The Interactions of Minerals, Proteins, and Phytic Acid in Rice Bran

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ABSTRACT

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The influences of the chemical environment on the mineral, protein, and phytic acid relationships in rice bran were examined. Adding increasing amounts of Fe(II), Fe(III), Zn(II), or Cu(II) ions as the sulfates to rice bran slurries affected the solubilities of the endogenous minerals, proteins, and phytates. The data from these solubility studies along with the results of sodium dodecyl sulfate gel electrophoresis, pH, and differential pulse

polarography experiments provided evidence for: association of iron, zinc, and copper ions with the albumins leading to the formation of insoluble mineral-albumin complexes; and the presence of potassium, magnesium, and calcium phytates, the solubilities of which were affected by pH and not by the addition of Fe(II), Fe(III), Zn(II), or Cu(II) ions.

During the last decade a trend has developed to incorporate bran into cereal products as a high-protein, high-fiber source. Until now, such use of rice bran has been hindered by its susceptibility to rancidity. The recent development of commercial processes for stabilizing rice bran foretells an upsurge of its use in cereal products.

The utilization of rice bran poses the question of the nutritional effect of its high phytic acid content. Phytic acid has been shown to interact 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).

In another paper (Champagne et al 1985) mineral, protein, and phytic acid relationships in rice bran were examined with regard to the solubility behavior of these components as a function of pH. This paper reports further study of the interactions of the phytates, minerals, and proteins in rice bran as influenced by the chemical environment. Effects of the addition of Fe(II), Fe(III), Zn(II), and Cu(II) ions on the solubilities of the substances in rice bran containing iron, zinc, copper, potassium, magnesium, calcium, phosphorus, and nitrogen have been examined.

MATERIALS AND METHODS

Bran Samples and Mineral Sources

Bran samples and mineral sources were identical to those described and prepared as outlined elsewhere (Champagne et al 1985).

Sodium Dodecyl Sulfate Gel Electrophoresis Reagents

Glacial acetic acid, methanol, glycerol, sodium phosphate monobasic, and sodium phosphate dibasic were reagent grade chemicals. Bio-Rad electrophoresis purity reagents used were: sodium dodecyl sulfate (SDS), acrylamide, 2-mercaptoethanol, Bis (N,N′-methylenebisacrylamide), TEMED (N,N,N′,N′-tetramethyl-ethylenediamine), ammonium persulfate, Coomassie Brilliant Blue R-250, and bromophenol blue. The electrophoresis gel tubes were soaked in Photo-Flo 200 solution from Eastman Kodak Company to facilitate gel removal. A kit of molecular weight markers containing seven proteins having molecular weights ranging from 14,200 to 66,000 was obtained from Sigma Chemical Company.

Supporting Electrolytes

All reagents used in preparing the supporting electrolytes were reagent grade. The iron content of these reagents was negligible. The supporting electrolytes were prepared as follows: 1) $0.1 N$ ammonium citrate, pH 6 was prepared from $0.1 N$ citric acid and NH_4OH titrated to pH 6. 2) $0.1 N$ sodium acetate, pH 4.5 was prepared from $0.1 N$ sodium acetate and $0.1 N$ acetic acid titrated to pH 4.5. 3) A solution of $0.1 N$ EDTA and $0.8 N$ sodium acetate, pH

5.2 was prepared from $0.1 N$ EDTA, $0.8 N$ sodium acetate, and $0.1 N$ acetic acid titrated to pH 5.2.

Equipment

Inductively coupled plasma (ICP) analyses and pH determinations were conducted as described in Champagne et al (1985). A Technicon Auto Analyzer II was used for ammonia determination. A Bio-Rad Laboratories model 155 electrophoresis cell with a Buchler 3-1500 constant power supply was used for SDS gel electrophoresis separations. The bands of the gels were scanned with a Gelman ACD-18 automatic computing densitometer. Differential pulse polarography experiments were performed using a Princeton Applied Research model 174 A polarograph in the differential pulse mode.

Sample Preparations

Aqueous aliquots containing 0.01–50 mg Fe(II), FE(III), Zn(II), or Cu(II) ions as the sulfates were added to 5-g samples of rice bran in polyethylene centrifuge bottles. The volumes were adjusted to 100 ml with distilled deionized water. One group of samples was treated without pH control, and for a second group pH values were adjusted to the 7.05–7.15 range with $0.1 N$ NaOH. This second group of samples was divided into two sets. The pH values of the first set were adjusted immediately after adding the exogenous minerals. The second set was incubated for 30 min at $20°C$ on the rotary shaker before pH adjustment. The samples with uncontrolled pH and those with pH adjusted before incubation were incubated for 60 min on the rotary shaker. The samples with pH adjusted after 30 min of incubation were incubated an additional 30 min. Immediately following incubation, the slurries were centrifuged for 30 min at $19,600 \times g$ and the supernatants decanted and saved. The resultant pH values of the supernatants were determined and the supernatants stored at $10°C$.

Sample Analyses

Determination of mineral concentrations. Concentrations of iron, zinc, and copper 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 determination. 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.

Soluble protein composition. SDS gel electrophoresis was performed on the bran supernatants to determine the molecular weights and the relative amounts of the subunits comprising the soluble proteins, in accordance with the method of Weber and Osborn (1969). The bands of the gels were scanned with a densitometer.

