

Isolation and Identification of Soluble Magnesium and Potassium Phytates from Rice Bran

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ABSTRACT

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Soluble magnesium and potassium phytates and inorganic magnesium and potassium phosphates in rice bran were isolated on Sephadex G-25-50. The eluting phosphorus, magnesium, and potassium components were identified by inductively coupled plasma spectroscopy. The isolated soluble magnesium and potassium phytates had low metal to phytate ratios: approximately two magnesium ions and one potassium ion per phytate

molecule at endogenous pH (7.1). The compositions of these soluble metal-phytate species were found to be pH-dependent. The number of magnesium and potassium ions associated per phytate molecule decreased with decreasing pH. At pH 2.7, no potassium ions were found associated with the soluble phytate, and the magnesium-to-phytate ratio was less than 1:1.

Most metal-phytate complexes are insoluble at intestinal pH (approximately pH 6.0-7.4). This insolubility of the metal-phytates has led to the proposal that phytic acid can cause reduced mineral bioavailability (Cheryan 1980, Graf 1983). In an earlier study of the solubility behaviors of the minerals and phytic acid in rice bran, it was shown that at pH 7.1 approximately 86, 88, and 64% of the phosphorus, magnesium, and potassium components, respectively, were insoluble (Champagne et al 1985a). The solubility behaviors of magnesium and potassium as a function of pH were shown to follow that of the phosphorus component; namely, low solubility at physiologic pH and high solubility below pH 5 (Champagne et al 1985a,b). Because approximately 90% of the phosphorus in rice bran is phytic phosphorus (Juliano 1972), these results provide evidence for the presence of magnesium and potassium phytates.

The solubility characteristics as a function of pH of systems containing metal ions and phytic acid at various molar ratios have been studied (Jackman and Black 1951, Oberleas and Moody 1981, Tangkongchitr et al 1982, Evans and Pierce 1981, Cheryan et al 1983, Grynspan and Cheryan 1983). Only a few studies have examined the composition of the *soluble* metal-phytates. In 1976, Morris and Ellis reported the isolation of monoferric phytate from wheat bran. Recently, Graf (1983) has determined the existence of two soluble Ca^{+2} -phytate species, Ca_1 - and Ca_2 -phytate, in a model system by Ca^{+2} -selective potentiometry.

In the investigations reported here, the water-soluble phosphorus, magnesium, and potassium components in rice bran were examined by gel filtration on Sephadex G-25-50 followed by inductively coupled plasma spectroscopy (ICP) of the eluting fractions. The Sephadex G-25-50 packing was found to be suitable for isolating the soluble magnesium and potassium phytates from the soluble inorganic magnesium and potassium phosphates. ICP analyses of the eluting fractions were used to quantify their phosphorus, magnesium, and potassium compositions. The fractionation of inositol polyphosphates on Sephadex has been previously achieved (Steward and Tate 1969, 1971).

MATERIALS AND METHODS

Bran Samples

Rough rice of the LaBelle variety was obtained from the 1983 crop of the Louisiana Agricultural Experiment Station (Rice Research) in Crowley, LA. It was dehulled by a Satake dehuller. The bran (approximately 6% removal by weight from brown rice kernel) was collected by milling using a McGill rice miller.

Reagents

Sodium phytate (>98% pure) and Sephadex G-25-50 (excludes molecular weights over 5,000) were obtained from Sigma Chemical Co. Phosphorus, magnesium, and potassium standards were prepared using certified atomic absorption reference solutions from Fisher Scientific Co. Blue Dextran, a commercial anhydroglucose polymer (Pharmacia Fine Chemicals) with a molecular weight of two million was used for determining the void volume of the Sephadex column.

Equipment

A 26 mm × 40 cm column from Pharmacia Fine Chemicals was employed for the gel filtration separations. A Buchler Fractometre 200 automatic fraction collector in the drop-counting mode was used to collect fractions from the gel filtration column. Phosphorus, magnesium, and potassium concentrations were determined using an Applied Research Laboratories model 34100 ICP spectrometer.

Sample Preparations

Five-gram samples of rice bran were incubated for 1 hr in polyethylene bottles containing 100-ml solutions of distilled-deionized water with 0-25 ml of 0.18N HCl. Immediately following incubation, these slurries were centrifuged for 30 min at 19,600 × g and the supernatants decanted. These supernatants were filtered through 0.45-μm membrane filters, and the pH values of the filtrates were determined.

