Solubility Behaviors of the Minerals, Proteins, and Phytic Acid in Rice Bran with Time, Temperature, and pH

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ABSTRACT

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Solubility data are presented as a function of time, temperature, and pH for eight elements in rice bran: iron, zinc, copper, potassium, magnesium, calcium, nitrogen, and phosphorus. The greatest increase in solubilization of the endogenous minerals and proteins with time of incubation was during the first 30–40 min at 20 and 60°C, with copper and phosphorus being exceptions. With the exception of endogenous zinc, increasing the temperature from 20 to 60°C resulted in increased solubilization. Endogenous zinc was found to be less soluble at 60 than at 20°C at times greater than 60 min. Solubility profiles of phosphorus and nitrogen components as a function of pH did not correspond, except in the pH 1–2 range, which shows that association between protein and phytic acid occurred only in this range. Potassium, magnesium, and calcium solubilities as a function of pH corresponded to those of the phytic acid salts of these minerals. A possible association of copper and zinc with the proteins in the pH 6.8–10.3 and 6.2–10.3 ranges, respectively, was indicated by the behavior of their solubility curves.

During the last 20 years, research on the interactions of minerals, proteins, and phytic acid in oilseeds has attracted much attention. It has been established that phytic acid interacts with minerals and proteins to reduce mineral bioavailability and alter protein functionality (Cheryan 1980, Cosgrove 1980, Erdman 1979, O'Dell 1979, Reddy et al 1982).

The protein-phytic acid solubility relationships as a function of pH have been investigated for soybeans (Fontaine et al 1946, de Rham and Jost 1979, Okubo et al 1975, Omosaiye and Cheryan 1979, Prattley et al 1982a,b), wheat (Hill and Tyler 1954c), peanuts (Fontaine et al 1946), cottonseed (Fontaine et al 1946), and rapeseed (Gillberg and Tornell 1976). Only a few studies have examined the effects of pH on the solubilities of the mineral components and on the mineral-phytate and mineral-protein relationships in oilseeds (Hill and Tyler 1954a–c, Rendleman 1982, Rendleman and Grobe 1982, Prattley et al 1982a,b). No previous work seems to have been published on the influence of pH on mineral, protein, and phytate solubilities and interactions in rice bran. Thus, in these studies the solubility behaviors of iron, zinc, copper, potassium, magnesium, calcium, phosphorus, and nitrogen in rice bran have been examined as a function of pH.

MATERIALS AND METHODS

Bran Samples

Rough rice of the LaBelle variety was obtained from the 1982 crop of the Louisiana Agricultural Experiment Station (Rice Research) in Crowley, LA. It was dehulled by a Satake dehuller. The bran (0–6%) was collected by milling using a McGill rice miller.

Mineral Sources

Ferrous, ferric, and zinc sulfate were obtained from Mallinckrodt, Inc. Cupric sulfate was obtained from Fisher Scientific Company. All of these were reagent grade chemicals. Iron, zinc, copper, phosphorus, potassium, magnesium, and calcium standards were prepared using certified atomic absorption reference solutions from Fisher Scientific. Nitrogen standards were prepared from an ammonium sulfate primary standard (Fisher Scientific no. A-938). The ammonium sulfate was dried for 2 hr at 105° C before being used.

Equipment

Inductively coupled plasma (ICP) analyses were conducted using an Applied Research Laboratories model 34100 ICP spectrometer. A Technicon Auto Analyzer II was used for ammonia determination. An Orion Research Microprocessor Ionanalyzer 901 was used to determine pH.

Sample Preparations

Effects of time and temperature on the solubilization of endogenous minerals and protein and the binding of exogenous minerals. Samples containing the equivalent of 20–25 mg of Fe(II), Fe(III), Zn(II), or Cu(II) ions from the sulfate salts and 5 g of rice bran were incubated in polyethylene bottles each containing 100 ml of distilled deionized water. The bottles were incubated on a rotary shaker for 5, 10, 15, 30, 60, 120, and 180 min at 20 and 60°C. Control samples without added minerals were treated identically. Immediately following incubation these slurries were centrifuged for 30 min at 19,600 × *g* and the supernatants decanted and saved. These supernatants were stored at 10°C.

Effects of pH on the solubilization of endogenous minerals. To determine the effects of pH, aliquots of either 0.18*N* HCl or 0.10*N* NaOH were added to 5-g samples of rice bran, and the final

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volumes were immediately adjusted to 100 ml with distilled deionized water. These samples were incubated on a rotary shaker for 60 min at 20°C. Immediately following incubation and centrifugation at 19,600 × *g* for 30 min, the resultant pH values of the supernatants were determined. Control samples (pH 7.1) were treated identically.