Studies of protein-mineral interactions by differential pulse polarography. Differential pulse polarography experiments were performed by titrating the rice bran control supernatant into systems containing one of the supporting electrolytes spiked with 5 ppm of Fe(III), Zn(II), or Cu(II) ions. The resultant reducible Fe(III), Zn(II), or Cu(II) content of the systems was monitored.

Reducible solubilized iron, copper, and zinc concentrations were determined on aliquots of the rice bran supernatants that contained these exogenous ions.

Data analysis. The figures were plotted by SAS using either spline methods SPLINE or SM_{xx}, or quadratic, cubic, or linear regressions with 95% confidence limits. The spline methods smooth points in a plot (Goodnight 1981).

RESULTS AND DISCUSSION

The phosphorus and nitrogen results can be considered representative of the behaviors of phytic phosphorus and protein, respectively. In rice bran, approximately 90% of the phosphorus is phytic phosphorus and almost all of the nitrogen is present as protein (Juliano 1972). The protein content of the supernatants was expected to be primarily water-soluble albumins, which constitute approximately 37% of the bran proteins (Juliano 1972).

Preliminary experiments showed that a representative incubation time and temperature for mineral solubilization and binding and for protein solubilization was 60 min at 20°C (Champagne et al 1985), so these conditions were selected. The rice bran supernatants obtained here and in other studies (Champagne et al 1985) were not true solutions. Upon centrifugation the aqueous rice bran slurries yielded supernatants of decreasing turbidity as increasing amounts of Fe(II), Fe(III), Zn(II), or Cu(II) ions were added. The turbidity of oilseed extracts has been linked to the presence of finely divided insoluble phytic acid derivatives (Gillberg and Tornell 1976, Cheryan et al 1983). This was suspected to be the case also 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.

Addition of Exogenous Minerals to Rice Bran

Effects of addition of exogenous minerals on solubilities of endogenous minerals and protein were studied by adding increasing amounts of Fe(II), Fe(III), Zn(II), or Cu(II) ions (0.002–10 mg/g bran) to aqueous slurries of rice bran. An examination of the solubility curves in Figures 1–7 revealed that when less than 0.1 mg of Fe(II), Fe(III), Zn(II), or Cu(II) ions was added per gram of bran, there was generally little or no change in the solubilities of the endogenous minerals and protein. Adding greater amounts of these ions, however, affected endogenous mineral and protein solubilization.

Addition of Fe(II) and Fe(III) Ions

Addition of 0.1–10 mg Fe(II) or Fe(III) ions per gram of bran decreased the solubilities of endogenous zinc and copper and of protein (Fig. 1). Note that the solubility curves were parallel for zinc, copper, and protein; the two sets of curves obtained from the addition of Fe(II) and Fe(III) ions were superposable. Thus, the oxidation state (+2 or +3) of the added iron ions did not affect the resultant solubilities of these endogenous minerals and protein. However, the oxidation state of added iron ions markedly influenced endogenous phosphorus solubility. Addition of 0.1–10 mg Fe(II) ions per gram of bran decreased phosphorus solubility and produced a curve that followed the zinc, copper, and protein solubility curves (Figs. 1 and 2). In contrast, the addition of 0.1–10 mg Fe(III) ions per gram of bran led to a twofold increase in phosphorus solubilized (Fig. 3). Similarly, effects of the addition of iron ions on solubilities of endogenous calcium, magnesium, and potassium depended on the oxidation state of the iron ions. Addition of 0.1–10 mg Fe(III) ions led to an increase in endogenous calcium, magnesium, and potassium solubilities, an increase similar to that observed for phosphorus following the addition of Fe(III) ions (Fig. 3). In contrast, adding 0.1–2 mg of Fe(II) ions per gram of bran led to a decrease in calcium and magnesium solubilities, a decrease similar to that observed for phosphorus solubility following the addition of Fe(II) ions (Fig. 2). Adding greater amounts of Fe(II) ions, however, led to no further decrease in calcium solubility and an increase in magnesium solubility. The
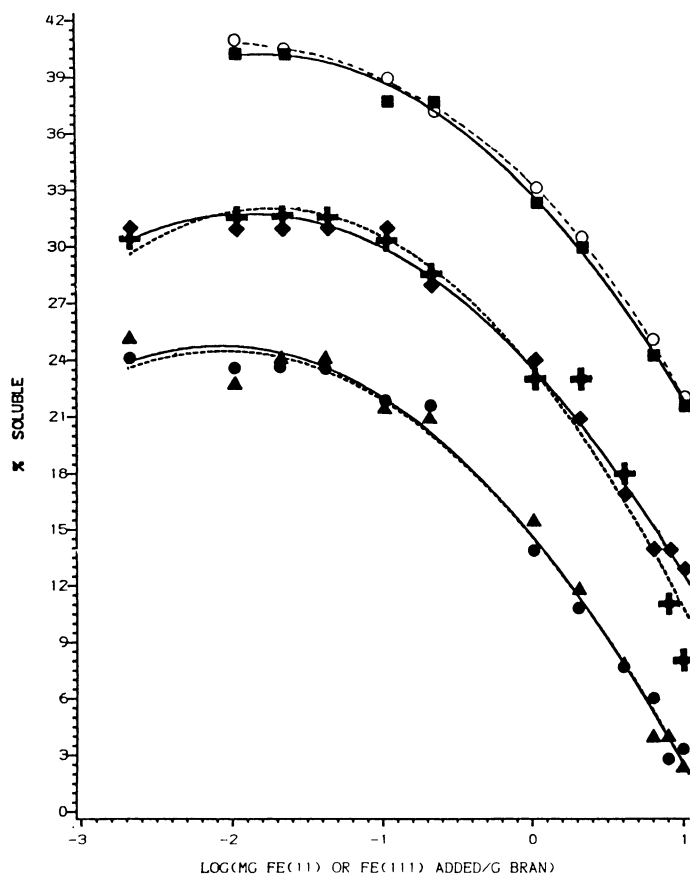

