Identification and Properties of "Phytate" in Cereal Grains and Oilseed Products

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The "phytate" content of selected cereal grains and oilseed products was determined by the commonly used method which involves precipitation from acid solution with ferric iron. To identify the chemical nature of the iron precipitable compounds, they were chromatographed on a Dowex-1 column and eluted with a linear ammonium formate gradient. This procedure separated the *myo*-inositol phosphate esters from the mono- through the hexaphosphates. Only the hexaphosphate was detected in extracts of mature seeds of corn, wheat, rice, soybean, and sesame. The phytate of corn germ, soybean meal, and soybean flakes was soluble in water to the extent of 70% or greater whereas that in sesame meal and isolated soybean protein was only slightly soluble. Pure inositol hexaphosphate in aqueous solution at pH 6 was readily destroyed by autoclaving but that in rice, wheat, and sesame meal was more stable, 5–25% loss in 2 hr at 115°. Approximately 70% of the phytate in isolated soybean protein was lost during a 2-hr period of autoclaving.

The occurrence of an organic form of phosphorus, commonly referred to as phytic acid, in plant seeds has long been recognized (Posternak, 1903), but recent nutritional observations have renewed interest in the inositol polyphosphates. Inositol hexaphosphate complexes with many mineral elements, in some cases rendering them insoluble and biologically unavailable. For example, it decreases the availability of dietary zinc (O'Dell, 1969), calcium (Harrison and Mellanby, 1939), and magnesium (Roberts and Yudkin, 1960). Its effect on iron is less clear. There is evidence that under some conditions, it decreases iron availability (Sharpe et al., 1950) and, under others, it has no effect (Morris and Ellis, 1975).

Phytate is defined as *myo*-inositol hexaphosphate and it is commonly determined in biological materials as the ferric iron precipitable phosphorus. Under the acid conditions used, inositol phosphate but not inorganic phosphate is precipitated. The chemical composition of the phosphate precipitated is not defined by this determination. Thus, the "phytate" in foodstuffs, as usually determined, could be a mixture of inositol polyphosphates. Cosgrove (1966) has shown that the "phytic acid" of soil is a complex mixture of polyphosphates, including esters of inositol other than *myo*-inositol. Johnson and Tate (1969) have shown that the "phytate" isolated from avian blood cells is primarily 1,3,4,5,6-*myo*-inositol pentaphosphate.

Neither the biological effects of the various polyphosphates nor their concentrations in natural products are well known. There is no phytate present in the early stages of seed development but at maturity it usually accounts for more than 60% of the total phosphorus. Only inositol hexaphosphate has been found in mature rice (Saio, 1964).

The purpose of this research was to define the chemical nature of the iron precipitable phosphorus, "phytate", in selected natural products and to determine its solubility and heat stability.

MATERIALS AND METHODS

Source of Materials. The corn samples, produced by the Pioneer Hi-Bred Corn Co., were of two types, a "high lysine" hybrid containing 0.44% lysine and a commercial hybrid containing 0.30% lysine. The soft wheat (Arthur variety) was produced by the Missouri Agricultural Experiment Station, the rice was donated by the Arkansas Rice Growers Cooperative, the sesame meal was donated by the John Kraft Sesame Corporation, and the soybean flakes were given by the USDA Northern Regional Laboratory. The corn germ was prepared by pilot scale dry milling of the corn samples, courtesy of Quaker Oats Company. All other products analyzed were of commercial origin. Sodium phytate, inositol hexaphosphate, from corn, type V (98% purity), was obtained from Sigma Chemical Co., St. Louis, Mo.

Analytical Methods. Phytate was determined by the methods of Early and DeTurk (1944). Finely ground samples were extracted for 18–24 hr with a solution containing 1.2% HCl and 10% Na_2SO_4. Ten milliliters of the extract was diluted with 10 ml of water and treated with 5 ml of 0.4% $FeCl_3$ in 0.6% HCl containing 5% Na_2SO_4. The phosphorus content of the insoluble ferric salt was determined colorimetrically after digestion with 3 ml of sulfuric and 5 ml of nitric acid. Phytic acid was calculated on the assumption that it contains 28.2% of phosphorus.

myo-Inositol was estimated by the colorimetric method of Lornitzo (1968). This method involves oxidation of inositol to inosose and its estimation by reaction with furfural.