Sample Analyses

Determination of mineral concentrations. Iron, zinc, and copper concentrations were determined by ICP on HNO_3-$HClO_4$ (3:1) digests of bran supernatants and of bran. Reported values represent averages from triplicate readings.

Protein determinations. Bran and bran supernatants were analyzed for protein by determination of total Kjeldahl nitrogen. Samples were subjected to a sulfuric acid digest using a catalyst of potassium sulfate and cupric sulfate (10:0.3), diluted, and placed on an auto analyzer for ammonia determination. A nitrogen to protein conversion factor of 5.95 was used.

Handling of Data

The figures were plotted by SAS using a spline method SM_{xx}. Spline methods smooth points in a plot (Goodnight 1981).

RESULTS AND DISCUSSION

Effects of Time and Temperature on the Solubilization of Endogenous Minerals and Protein and the Binding of Exogenous Minerals

Assessment of the effects of time and temperature on mineral and protein solubilization and mineral binding allowed a representative time and temperature for sample incubation to be chosen for the pH studies. Figures 1 a and b depict the effects of time and temperature on the solubilities of endogenous substances containing iron, zinc, copper, phosphorus, and nitrogen (protein) in rice bran. Data points represent averages from triplicate samplings. The average standard deviations of the data points were <0.4% for Fe, Zn, P, Ca, Mg, and K; <2.0% for Cu; and <1.0% for N.

The greatest increase in solubilization of the endogenous minerals and proteins with time of incubation was during the first 30–40 min at 20 and 60°C, with copper and phosphorus being exceptions. Phosphorus solubility increased sharply during the first 120 min of incubation at 20 and 60°C. Copper showed a steady increase in solubility at 20°C for incubation times up to 180 min. With the exception of endogenous zinc, increasing the temperature from 20 to 60°C resulted in increased solubilization. Endogenous zinc was found to be less soluble at 60 than at 20°C at times greater than 60 min.

Statistical evaluation of the data by piecewise linear regression showed that solubilities of minerals and proteins continued to increase significantly with longer (30–180 min) extraction times, with two exceptions: 1) the solubility of copper at 60°C remained constant (hypothesis of zero slope could be accepted); and 2) the solubility of zinc at 60°C significantly decreased. It is not obvious why the solubility behavior of zinc contrasted with that of nitrogen, phosphorus, iron, and copper components with increased time at 60°C. Incubation at 60°C could possibly have increased the number of zinc binding sites of an insoluble binder or binders (i.e., proteins, fiber, phytates, etc., that are insoluble at pH 7.1).

As shown in Table I, after 10 min of incubation maximum insolubilization was approached for exogenous iron, zinc, and copper ions introduced as aqueous solutions of the sulfates. There was no further increase in insolubilization for incubation times up to 120 min. Increasing the incubation temperature from 20 to 60°C