Fig. 1. Effects of the addition of Fe(II) (—) and Fe(III) (---) ions on Cu (■, O), N (◆, ➕), and Zn (▲, ●) solubilities without pH adjustment.
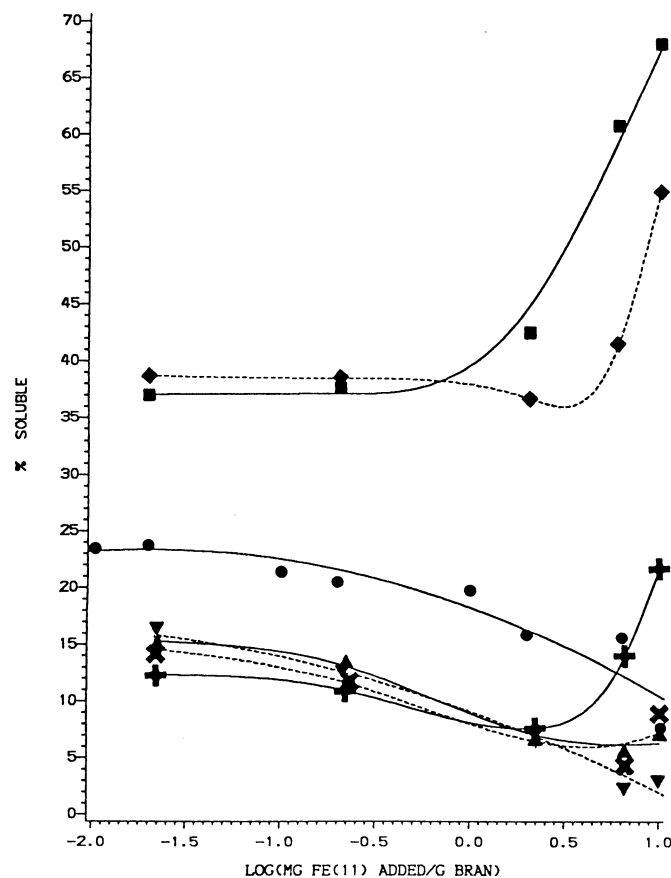

Fig. 2. Effects of the addition of Fe(II) ions on K (■, ◆), P(●), Mg (➕, ✕), and Ca (▲, ▼) solubilities with (---) and without (—) pH adjustment to 7.1.

solubility curve of potassium resulting from the addition of Fe(II) ions differed from those of calcium and magnesium. Potassium solubility increased with the addition of 0.1–10 mg of Fe(II) ions per gram of bran (Fig. 2).

Addition of Zn(II) Ions

Adding 0.1–10 mg of Zn(II) ions per gram of bran to the aqueous slurries resulted in a protein solubility curve (Fig. 4) similar to that obtained by adding iron ions (Fig. 1). However, the resultant endogenous copper solubility curve (Fig. 4) was unlike that from the addition of iron ions (Fig. 1). Adding 0.1–2 mg of Zn(II) ions per gram of bran decreased copper solubilized but adding greater quantities of Zn(II) ions led to an increase in copper solubilized. Addition of 0.1–4 mg of Zn(II) ions per gram of bran produced a slight decrease in endogenous iron solubilized. When larger amounts of Zn(II) ions were added, up to 10 mg/g bran, no further change was observed in endogenous iron solubilization (Fig. 4). Endogenous phosphorus, potassium, magnesium, and calcium decreased in solubility with the addition of up to 1 mg of Zn(II) ions per gram of bran. The solubilities of these minerals, except phosphorus, increased with the addition of 1–10 mg of Zn(II) ions per gram of bran (Fig. 5). Phosphorus solubility increased with additions from 1 to 8 mg Zn(II) ions per gram of bran and then decreased with further additions up to 10 mg/g bran. The magnesium, potassium, and calcium solubility curves paralleled the phosphorus curve for additions of 0.02–8 mg of Zn(II) ions per gram of bran.

Addition of Cu(II) Ions

The addition of 0.1–4 mg of Cu(II) ions per gram of bran caused a marked decrease in the amount of endogenous zinc and protein solubilized, but endogenous iron solubility was unaffected (Fig. 6). The resultant zinc and protein solubility curves roughly paralleled each other. Phosphorus, potassium, and magnesium solubilities

increased with the addition of approximately 1–10 mg of Cu(II) ions per gram of bran (Fig. 7). The addition of up to 10 mg Cu(II) ions per gram of bran had little effect on calcium solubility.

