as indicated below:

TABLE III.

Time of heating. 125–135°. Hrs.	Solvent.	Time of extraction. Hrs.	Inorganic phosphorus in extract. Per cent.	Total phosphorus in extract. Per cent.	Total phosphorus as inorganic. Per cent.
2	0.2% HCl	24	0.239	1.100	21.78
10	0.2% HCl	24	0.248	1.038	23.89
10	0.1% HCl	48	0.692	1.089	63.56
10	Water	48	0.500	1.073	45.92
10	2.0% HCl	24	0.192	1.193	16.10

Wheat bran was heated to 165° for 15 minutes and extracted as indicated below:

TABLE IV.

Solvent.	Time of extraction. Hrs.	Inorganic phosphorus in extract. Per cent.	Total phosphorus in extract. Per cent.	Total phosphorus as inorganic. Per cent.
0.2% HCl	24	0.400	1.061	37.71
0.1% HCl	48	1.051	1.246	84.36

Ten grams of bran were extracted for 48 hours with 100 cc. of solvent with and without the addition of 5 cc. of a 40% solution of formaldehyde. The results below indicate that the addition of formaldehyde was without effect on the activity of the enzyme.

TABLE V.

Solvent.	Inorganic phosphorus in extract. Per cent.	Total phosphorus in extract. Per cent.	Total phosphorus as inorganic. Per cent.
0.1% HCl	1.097	1.203	91.21
0.1% HCl with formaldehyde	1.033	1.156	89.42
Water	0.801	1.186	67.60
Water with formaldehyde	0.557	0.810	68.73

10 g. of wheat embryo were extracted with 100 cc. of dilute hydrochloric acid as indicated below:

TABLE VI.

Solvent.	Time of extraction. Hrs.	Inorganic phosphorus in extract. Per cent.	Total phosphorus in extract. Per cent.	Total phosphorus as inorganic. Per cent.
0.1% HCl	6	0.362	0.627	57.78
0.1% HCl	24	0.719	0.875	82.17
0.1% HCl	48	0.730	0.852	85.62
0.2% HCl	24	0.507	0.757	66.91
0.4% HCl	24	0.109	0.512	21.07
0.5% HCl	24	0.100	0.500	20.00
0.6% HCl	24	0.088	0.561	15.69
1.0% HCl	24	0.095	0.749	12.64

Summary.

In hydrochloric acid of 0.2% concentration the hydrolysis effected by the enzyme in wheat bran as indicated by the production of inorganic phosphoric acid is inhibited, and is only about one-third as great as in the case of 0.1% hydrochloric acid, the concentration of acid in which the enzyme exhibits its maximum activity.

Dry heat increases the amount of inorganic phosphorus extracted from wheat bran without apparently destroying the enzyme.

Formaldehyde has no effect on the activity of the phytase in wheat bran.

The phytin-splitting enzyme is present in wheat embryo. It exerts its maximum activity in the presence of one-tenth per cent. HCl, and is inhibited by two-tenths per cent. HCl. The enzyme is practically destroyed by four-tenths per cent. HCl.

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MADISON, WIS.