Column Preparation

Columns were prepared and packed after equilibration with eluant (distilled-deionized water, 0.1M sodium chloride, or 0.1M lithium chloride), in accordance with the instructions given in a booklet by Pharmacia Fine Chemicals (1975). A constant elution rate (approximately 4-5 min/5 ml) was established for each column before sample analysis. The void volume of each column was taken to be the elution volume of Blue Dextran, approximately 60-70 ml.

Sample Analyses

Each sample was layered on a polyamide net that covered the gel surface, and 40 5-ml (128 drops) fractions were collected. The fractions were analyzed directly by ICP for total phosphorus, magnesium, and potassium.

Between sample analyses, each column was flushed with 100 ml of eluant.

RESULTS AND DISCUSSION

Soluble Components Extracted at Endogenous pH: Distilled-Deionized Water as Eluant

A typical gel filtration chromatogram, using distilled-deionized water as eluant, is shown in Figure 1 for the separation of the water

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soluble phosphorus, magnesium, and potassium components in the rice bran slurry at endogenous pH (7.1). Two phosphorus-containing peaks were seen; the first peak (maximum: fraction 18) and second peak (maximum: fraction 24) positions were found to contain phytic phosphorus and inorganic phosphate, respectively, when compared to chromatograms of standard solutions. Approximately 86% of the soluble phosphorus was determined to be phytic phosphorus, whereas 13% was inorganic phosphate (Table I).

The maximum of the first magnesium peak and that of the first potassium peak were found to coincide with the maximum of the phytic phosphorus peak. The maximum of the second magnesium peak and that of the second potassium peak were one fraction later in eluting than the maximum of the inorganic phosphate. This trailing of the magnesium and potassium peaks was seen also in the separation of a solution of K_2HPO_4 on the Sephadex G-25-50 column. The maximum of the potassium peak was one fraction behind that of the phosphorus peak.

The magnesium and potassium peaks were skewed, with diffuse front edges and sharp back edges, as shown in Figure 1. The phosphate peak also was skewed, but the front edge was sharp while the back edge was diffuse. Neddermeyer and Rogers (1968) observed peak profiles for inorganic salts eluted with water from Sephadex G-10 and G-25 similar to those observed here. These investigators attributed the skewing to a Donnan salt-exclusion effect that arises between the ionic solute and a small number of fixed carboxylate anions within the Sephadex matrix. The Donnan salt-exclusion effect restricts the penetration of ions into the charged matrix. On the front side of the peak, the charged sites hindered the diffusion of the potassium and magnesium ions into the gel, whereas on the back side they enhanced diffusion out of the gel interior. The Donnan salt-exclusion effect on the negative phosphate anions was the opposite. This effect would explain the

maxima of the potassium and magnesium peaks not coinciding with the phosphate peak maximum. Thus, there is strong evidence that the magnesium and potassium components were associated with the inorganic phosphate component.

Quantitation of the chromatograms revealed that, on the average, approximately two magnesium ions and one potassium ion were associated per phytate molecule. On the average, one potassium ion and 0.5 magnesium ion were associated per molecule of inorganic phosphate. Approximately 75% of the soluble magnesium was associated with the phytate, and 25% was associated with the inorganic phosphate (Table II). Approximately 13 and 22% of the soluble potassium was associated with the phytate and inorganic phosphate, respectively. Approximately 65% of the soluble potassium did not elute in the first 40 fractions (200 ml) collected, whereas the potassium from a solution of potassium chloride put on the column eluted in 34 fractions (170 ml) with 98% recovery. Because molecules (or ions) elute from the Sephadex bed in order of decreasing molecular (or ionic) size, collecting 40 fractions should have been sufficient for the recovery of the soluble potassium extracted from the rice bran. Thus, the potassium that did not elute in the first 40 fractions was probably in the form of un-ionized species that were adsorbed by the column bed.

pH Effects

The effects of decreasing the pH of the rice bran slurries on the composition of the eluted soluble phosphorus, magnesium, and potassium components were examined. Results are summarized in Tables I-III.

There was an increase in the percentage of soluble phosphorus that was phytic phosphorus, whereas the percentage of inorganic phosphate decreased as the pH of the rice bran slurries decreased (Table I). The inorganic phosphate eluted earlier at pH 4.5 and pH

TABLE I
Composition of the Soluble Phosphorus Component in Rice Bran as a Function of pH

pH	Phytic P (%)	Inorganic P (%)
7.1	86	13
6.3	89	10
4.5	91	7
2.7	95	3

TABLE II
Composition of the Soluble Magnesium and Soluble Potassium Components in Rice Bran as a Function of pH

pH	% Associated to		
	Phytic P	Inorganic P	% Not Eluting
Soluble Magnesium			
7.1	75	25	0
6.3	62	15	23
4.5	42	49	9
2.7	18	38	45
Soluble Potassium			
7.1	13	22	65
6.3	16	11	73
4.5	13	7	80
2.7	0	0	100