Effects of the Addition of Mineral Ions on Molecular Weight Protein Fractions

In order to examine further the effects of the addition of mineral ions on protein solubility, SDS gel electrophoresis was performed on the bran supernatants. These results revealed that the solubilized protein content of the control supernatants (pH 7.1) consisted of four major molecular weight fractions in the 14,200–66,000 range: 14,800, 23,000, 32,300, and 45,000 in a ratio of approximately 5:12:8:8. These four major molecular weight protein fractions were consistent with those for rice albumin. Padhye and Salunkhe (1979) identified six major protein fractions in rice albumin obtained from milled rice with molecular weights 7,400, 15,300, 20,300, 31,600, 43,700, and 135,000. Densitometric scans of the SDS electrophoretic gels showed that addition of Fe(II), Fe(III), Zn(II), or Cu(II) ions led to partial insolubilization of all four major molecular weight fractions. The percentage of

TABLE I
Effect of the Addition of Fe(II) Ions (10 mg/g bran) on the Solubilities of Four Major Protein Fractions in Rice Bran

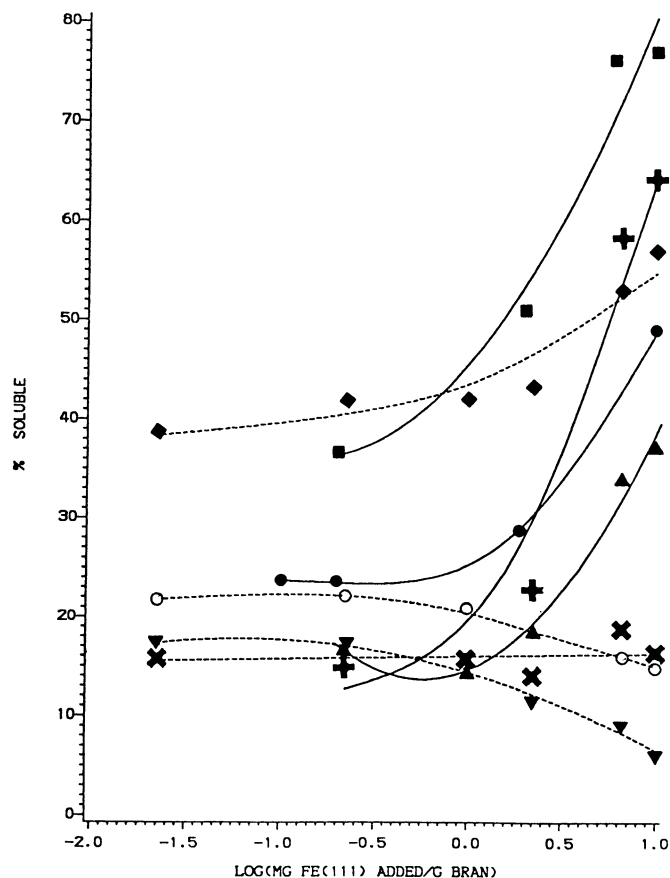| Protein Fraction (mol wt)	% Insolubilized	
	With pH Adjustment	Without pH Adjustment
14,800	70	68
23,000	80	80
32,300	84	85
45,000	92	92

Fig. 3. Effects of the addition of Fe(III) ions on K (■,◆), P (●, o), Mg (✚, ✗), and Ca (▲,▼) solubilities with (---) and without (—) pH adjustment to 7.1.
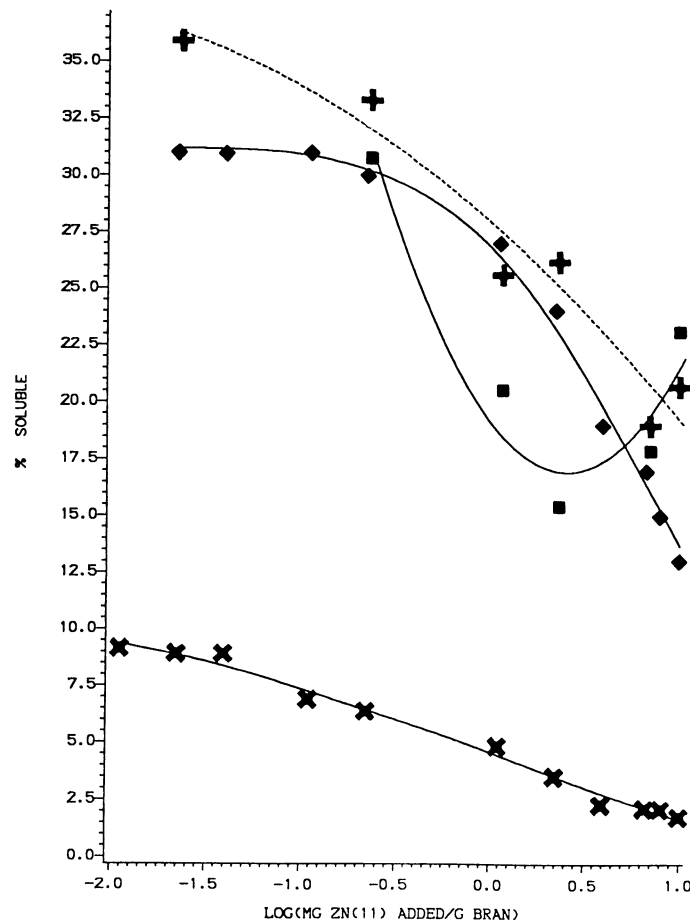