Chromatographic Separation of Inositol Polyphosphates. Standard chromatograms were prepared by chromatography of partial hydrolysates of sodium phytate. Hydrolysis was performed at pH 5.2 according to the procedure of Desjobert and Petek (1956). Refluxing for 12 hr released 27% of the phosphorus and produced some of all the phosphate esters of inositol ranging from the mono- to the hexaphosphate. After 33 hr of refluxing 68% of the phosphorus was released. The proportion of the lower phosphate esters increased while the hexaphosphate was nearly exhausted.

To prepare the inositol phosphates precipitated by iron for column chromatography, the precipitate was washed twice with 3 ml of 0.5% HCl and then suspended in 5 ml of 1 *N* NaOH. The $Fe(OH)_3$ formed was removed by centrifugation and washed with 3 ml of 1 *N* NaOH. The combined supernates were adjusted to pH 7.0 with dilute HCl before chromatography.

An aliquot (10–25 mg of organic phosphorus) of the hydrolysate or the solubilized iron precipitate, pH 7.0, was applied to a Dowex 1-X8 (100–200 mesh, formate) column (0.8 × 30 cm). The esters were eluted by use of a linear gradient of ammonium formate, 0.24–1.2 *M*, pH 7.0. A total volume of 1300 ml was collected in 5-ml aliquots.

The inositol phosphates were detected qualitatively by addition of 1 ml of 12 *N* HCl followed by 0.5 ml of 1.2% $FeCl_3$ in 1.2% HCl–5% Na_2SO_4. The tubes were then heated at 40° for 20 min and cooled in ice. A white precipitate

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Table I. Phytate Concentration in Whole Grain Cereals and Oilseed Fractions[a]

Sample	Phytic acid: Phosphorus, %	Phytic acid: Calcd,[c] %
Corn, comm. hybrid	0.25 ± 0.01[b]	0.89
Corn, high lysine	0.28 ± 0.01	0.99
Wheat, soft	0.32 ± 0.003	1.15
Rice, brown	0.25 ± 0.001	0.89
Soybean Meal, comm.	0.40 ± 0 002	1.42
Soybean flakes, defatted	0.43 ± 0.002	1.52
Isolated soybean protein	0.43 ± 0.008	1.52
Sesame meal, defatted	1.46 ± 0.05	5.18

[a] At least four analyses for each sample; reported on an air dry basis. [b] Standard error of the mean. [c] Calculated phytic acid assuming 28.2% phosphorus in the molecule.

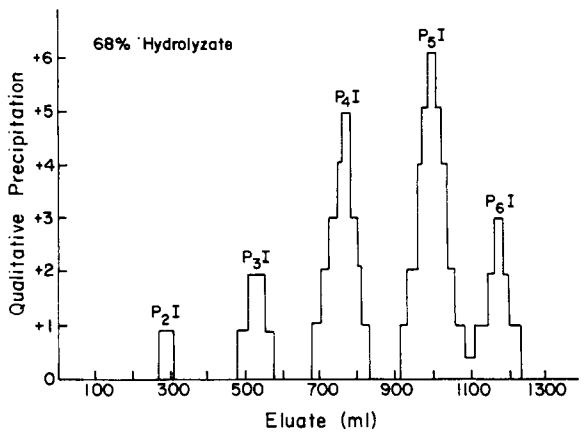

Figure 1. Chromatographic separation of the hydrolysis products of *myo*-inositol hexaphosphate on a Dowex 1-X8 (formate) column. Eluted with a linear gradient (0.24 to 1.2 *M*) of ammonium formate made from 625 ml each of 0.24 and 1.2 *M* formate buffers (pH 7.0). Compounds detected qualitatively by precipitation with ferric chloride in dilute HCl. Amount of precipitate estimated on a scale of 0 to 6.