TABLE III
The Number of Magnesium and Potassium Ions Associated per Soluble Phytate Molecule and per Inorganic Phosphate Molecule as a Function of pH

pH	Mg/Phytate	Mg/Inorganic P	K/Phytate	K/Inorganic P
7.1	2.0	0.5	1.1	1.0
6.3	1.9	0.8	0.8	0.9
4.5	1.4	3.6	0.5	0.6
2.7	0.7	8.8	0.0	0.0

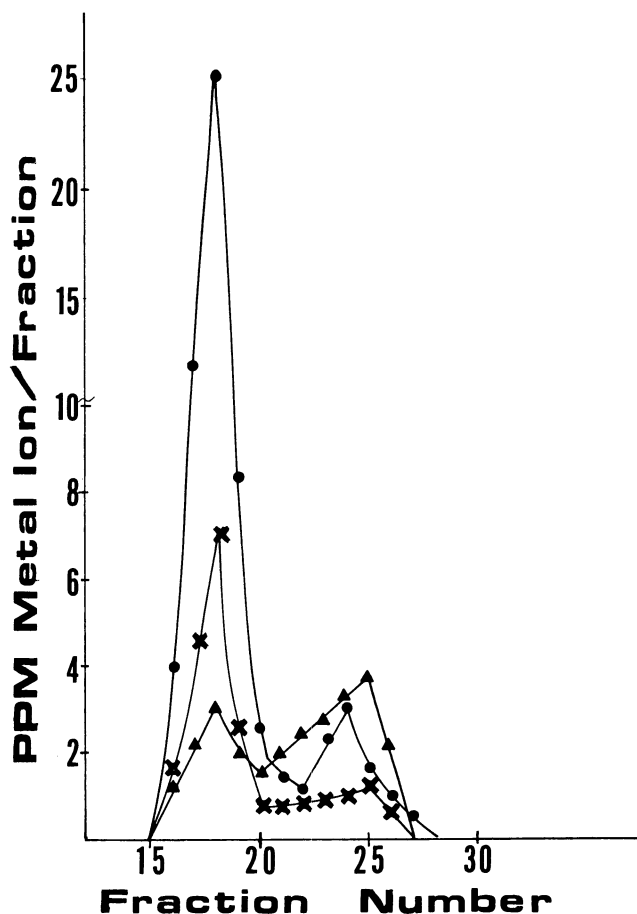


Fig. 1. Typical gel filtration chromatogram for the separation of the soluble phosphorus (●), magnesium (x), and potassium (▲) components in rice bran at endogenous pH (7.1) using water as eluant.

2.7. Its peak maximum was found in fraction 21 rather than in fraction 24. The maxima of the second magnesium and potassium peaks still eluted in fraction 25 at these lower pH values. Solutions of K_2HPO_4 adjusted to pH 4.5 and pH 2.7 behaved similarly on the Sephadex column; the inorganic phosphate peak maximum was in fraction 21, and the potassium peak maximum was in fraction 25. Thus, the magnesium, potassium, and inorganic phosphate components appear to be only loosely associated at these lower pH values.

The percentage of soluble magnesium and potassium associated with the phytic phosphorus decreased as the pH of the rice bran slurries decreased (Table II). Likewise, the average number of magnesium and potassium ions associated per phytate molecule decreased with decreasing pH (Table III). The magnesium-to-phytate ratio decreased from approximately 2:1 to less than 1:1 over the 7.1–2.7 pH range. Although there was approximately one potassium ion per phytate molecule at endogenous pH, no potassium ions were found to be associated with the soluble phytate at pH 2.7.

Soluble Components Extracted at Endogenous pH: Lithium and Sodium Chloride as Eluants

A typical gel-filtration chromatogram is shown in Figure 2 for the separation of the soluble phosphorus, magnesium, and potassium components in the rice bran slurry extracted at endogenous pH (7.1), using either 0.1 M lithium or sodium chloride as eluant. The peak profiles were more nearly symmetrical when these eluants were used instead of water. Neddermeyer and Rogers (1968) concluded that the Donnan salt-exclusion effect is eliminated and elution behavior becomes normal when the eluant has an ionic strength exceeding 0.02.