Fig. 4. Effects of the addition of Zn(II) ions on Cu (■), N (◆), and Fe (✗) solubilities without pH adjustment. The dashed line shows the effect of pH adjustment to 7.1 on resultant Cu (✚) solubility.
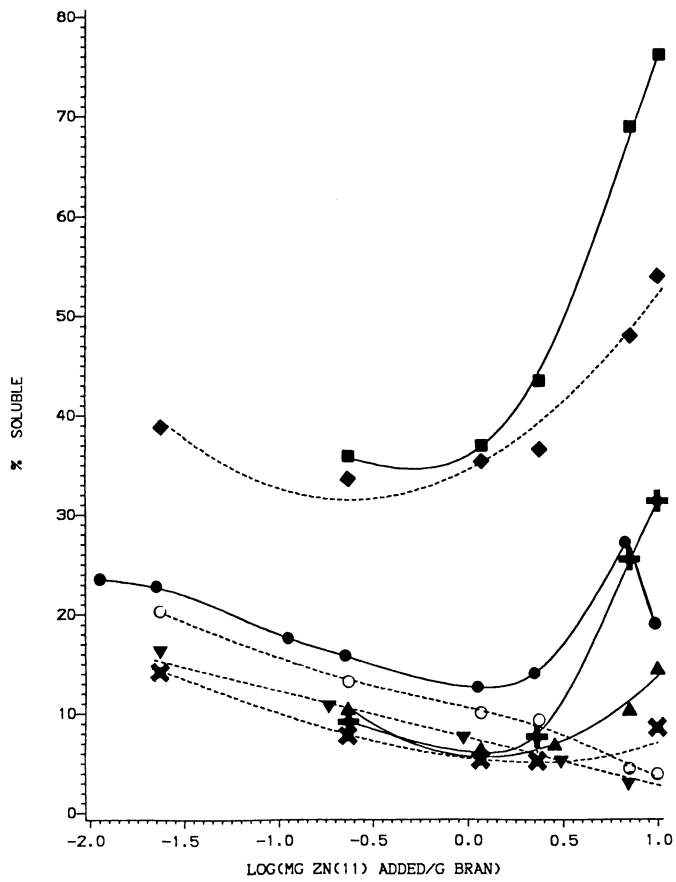
Fig. 5. Effects of the addition of Zn(II) ions on K (■,◆), P(●, o), Mg (✚, x), and Ca (▲,▼) solubilities with (---) and without (—) pH adjustment to 7.1.
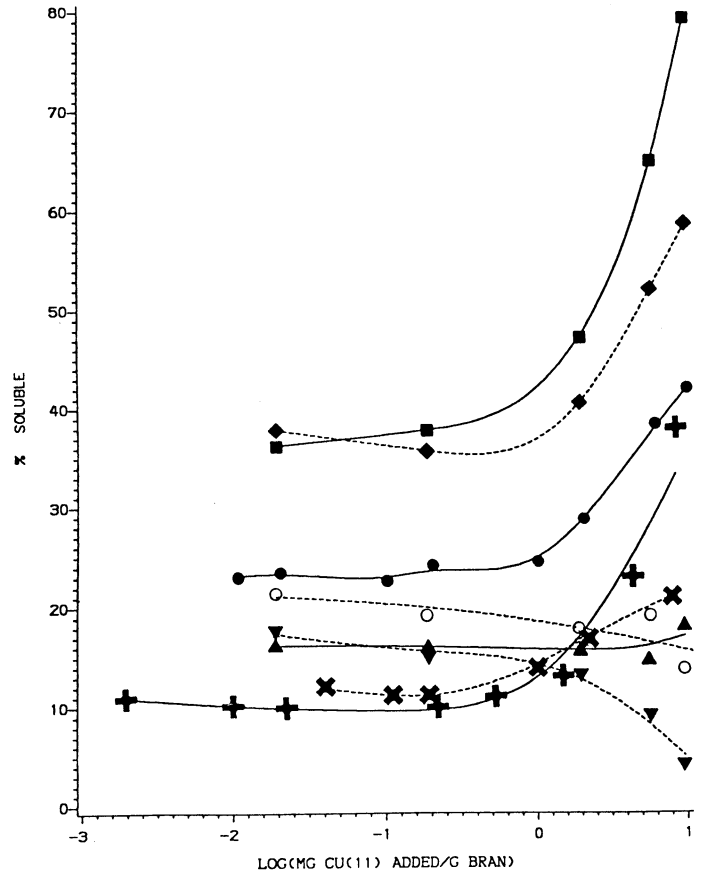