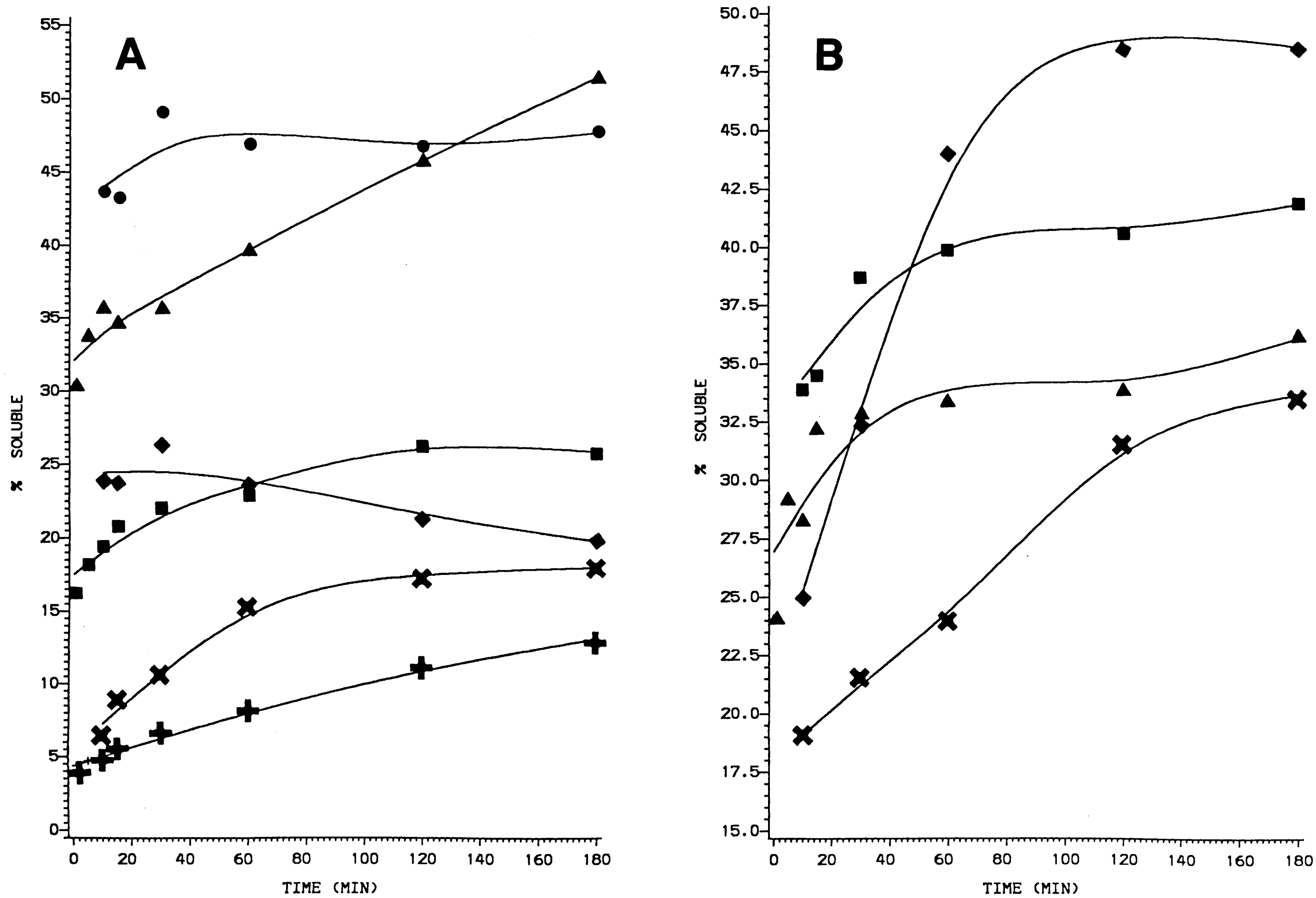

Fig. 1. A, Effects of time and temperature of bran incubation on iron (✚20°C, ✖60°C), zinc (■ 20°C, ◆ 60°C), and copper (▲ 20°C, ● 60°C) solubilities. **B,** Effects of time and temperature of bran incubation on phosphorus (✖20°C, ◆ 60°C) and nitrogen (▲ 20°C, ■ 60°C) solubilities.

increased the insolubilization of exogenous iron, zinc, and copper ions slightly.

From this study of the effects of time and temperature on mineral solubilization and binding and on protein solubilization, a representative time (60 min) and temperature (20° C) for incubation were chosen for the pH studies.

Supernatant Turbidity

In the above experiments (at endogenous pH 7.1), and in other extraction experiments carried out at pH values between 6.5 and 10.3, the supernatants obtained were not true solutions. Upon centrifugation the aqueous rice bran slurries yielded supernatants of increasing turbidity as the pH increased from approximately 6.5 to 10.3. Several authors (Fontaine et al 1946, Saio et al 1967) have suggested that the turbidity of soybean and cottonseed protein extracts is connected with the presence of phytic acid. Gillberg and Tornell (1976) and Cheryan et al (1983) confirmed that the turbidity of such extracts was caused by finely divided insoluble phytic acid derivatives. This was suspected to also be the case here with rice bran extracts. However, in this study, when centrifugations were done at 19,600 $\times g$ for 30 min, reproducible mineral and nitrogen assays were obtained.