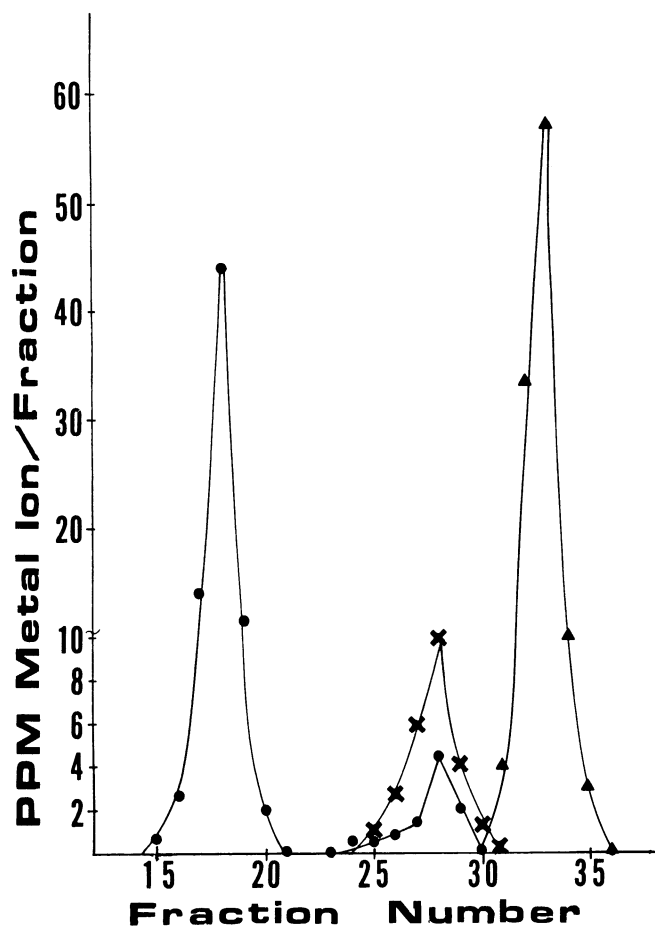


Fig. 2. Typical gel filtration chromatogram for the separation of the soluble phosphorus (●), magnesium (x), and potassium (▲) components in rice bran at endogenous pH (7.1) using either 0.1 M lithium or sodium chloride as eluant.

The maximum of the inorganic phosphate peak was found in fraction 29, which is five fractions later than the position of this peak maximum when water was used as eluant. The ratio of phytic phosphorus to inorganic phosphate was not affected by the choice of eluant. However, using either 0.1 M lithium or sodium chloride as eluant led to the soluble magnesium and potassium components no longer being associated with the soluble phytic phosphorus component. The maximum of the soluble magnesium peak coeluted with that of the inorganic phosphate, whereas the potassium eluted five fractions later. The maximum of the soluble potassium corresponded to that obtained when a solution of potassium chloride was passed through the column.

It appears that the lithium and sodium ions displaced the magnesium and potassium components from the soluble phytic phosphorus molecule. The implication of this result is that the order of stability of these metal-phytate complexes (or associations) is $Li^+, Na^+ > K^+, Mg^{++}$. This is in agreement with the order of stability of the alkali metal complexes with the related ligand pyrophosphate, which is $Li^+ > Na^+ > K^+$ (Lambert and Watters 1957).

CONCLUSIONS

Gel filtration on Sephadex G-25-50, followed by ICP analyses of the eluting fractions, was found to be a suitable method for the isolation and identification of the water-soluble phosphorus, magnesium, and potassium components of rice bran. The isolated soluble magnesium and potassium phytates had low metal-to-phytate ratios: approximately two magnesium ions and one potassium ion per phytate molecule at endogenous pH (7.1). The soluble metal-phytates reported by Morris and Ellis (1976) and by Graf (1983) similarly had low metal-to-phytate ratios. Graf (1983) raised the question that possibly all metal-phytate complexes having a low metal-to-phytate ratio may be soluble at intestinal pH. The nutritional consequence of this occurrence is that optimal intestinal uptake of cations could occur not only at high metal-to-phytate ratios, but also when these ratios were very low. For rice bran, the results of these investigations show that the water-soluble phosphorus, magnesium, and potassium components extracted at intestinal pH values are in nutritionally desirable forms, i.e., as metal-phytates with low metal-to-phytate ratios and as inorganic phosphates.

The compositions of the soluble magnesium and potassium phytate species isolated from the rice bran were found to be pH dependent. The number of magnesium and potassium ions associated per phytate molecule decreased with decreasing pH. Because the solubilities of the magnesium and potassium components parallel that of the phosphorus component as a function of pH (Champagne et al 1985a,b), a change in extractability of phytic acid with a change of pH would not account for the change in distribution of the ions.