Fig. 7. Effects of the addition of Cu(II) ions on K (■,◆), P (●, o), Mg (✚, x) and Ca (▲,▼) solubilities with (---) and without (—) pH adjustment to 7.1.
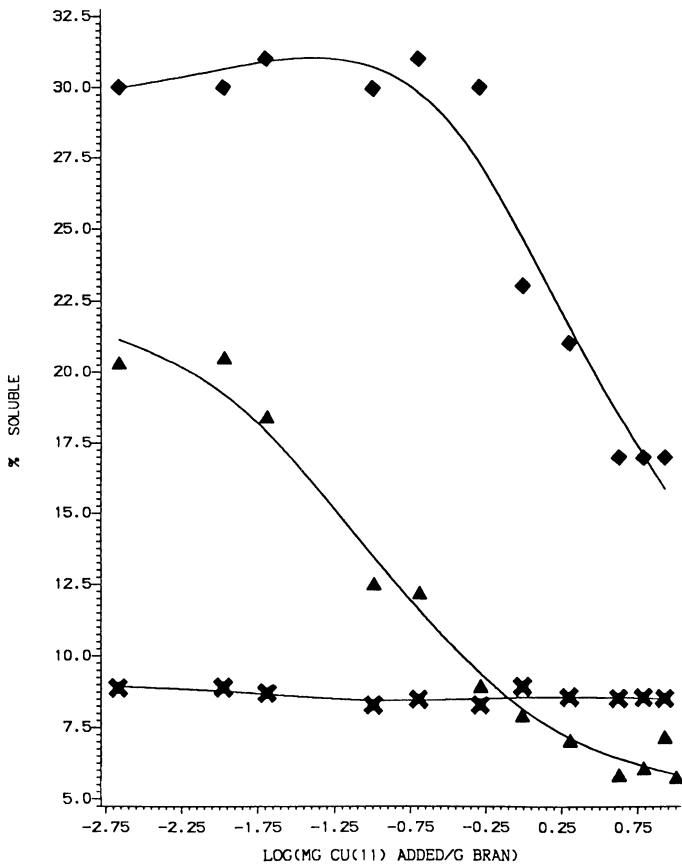

Fig. 6. Effects of the addition of Cu(II) ions on N (◆), Zn (▲), and Fe (x) solubilities without pH adjustment.
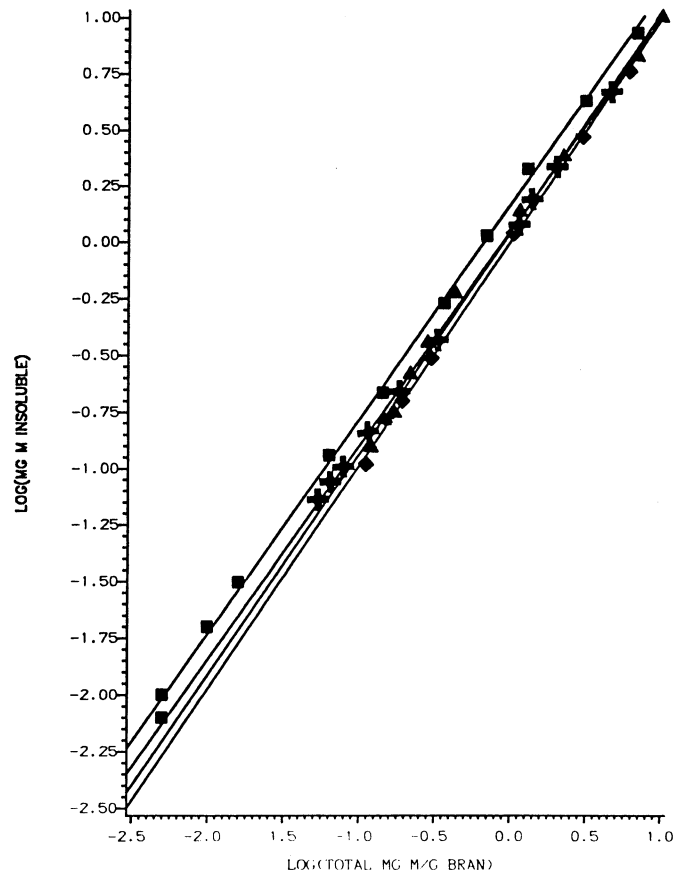

Fig. 8. Fe, Zn, and Cu insolubilized (bound) after addition of Fe(II)(✚), Fe(III) (■), Zn(II) (▲), and Cu(II) (◆) ions to the rice bran slurries.

insolubilization was greater for the protein fractions of higher molecular weight. Table I lists estimates for the percentage of each molecular weight fraction insolubilized by the addition of Fe(II) ions.

Binding of Exogenous Fe(II), Fe(III), Zn(II), and Cu(II) Ions

Figure 8 depicts the amount of iron, zinc, and copper rendered insoluble (bound) after these ions were added to the rice bran slurries. There was a linear increase in the amount of mineral insolubilized as the total amount of mineral (milligrams of mineral added + milligrams of endogenous mineral) present increased. Note that the increase was the same for the iron, zinc, and copper species.

Parallelism of Solubility Curves

The parallelism of the zinc, copper, and protein solubility curves resulting from the addition of Fe(II) and Fe(III) ions (Fig. 1) and the zinc and protein solubility curves resulting from the addition of copper (II) ions (Fig. 6) can be interpreted if an association of these endogenous minerals with the soluble proteins takes place. The observed decreasing solubilities of these endogenous minerals, and of protein, arising from the addition of the mineral ions can be explained as having resulted from either effects of the added mineral salts on ionic strength (leading to salting-out of soluble endogenous mineral-protein complexes), or the exogenous mineral ions interacting with endogenous minerals and proteins to yield insoluble mineral-protein complexes.