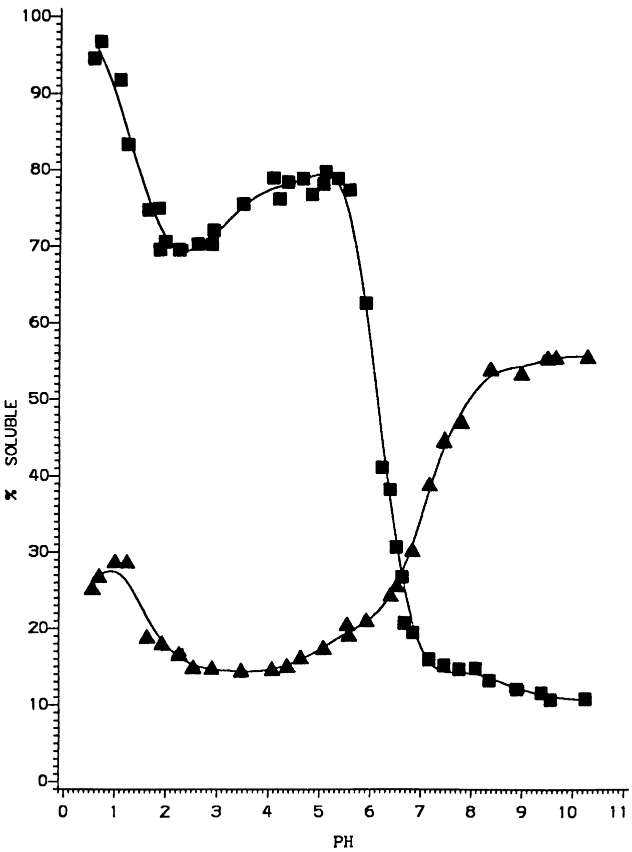

Fig. 2. Solubility behaviors of nitrogen (▲) and phosphorus (■) as a function of pH in rice bran slurries incubated at 20° C for 60 min.

TABLE I
Effect of Time and Temperature on the Insolubilization of Exogenous Iron, Zinc, and Copper Ions

Time (min)	Insolubilized (%)							
	Fe (II)		Fe (III)		Zn (II)		Cu (II)	
	20°C	60°C	20°C	60°C	20°C	60°C	20°C	60°C
2	31	···	86	···	88	···	93	···
5	60	···	90	···	96	···	93	···
10	92	94	95	95	98	98	93	93
30	93	98	94	96	98	98	93	94
60	93	97	94	98	98	99	93	94
120	94	98	94	96	98	98	93	94

Effects of pH on the Solubilization of Endogenous Minerals and Protein

Figures 2–4 show the solubility behaviors of iron, copper, zinc, magnesium, calcium, potassium, nitrogen, and total phosphorus in rice bran as a function of pH. Because approximately 90% of the phosphorus in rice bran is phytic phosphorus (Juliano 1972), the total phosphorus curve represents the behavior of phytic phosphorus. The nitrogen results can be considered to represent the solubilities of protein, since almost all of the nitrogen in rice bran is present as protein. The protein content of the supernatants was expected to consist primarily of water-soluble albumins, which constitute approximately 37% of the bran proteins (Juliano 1972).

As illustrated in Figure 2, the solubility of total phosphorus as a function of pH did not correspond to that of nitrogen in the pH 2.0–10.3 range. Total phosphorus solubility increased from a minimum of 11.5% at pH 10.3 to a maximum of 80% in the pH 4.1–5.2 range. In contrast, nitrogen solubility decreased from a maximum of 55% at pH 10.3 to a minimum of approximately 15% in the pH 2.4–4.1 range.

This solubility behavior contrasts with that observed for rapeseed (Gillberg and Tornell 1976), peanuts (Fontaine et al 1946), and soybeans (Fontaine et al 1946, de Rham and Jost 1979). In these systems the solubility of phytic phosphorus somewhat parallels that of the proteins, as exemplified for defatted soy flour in Figure 5. This solubility profile of phytic phosphorus in soy flour differs from that seen in the absence of protein (Cheryan 1980) or protein binding (Hill and Tyler 1954c), suggesting an interaction between phytic phosphorus and protein. It has been shown (Cosgrove 1966, Okubo et al 1976, de Rham and Jost 1979) that the anionic phosphate groups of phytate bind strongly to the cationic groups of protein below the pH of the "true" isoelectric point of the apo-protein moiety. At pH values above the isoelectric point