There has been a lack of agreement about the precise state of existence of the soluble phytate salts at low pH (Cheryan 1980). Some investigators believe that the metal-phytates so formed are completely dissociated, whereas others contend that they exist as soluble un-ionized salts (Allred et al 1964, Hill and Tyler 1954, Omosaiye and Cheryan 1979, Smith and Rackis 1957). Our investigations support the former contention, that the magnesium and potassium phytates in rice bran are labile and dissociate at low pH.

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LITERATURE CITED

- ALLRED, J. B., KRATZER, F. H., and PORTER, J. W. G. 1964. Some factors affecting the *in vitro* binding of zinc by isolated soybean protein and by β -casein. *Br. J. Nutr.* 18:575.
 CHAMPAGNE, E. T., RAO, R. M., LUZZO, J. A., ROBINSON, J. W.,

- GALE, R. J., and MILLER, F. 1985a. Solubility behavior of the minerals, proteins, and phytic acid in rice bran with time, temperature, and pH. *Cereal Chem.* 62:218.
- CHAMPAGNE, E. T., RAO, R. M., LIUZZO, J. A., ROBINSON, J. W., GALE, R. J., and MILLER, F. 1985b. The interactions of minerals, proteins, and phytic acid in rice bran. *Cereal Chem.* 62:231.
- CHERYAN, M. 1980. Phytic acid interactions in food systems. *CRC Crit. Rev. Food Sci. Nutr.* 13:297.
- CHERYAN, M., ANDERSON, F. W., and GRYNSPAN, F. 1983. Magnesium-phytate complexes: Effect of pH and molar ratio on solubility characteristics. *Cereal Chem.* 60:235.
- EVANS, W. A., and PIERCE, A. G. 1981. Calcium-phytate complex formation studies. *J. Am. Oil Chem. Soc.* 58:850.
- GRAF, E. 1983. Calcium binding to phytic acid. *J. Agric. Food Chem.* 31:851.
- GRYNSPAN, F., and CHERYAN, M. 1983. Calcium phytates: Effect of pH and molar ratio on in-vitro solubility. *J. Am. Oil Chem. Soc.* 60:1761.
- HILL, R., and TYLER, C. 1954. The effect of decreasing acidity on the solubility of calcium, magnesium, and phosphorus in bran and certain pure salt solutions. *J. Agric. Sci.* 44:311.
- JACKMAN, R. H., and BLACK, C. A. 1951. Solubility of iron, aluminum, calcium, and magnesium inositol phosphate at different pH values. *Soil Sci.* 72:179.
- JULIANO, B. O. 1972. The rice caryopsis and its composition. Page 16 in: *Rice Chemistry and Technology*, 1st ed. D. F. Houston, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.
- LAMBERT, S. M., and WATTERS, J. I. 1957. The complexes of pyrophosphate ion with alkali metal ions. *J. Am. Chem. Soc.* 79:4262.
- MORRIS, E. R., and ELLIS, R. 1976. Isolation of monoferric phytate from wheat bran and its biological value as an iron source to the rat. *J. Nutr.* 106:753.
- NEDDERMEYER, P. A., and ROGERS, L. B. 1968. Gel filtration behavior of inorganic salts. *Anal. Chem.* 40:755.
- OBERLEAS, D., and MOODY, N. 1981. In-vitro interaction of phytate with trace elements. Page 129 in: *Trace Element Metabolism in Man and Animals*. J. M. Howell, J. M. Gawthorne, and C. L. White, eds. (TEMA-4) Aust. Acad. Sci.: Canberra City, Australia.
- OMOSAIYE, O., and CHERYAN, M. 1979. Low-phytate, full-fat soy protein product by ultrafiltration of aqueous extracts of whole soybeans. *Cereal Chem.* 56:58.
- PHARMACIA FINE CHEMICALS. 1975. *Gel Filtration—Theory and Practice*. Pharmacia: Uppsala, Sweden.
- SMITH, A. K., and RACKIS, J. J. 1957. Phytin elimination in soybean protein isolation. *J. Am. Chem. Soc.* 79:633.
- STEWART, J. W., and TATE, M. E. 1969. Gel chromatography of inositol polyphosphates and the avian haemoglobin-inositol pentaphosphate complex. *J. Chromatog.* 45:400.
- STEWART, J. W., and TATE, M. E. 1971. Gel chromatography of soil organic phosphorus. *J. Chromatog.* 60:75.
- TANGKONGCHITR, U., SEIB, P. A., and HOSENEY, R. C. 1982. Phytic acid. III. Two barriers to the loss of phytate during breadmaking. *Cereal Chem.* 59:216.

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