Ionic Strength Effects on Protein Solubility

Neutral salts containing di- or trivalent ions of high ionic strength are effective in salting-out proteins (White et al 1978). Ionic strength increased with the amount (0.002–10 mg/g bran) of exogenous Fe(II), Fe(III), Zn(II), or Cu(II) ions added to aqueous slurries of rice bran. However, addition of 10 mg of any of these mineral ions to the aqueous slurries resulted in theoretical increases

no greater than $\mu = 0.036$. Salting-out of the soluble proteins would not be expected to occur with such a small increase in ionic strength. Thus, ionic strength effects can be ruled out.

Binding of Zinc, Copper, and Iron Ions by Albumins

The soluble protein content of the rice bran supernatants was expected to consist primarily of the albumins. Studies have shown that zinc, copper, and iron ions bind to human serum albumin (Gurd 1954; Gurd and Goodman 1952; Gurd and Wilcox 1956; Tanford 1951, 1952; Klotz and Curme 1948; Klotz et al 1950; Klotz and Fiess 1951; Coddington and Perkins 1960; Breslow 1973; McMenamy 1977) and bovine serum albumin (Rao and Lal 1958a,b; Camon 1956). It has been established that the principle mode of Zn(II) binding between pH 5.5 and 7.5 is a 1:1 interaction between Zn(II) and the imidizole group of the histidine residues (Gurd and Goodman 1952, Breslow 1973). The zinc-albumin complexes are soluble at 0°C in the pH 5–6.7 range. At temperatures above 5–10°C precipitation occurs very rapidly (5–6 min) (Gurd 1954). Copper (II) binding at near neutral pH occurs at the NH-terminal-penultimate histidine complex site (Shearer et al 1967) and weakly at the single SH group (Klotz et al 1955).

A consequence of these metal ions binding to albumin is displacement of protons. Gurd and Goodman (1952) demonstrated that increasing the concentration of zinc ions (i.e., increasing the number of protein bound ions) led to a decrease in equilibrium pH. A comparable decrease in pH was observed when increasing amounts of Fe(II), Fe(III), Zn(II), or Cu(II) ions were added to the rice bran slurries. The equilibrium pH of the rice bran slurries decreased from 7.1 (endogenous pH) to 5.2 (Fig. 9). The decrease in pH was identical for equivalent amounts of the four exogenous ions added.

Effects of pH on Mineral and Protein Solubilities

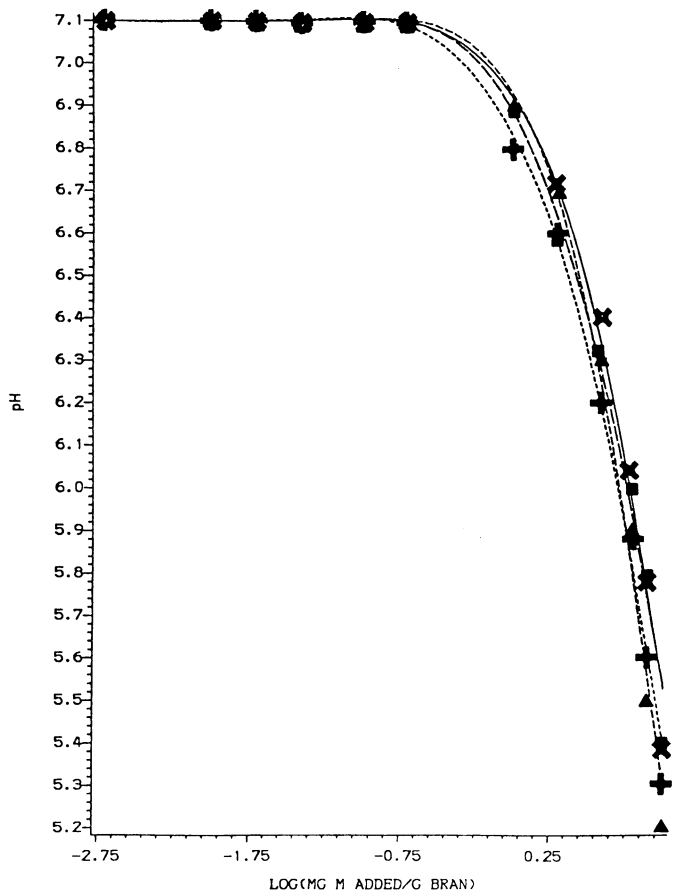To investigate the effects of pH on resultant mineral and protein