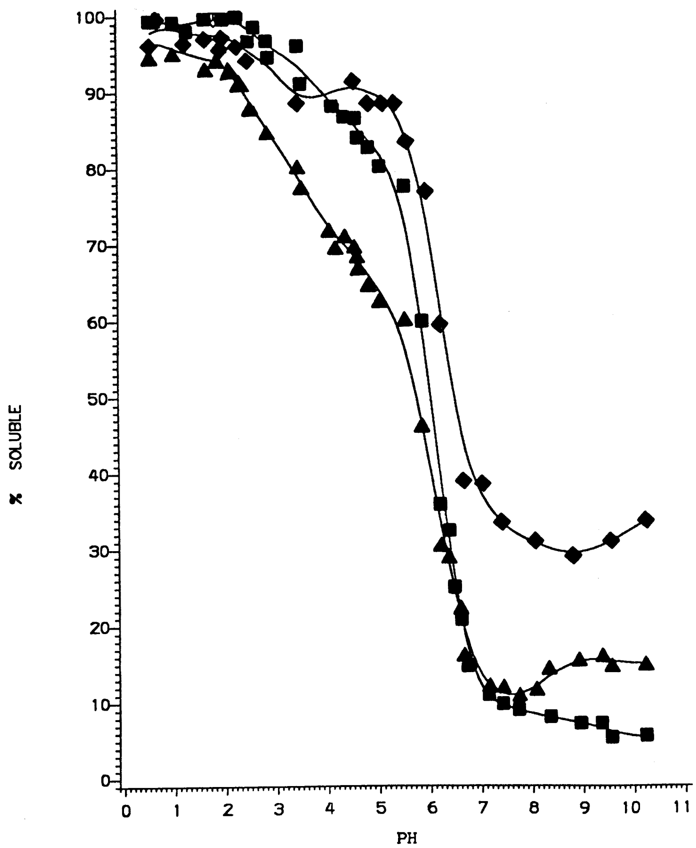

Fig. 3. Solubility behaviors of potassium (◆), magnesium (■), and calcium (▲) as a function of pH in rice bran slurries incubated at 20° C for 60 min.

binding occurs through a salt linkage or an alkaline-earth ion bridge to the negatively charged protein. At high pH (above 11) phytate-protein binding is disrupted, and the liberated phytate precipitates in the presence of sufficient calcium or magnesium.

The solubility profile of total phosphorus in rice bran as a function of pH (Fig. 2) is similar to that observed by Hill and Tyler (1954a) for wheat bran (Fig. 6). For wheat and rice bran extracts, the amount of soluble total phosphorus increased sharply between pH 6.2 and 5.0. It then decreased gradually to a minimum at approximately pH 2.0, before rapidly increasing again at lower pH values. Hill and Tyler did not report tne effects of pH on nitrogen solubility in wheat bran. Information allowing a comparison of the nitrogen solubility profile of rice bran with that of wheat bran was not found in the literature.

The solubility profile of nitrogen in rice bran as a function of pH (Fig. 2) resembles that found for cottonseed meal (Fontaine et al 1946) (Fig. 7). With extracts of cottonseed meal or rice bran, the solubility of total phosphorus did not corrrespond to that of nitrogen in the pH 2.8–10.3 range. In the pH 2.0–1.0 and 2.8–1.5 ranges for rice bran and cottonseed meal, respectively, there was a sharp increase in both total phosphorus and nitrogen solubilities. In wheat bran there was also a sharp increase in total phosphorus solubility at pH values below 2. There appears to be some association between protein and phytic acid in wheat bran (Hill and Tyler 1954c), in cottonseed meal (Fontaine et al 1946), and in rice bran for these pH ranges. At pH values lower than approximately 1.5 for cottonseed meal and 1.0 for rice bran, nitrogen solubility decreased and total phosphorus solubility increased. Fontaine et al (1946) have suggested that this solubility effect at pH values lower than 1.5 arises from the complete displacement of the phytic acid ion by the chloride ion.

The solubility of potassium, magnesium, and calcium as a function of pH corresponded to that of phosphorus in the 4.0–10.3 pH range (Fig. 3). These solubility curves for magnesium, potassium, and calcium as a function of pH are similar to those found in the literature for their corresponding salts of phytic acid (Jackman and Black 1951, Saio et al 1967, McKinney et al 1949, Smith and Rackis 1957). In general, magnesium, calcium, and potassium salts of phytic acid tend to be highly soluble at low pH and insoluble at high pH. A rapid drop in solubility has been observed at pH 5.5–6.0 for calcium phytate (Jackman and Black 1951, Saio et al 1967, McKinney et al 1949) and at 7.2–8.0 for magnesium phytate (Smith and Rackis 1957). A rapid drop in solubility was seen in these experiments in the 5.6–7.0 pH range for magnesium, potassium, and calcium species. The magnesium and calcium solubility curves for rice bran resemble the solubility profiles of magnesium and calcium in wheat bran (Hill and Tyler 1954a).