Table II. Ratio of Phosphorus to Inositol in Fractions Obtained from Dowex 1 Chromatography (Figure 1) of the 68% Hydrolysate of Inositol Hexaphosphate

Fraction	Ester identified	P/inositol: Theory	P/inositol: Obsd
Init.	Hexaphosphate	6	5.9
P_6I	Hexaphosphate	6	5.7
P_5I	Pentaphosphate	5	4.8
P_4I	Tetraphosphate	4	4.1
P_3I	Triphosphate	3	2.6
P_2I	Diphosphate	2	1.7

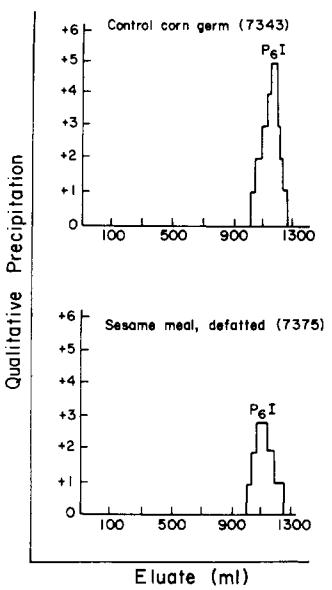

Figure 2. Chromatographic identification of the iron precipitable inositol phosphate extracted from corn germ and sesame meal with dilute HCl and Na_2SO_4.

formed in tubes containing any of the inositol esters from the di- to the hexaphosphates. However, iron salts of the mono-, di-, and triphosphates were too soluble to afford reliable quantitation. For the higher phosphate esters the precipitate from the peak tubes could be combined for quantitative determination.

Solubility and Stability of Phytate in Natural Products. To determine extractability of phytate from natural products, 8 g of sample was suspended in 200 ml of distilled water and stirred at room temperature for 18 hr. The pH was measured and then adjusted to pH 1.0–1.5 with HCl. Phytate was then precipitated with $FeCl_3$ and the quantity compared with that extracted by the usual procedure.

Stability of the phytate to autoclaving was determined by heating a slurry of the product (8 g plus 2 ml of H_2O) at 115° for 30 min. In other trials slurries were autoclaved for periods ranging from 15 to 240 min.

RESULTS

Phytate Concentrations. The phytate concentrations determined by the iron precipitation method are shown in Table I. The cereal grains contained approximately 1% of phytic acid whereas soybean meal and isolated soybean protein contained about 1.5%. The defatted sesame meal contained more than 5% phytic acid. Thus, sesame seed is a rich source of phytate and when the meal is used as a protein supplement it has a strong potential for binding trace elements.

Separation of the Inositol Polyphosphates and Identification of the Iron Precipitable Phosphorus in Natural Foodstuffs. Figure 1 shows the gradient elution pattern of a 33-hr hydrolysate of inositol hexaphosphate. Height of the blocks represents an estimate of the amount of precipitate in each tube on a scale of 0 to 6. The precipitates within a given peak were combined, the total phosphate estimated, and the inositol phosphates recovered by hydrolysis of the ferric salt in alkaline solution.

The chemical composition of each fraction was determined by estimation of its inositol and phosphorus compositions. Results of these analyses are shown in Table II. Within experimental error each fraction had the phosphorus to inositol ratio predicted from their order of elution, beginning with the first qualitatively detectable peak, the diphosphate, and progressing through the hexaphosphate.

The iron precipitable portions of the acid extracts prepared from corn germ, wheat, rice, soybean flakes, and sesame meal were chromatographed on Dowex columns as described for the hydrolysates. In all samples examined only inositol hexaphosphate was detected. Essentially all of the phytate in corn occurs in the germ (O'Dell et al., 1972). Chromatograms of the commercial hybrid germ and of sesame meal phytates are shown in Figure 2. Similar chro-

Table III. Organic Phosphorus Recovery and Phosphorus to Inositol Ratios in Fractions Recovered from Dowex Columns

Sample	Recovery, %	P/inositol	
		Theory	Obsd
Phytate standard		6	5.9
Corn germ, comm.	86.7	6	5.8
Corn germ, high lysine	90.8	6	5.9
Wheat	88.6	6	5.9
Rice	79.3	6	5.5
Soybean flakes	79.8	6	5.6
Sesame meal	82.2	6	5.7

Table IV. Extractability of Phytate by Water and Stability of Phytate to Autoclaving for 30 min

Sample	pH of extract[a]	Phytate extracted[b]		Phytate stability,[c] %
		No heat, %	Autoclaved, %	
Corn germ	6.1	86	87	94
Sesame meal	6.8	13	5	96
Soybean meal	6.8	69	84	79
Soybean flakes	6.5	97	94	91
Soybean protein	4.9	0	0	98

[a] pH of aqueous extract made at room temperature, 8 g in 200 ml of water. [b] Phytate extracted by water with and without autoclaving at 115° for 30 min. Percentage based on phytate extracted by HCl-Na_2SO_4 solution. [c] Phytate extracted by HCl-Na_2SO_4 after autoclaving compared to that before autoclaving for 30 min.