The solubility of iron, zinc, and copper in rice bran as a function of pH for the pH range 0.6–10.3 is shown in Figure 4. Minima in the iron solubility curve occurred at pH 6.7–7.2 (6%) and at pH 4.4–4.8 (16%). Only a slight increase in iron solubility was observed at pH values higher than pH 7.2 (from 6–10%). As the pH decreased from 7.2 to the 4.4–4.8 range, a maximum solubility was observed at approximately pH 5.6 (20%). At pH values lower than 2.0, there

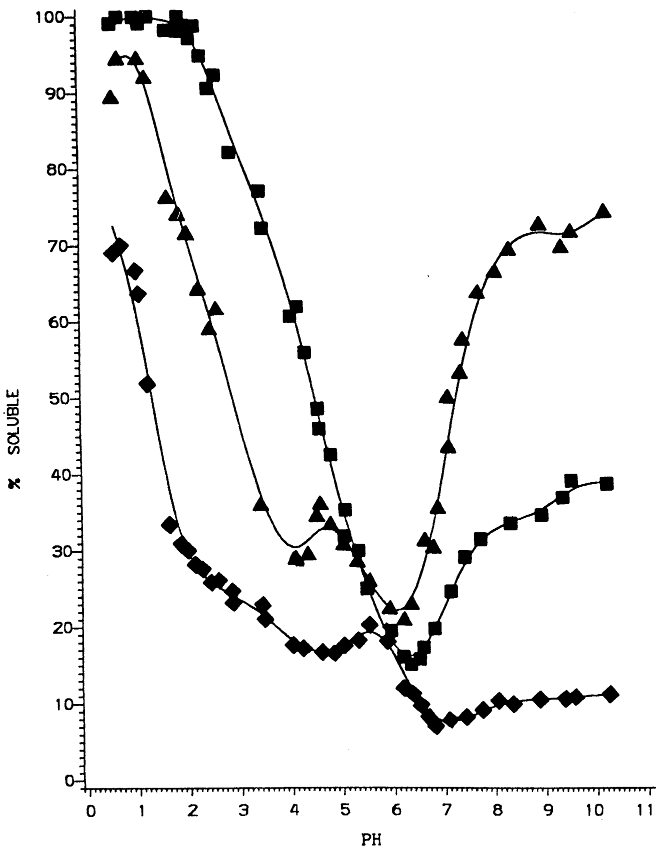

Fig. 4. Solubility behaviors of iron (◆), zinc (■), and copper (▲) as a function of pH in rice bran slurries incubated at 20°C for 60 min.

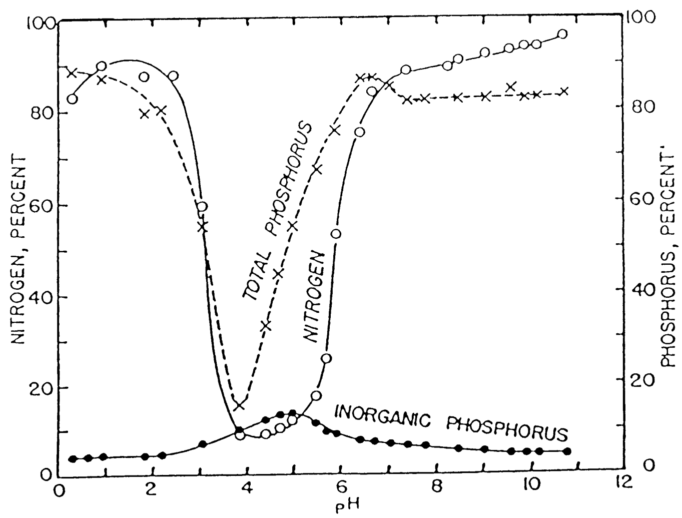

Fig. 5. Solubility behaviors of nitrogen and phosphorus in defatted soy flour. (From Fontaine et al 1946. J. Biol. Chem. 164:487. With permission of the American Society of Biological Chemists, Inc.)

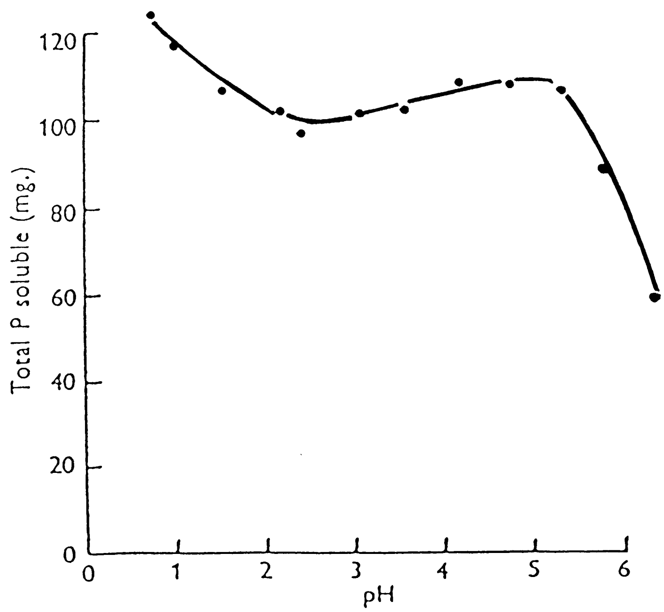

Fig. 6. Solubility behavior of phosphorus in wheat bran as a function of pH. (From Hill and Tyler 1954a. With permission of Cambridge University Press.)

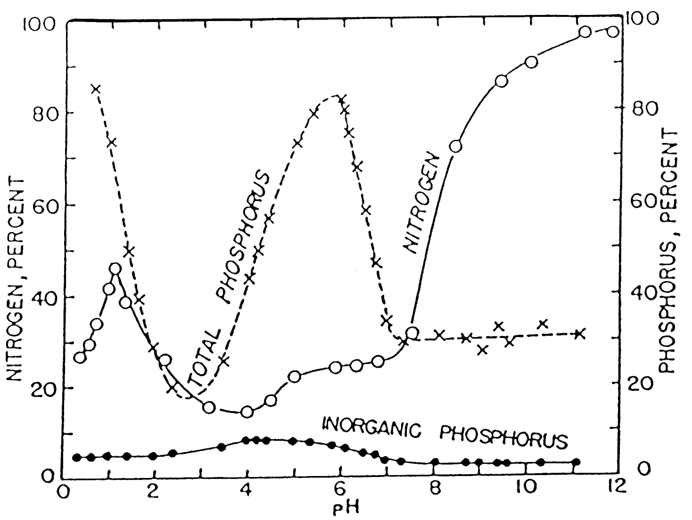

Fig. 7. Solubility behaviors of nitrogen and phosphorus in cottonseed meal. (From Fontaine et al 1946. With permission of the American Society of Biological Chemists, Inc.)

was a rapid increase in iron solubility (to approximately 70% at pH 0.6).

Minima in the copper solubility curve occurred at pH 6.2 (20%) and 4.2 (28%). From pH 6.2 to 10.3 and from pH 4.2 to 0.6, copper solubility increased to approximately 70 and 95%, respectively. A maximum in the curve representing 32% solubilized copper occurred at pH 4.6.

Minimum zinc solubility (14.5%) occurred at pH 6.2. In going from pH 6.2 to pH 10.3, there was only a gradual increase in zinc solubility to 36%. Zinc solubility increased rapidly to a value of approximately 100% in going from pH 6.2 to pH 0.6.

No relationship was apparent between the zinc, iron, and copper solubility curves and the phosphorus solubility curve over the pH 0.6–10.3 range. Precipitation methods for phytate determination are based on the insolubility of ferric phytate at acid pH (Oberleas 1971). If the soluble iron and phosphorus components were associated, a decrease in soluble iron with decreasing pH would be expected. However, a rapid increase in iron solubility was observed in the pH 2.0–0.6 range. The sharp increase in copper solubility in the pH 6.8–10.3 range indicated a possible association of copper with the soluble nitrogen component (protein). Zinc also followed the nitrogen solubility curve in the pH 6.2–10.3 range, which is evidence that zinc too is possibly associated with the protein.

CONCLUSIONS

Insight into the mineral, protein, and phytate relationships in rice bran was gained by examining the solubility behavior of these components as a function of pH. In rice bran, the solubility of total phosphorus as a function of pH did not correspond to that of nitrogen in the pH 2.0–10.3 range. Whereas in the pH 2.0–1.0 range the total phosphorus curve was seen to follow that of nitrogen, and it may be inferred that association between protein and phytic acid occurred in this pH range. The solubility profile of the total phosphorus and nitrogen components, as a function of pH, was found to resemble that for cottonseed meal but to contrast with that observed for rapeseed, peanut, and soybean systems. The solubility behavior of total phosphorus in rice bran was also seen to be very similar to that of total phosphorus in wheat bran.

Potassium, magnesium, and calcium solubilities as a function of pH in rice bran appeared to correspond to those of their phytic acid salts. No relationships between the soluble iron, zinc, and copper species and phytic acid were apparent from the pH data. However, a possible association of copper and zinc with protein was indicated by the behavior of the solubility curves in the pH 6.8–10.3 and 6.2–10.3 ranges, respectively.