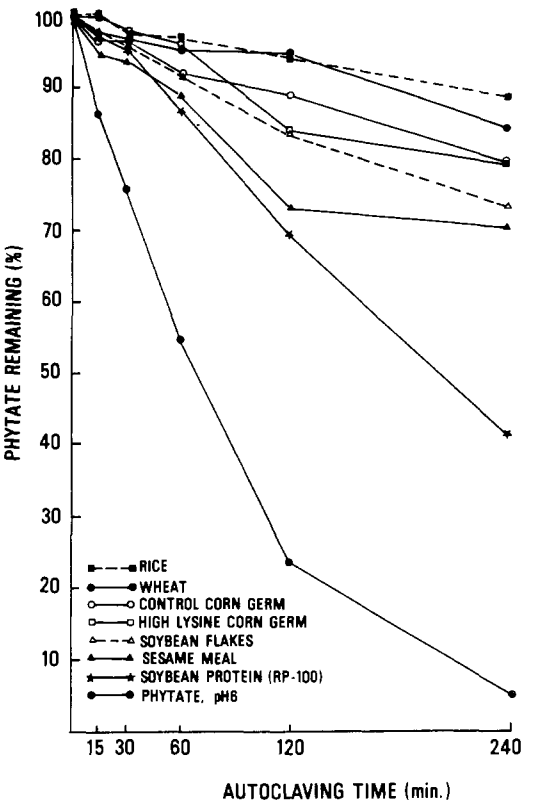

Figure 3. Rate of loss of "phytate" during autoclaving inositol hexaphosphate and moist slurries of various natural products at 115°.

matograms were observed for high lysine corn germ, soybean flakes, wheat, and rice. There was no evidence for more than one peak for any of the natural products.

The recovery of organic phosphorus from the columns loaded with iron precipitable phosphorus, and the phosphorus to inositol ratios are shown in Table III. The recovery was less than 100% for all samples including the standard phytate hydrolysate for which it was 81%. Total phytate within a peak was determined by combination of all tubes that gave a qualitative test. Small losses due to hydrolysis and lack of detection may account for the low recovery. Although the ratios of phosphorus to inositol were less than six they were, within experimental error, compatible with that of a hexaphosphate. From these results it may be concluded that the "phytate" in these products is *myo*-inositol hexaphosphate.

Solubility and Stability of Phytate in Natural Products. The extractability of phytate from a product is dependent upon its chemical environment, i.e., the type of protein and cations with which it is associated, and on the pH of the solvent. It is assumed here, as it is generally, that all of the phytate is extracted by a high concentration of H^+ and a high ionic strength. When water alone is the solvent, the final pH as well as the solubility depend on the product being extracted. The water solubility of phytate in various products is shown in Table IV. Also presented are data related to the effect of autoclaving on phytate extractability and stability. From these results it is clear that autoclaving has little effect on phytate solubility in water. Furthermore, autoclaving for 30 min at 115° has a minimal effect on the quantity of "phytate" in the products. However, it should be noted that the nature of the iron precipitable phosphorus may be changed Although the data are not shown here, autoclaving soybean flakes for 2 hr converted at least 10% of the inositol hexaphosphate to the pentaphosphate. After 4 hr, the tetraphosphate appeared and the quantity of penta- was almost equal to that of the hexaphosphate.

Nearly 90% of the phytate in corn germ and soybean flakes is soluble in water. However, none of the phytate bound to isolated soybean protein is soluble in water and only approximately 10% of that in defatted sesame meal is soluble. Lolas and Markakis (1975) found that more than 99% of the phytic acid in beans is soluble in water.

Since phytate in foods can be detrimental to the absorption of essential trace elements, such as zinc, it is desirable to know something about the rate of destruction of phytate during heat processing. To this end, various products were autoclaved in the moist state. Parenthetically, it can be stated that the amount of water added during autoclaving had little or no effect on the rate of phytate destruction. The rate of decrease of phytate with time of heating is shown in Figure 3. Autoclaving inositol hexaphosphate in aqueous solution (pH 6.0) resulted in nearly an 80% loss of iron precipitable phosphorus in 2 hr; approximately 50% was lost within 1 hr. The next most labile source of phytate is that in isolated soybean protein, 70% loss in 2 hr. The phytate in the other products is relatively stable. Losses in 2 hr varied from 25% in sesame meal to 5% in rice and wheat. Lease (1966) observed no decrease in the phytate content of sesame meal after autoclaving for 2 hr and only a 22% decrease after 4 hr. These results suggest that the rate of phytate destruction, probably by hydrolysis, is influenced by its protein and/or cation environment.