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LITERATURE CITED

CHERYAN, M. 1980. Phytic acid interactions in food systems. CRC Crit. Rev. Food Sci. Nutr. 13:297.

CHERYAN, M., ANDERSON, F. W., GRYNSPAN, F. 1983. Magnesium-phytate complexes: Effect of pH and molar ratio on solubility characteristics. Cereal Chem. 60:235.

COSGROVE, D. J. 1966. The chemistry and biochemistry of inositol polyphosphates. Rev. Pure Appl. Chem. 16:209.

COSGROVE, D. J. 1980. Inositol Phosphates: Their Chemistry, Biochemistry, and Physiology. Studies In Organic Chemistry 4. Elsevier Scientific Publishing Co.: New York.

de RHAM, O., and JOST, T. 1979. Phytate-protein interactions in soybean extracts and manufacture of low-phytate soy protein products. J. Food Sci. 44:596.

ERDMAN, J. W. 1979. Oilseed phytates: nutritional implications. J. Am. Oil Chem. Soc. 56:736.

FONTAINE, T. D., PONS, W. A., and IRVING, G. W. 1946. Protein-phytic acid relationships in peanuts and cottonseed. J. Biol. Chem. 164:487.

GILLBERG, L., and TORNELL, B. 1976. Preparation of rapeseed protein isolates. Dissolution and precipitation behavior of rapeseed proteins. J. Food Sci. 41:1063.

GOODNIGHT, J. 1981. SAS/Graph User's Guide. SAS Institute Inc.: Cary, NC.

HILL, R., and TYLER, C. 1954a. The effect of increasing acidity on the solubility of calcium, magnesium and phosphorus in certain cereals and pure salts. J. Agric. Sci. 44:293.

HILL, R., and TYLER, C. 1954b. 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.

HILL, R., and TYLER, C. 1954c. The reaction between phytate and protein. J. Agric. Sci. 44:324.

JACKMAN, R. H., and BLACK, C. A. 1951. Solubility of iron, aluminum, calcium, and magnesium inositol phosphates 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. American Association of Cereal Chemists: St. Paul, MN.

McKINNEY, L. L., SOLLARS, W. F., and SETZKORN, E. A. 1949. Studies on the preparation of soybean protein free from phosphorus. J. Biol. Chem. 178:117.

OBERLEAS, D. 1971. The determination of phytate and inositol phosphates. Methods Biochem. Anal. 20:87.

O'DELL, B. L. 1979. Effect of soy protein on trace mineral availability. Page 187 in: Soy Protein and Human Nutrition. H. C. Wilke, D. T. Hopkins, and D. H. Waggle, eds. Plenum Press: New York.

OKUBO, K., WALDROP, A. B., IACOBUCCI, G. A., and MYERS, D. V. 1975. Preparation of low-phytate soybean protein isolate and concentrate by ultrafiltration. Cereal Chem. 52:263.

OKUBO, K., MYERS, D. V., and IACOBUCCI, G. A. 1976. Binding of phytic acid to glycinin. Cereal Chem. 53:513.

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.

PRATTLEY, C. A., STANLEY, D. W., and VAN DE VOORT, F. R. 1982a. Protein-phytate interactions in soybeans. II. Mechanism of protein-phytate binding as affected by calcium. J. Food Biochem. 6:255.

PRATTLEY, C. A., STANLEY, D. W., SMITH, T. K., and VAN DE VOORT, F. R. 1982b. Protein-phytate interactions in soybeans. III. The effect of protein-phytate complexes on zinc bioavailability. J. Food Biochem. 6:273.

REDDY, N. R., SATHE, S. K., and SALUNKHE, D. K. 1982. Phytates in legumes and cereals. Page 1 in: Adv. Food Res. Vol. 28. C. O. Chichester, E. M. Mrak, and G. F. Stewart, eds. Academic Press: New York.

RENDLEMAN, J. A. 1982. Cereal complexes: binding of calcium by bran and components of bran. Cereal Chem. 59:302.

RENDLEMAN, J. A., and GROBE, C. A. 1982. Cereal complexes: binding of zinc by bran and components of bran. Cereal Chem. 59:310.

SAIO, K., KOYAMA, E., and WATANABE, T. 1967. Protein-calcium-phytic acid relationships in soybeans. I. Effect of calcium and phosphorus on solubility characteristics of soybean meal protein. Agric. Biol. Chem. 31:1195.

SMITH, A. K., and RACKIS, J. J. 1957. Phytin elimination in soybean protein isolation. J. Am. Chem. Soc. 79:633.

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