DISCUSSION

The term "phytate" is not well defined when it is applied to the organic phosphorus compounds in natural products determined by the acid ferric iron precipitation method. Many inositol phosphate esters are precipitated under the conditions commonly used. These compounds include the polyphosphates of inositol isomers other than *myo*-inositol. In this study it was shown that all of the *myo*-inositol phosphates from the di- through the hexaphosphate form insoluble iron complexes. However, the mono-, di-, and triphosphates are appreciably soluble and are not quantitatively precipitated. The monophosphate did not precipitate at the concentrations tested in this study. Mollgaard (1946) stated that inositol mono- and diphosphates are not measured analytically by the iron precipitation method. The present results agree that the diphosphate is not quantitatively precipitated. Whether or not a precipitate is observed depends on concentrations.

Although the effects of penta-, tetra-, and triphosphates on nutrient availability are not known, it appears that the lower phosphates do not occur at detectable concentrations in mature plant seeds of commerce. From the present study, it is clear that they might arise during processes which involve prolonged heat treatment. Enzyme hydrolysis will also give rise to a series of inositol polyphosphates. Tomlinson and Ballou (1962) treated phytic acid with phytase from wheat bran and isolated the *myo*-inositol 1- and 2-monophosphate, 1,2-diphosphate, 1,2,3-triphosphate, and 1,2,5,6-tetraphosphate. The pentaphosphates were present but were not characterized.

Phytate can be readily extracted from corn germ and soybean flakes but it is tightly bound in sesame meal and to isolated soybean protein. Water extraction does not offer a promising practical method of removing phytate from foodstuffs, but it will remove phytate from some products during processes in which water is added and then removed. For example, phytate is removed from corn products during the wet milling process.

These results confirm the usual assumption that the iron precipitable phosphorus in plant seeds is chiefly *myo*-inositol hexaphosphate. It is also apparent that the usual cooking procedures will not destroy an appreciable proportion of phytate in natural foodstuffs. Neither is it likely to materially change the nature of the "phytate". Some foodstuffs subjected to water extraction may lose appreciable quantities of phytate. The rate of destruction of inositol hexaphosphate by heat is low when it is associated with proteins and/or cations in natural products. The cations naturally associated with the phytate are not known, but as pointed out previously (O'Dell et al. 1972), phytate could not possibly exist in cereal grains totally as phytin, the calcium–magnesium salt, because there is not sufficient calcium present to account for the necessary stoichiometry.

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Synthesis and Flavor Properties of Some Alkyl-Substituted α-Pyrone Derivatives

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A general synthesis of 6-alkyl-α-pyrones has been achieved in ca. 40% overall yield by acylation of methyl 3-butenoate under Friedel–Crafts conditions followed by cyclization of the intermediate unsaturated keto esters at 490°C. The gas chromatographic retention indices (I_E values), ir, NMR, and mass spectral data for 6-alkyl- and 6-alkyl-4-methyl-α-pyrones are presented. The organoleptic properties of these alkyl-substituted α-pyrones and related compounds are discussed.

Lactones are of considerable significance to the fragrance and food industries due to their potent organoleptic characteristics which may convey either desirable or objectionable qualities. Much of this information has been summarized by Forss (1972).

International Flavors and Fragrances (IFF-R&D), Union Beach, New Jersey 07735.

Although the odor and taste properties of the saturated γ- and δ-lactones are well known, less organoleptic information is available on the unsaturated lactones, particularly the δ-lactones containing double bonds in the ring. (Although Chemical Abstracts classifies the six-membered δ-lactones as derivatives of 2(*H*)-pyran-2-one, the older terms α-pyrone and δ-lactone, preferred by most authors, are used in this paper.) Nobuhara (1968, 1969a,b) reported multistep syntheses of alkyl-substituted unsaturated δ-lactones in which the position and number of double bonds