Fig. 9. Equilibrium pH of rice bran slurries following addition of Fe(II) (x), Fe(III) (✚), Zn(II) (▲), and Cu(II) (■) ions.
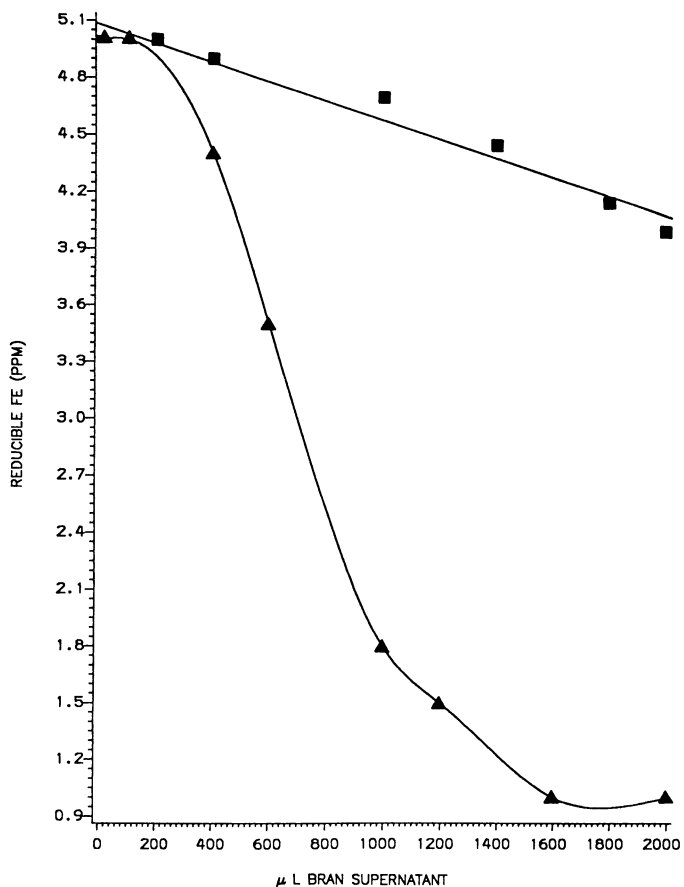

Fig. 10. Titrating rice bran supernatant (100 μl–2 ml) into 20 ml electrolyte spiked with 5 ppm of Fe(III). Systems: 0.1 g bran/ml into 0.1 N EDTA/0.8 N NaOAc (■); 0.25 g bran/ml into 0.1 N NH₄Citrate (▲).

solubilities, the pH of the rice bran slurries containing increasing amounts of added Fe(II), Fe(III), Zn(II), or Cu(II) ions (0.04–8 mg/g bran) were adjusted to a constant pH of 7.1 by two means: 1) immediately after addition of the exogenous mineral ion, and 2) after allowing the slurries to incubate for 30 min. To avoid possible competition between protein and buffer for complexing the mineral ions, pH was adjusted with NaOH instead of a buffer. These adjustments of pH (by both means) did not alter the amount of iron, zinc, or copper rendered insoluble (Fig. 8), nor the resulting zinc (Figs. 1 and 6), copper (Fig. 1), or protein (Figs. 1, 4, and 6) solubility curves observed when the pH was not controlled.

Adjusting the pH of the rice bran slurries to 7.1 did not change the SDS gel electrophoresis results. As for the experiments without pH control, all four major molecular weight fractions were partially insolubilized by the addition of Fe(II), Fe(III), Zn(II), or Cu(II) ions. The percentage of insolubilization was greater for the higher molecular weight fractions. Table I, which lists estimates for the percentage of each molecular weight fraction insolubilized by the addition of Fe(II) ions, permits a comparison of the results for conditions of constant pH and those of uncontrolled pH.

The prospects for insoluble hydroxide formation were increased as the pH was adjusted to 7.1 with NaOH. In the absence of complex formation, the onset of hydrolysis of Zn(II) and Cu(II) occurs at approximately pH 6, whereas Fe(III) ions are present as the hydroxides at pH values above 2 (Gurd and Wilcox 1956). Thus, the observations that pH adjustment to 7.1 did not change the resultant mineral and protein solubilities indicated that: 1) pH effects were not a factor and 2) the mineral-protein complexes were stable to hydrolysis of the metal ions for pH values to 7.1.

Protein-Mineral Interactions Studied by Differential Pulse Polarography

Differential pulse polarography experiments provided insight into the relationship between the exogenous minerals and the endogenous minerals and protein. The experiments discussed thus far involved the interactions of 5 g of bran with 0.1–50 mg of Fe(II), Fe(III), Zn(II), or Cu(II) ions. Differential pulse polarography experiments allowed the examination of the interactions of 5 ppm of Fe(II), Fe(III), Zn(II), or Cu(II) ions with small (100 μl to 2 ml) amounts of the rice bran control supernatant. Titrating from 100 μl to 2 ml of a control supernatant into an electrolyte spiked with 5 ppm of Fe(III), Zn(II), or Cu(II) ions resulted in a decrease in reducible mineral content (Figs. 10–12). The curves in Figures 11 and 12, resulting from the titration of Zn(II) and Cu(II) ions with the control supernatants in sodium acetate buffer, were in agreement with the diffusion current versus albumin concentration curves obtained from amperometric titrations of these ions with albumin in sodium acetate buffers (Saroff and Mark 1953). Thus, the data represented in Figures 10–12 support the postulate that the exogenous mineral ions interacted with albumin to yield insoluble mineral-albumin complexes.

Figures 10–12 illustrate the competition of the complexing/chelating electrolytes with the binders, presumably albumin, in the rice bran supernatant. The relative binding strengths of these agents and their respective reported stability constants (25°C, 0.1 ionic strength) (Martell and Sillen 1964, Martell and Smith 1982) were determined: For Cu(II), in decreasing order, EDTA/NaOAc (18.8), NH$_4$Citrate (5.9), albumin (3.7), and NaOAc (1.8); for Zn(II), in decreasing order, albumin (2.9), and NaOAc (1.0); and for Fe(III), in decreasing order, EDTA/NaOAc (25.0), albumin, and NH$_4$Citrate (4.9). The relative binding strengths of these agents agreed with reported stability constants.

Information discerning whether the soluble protein and the iron, zinc, and copper content of the supernatants were associated, was
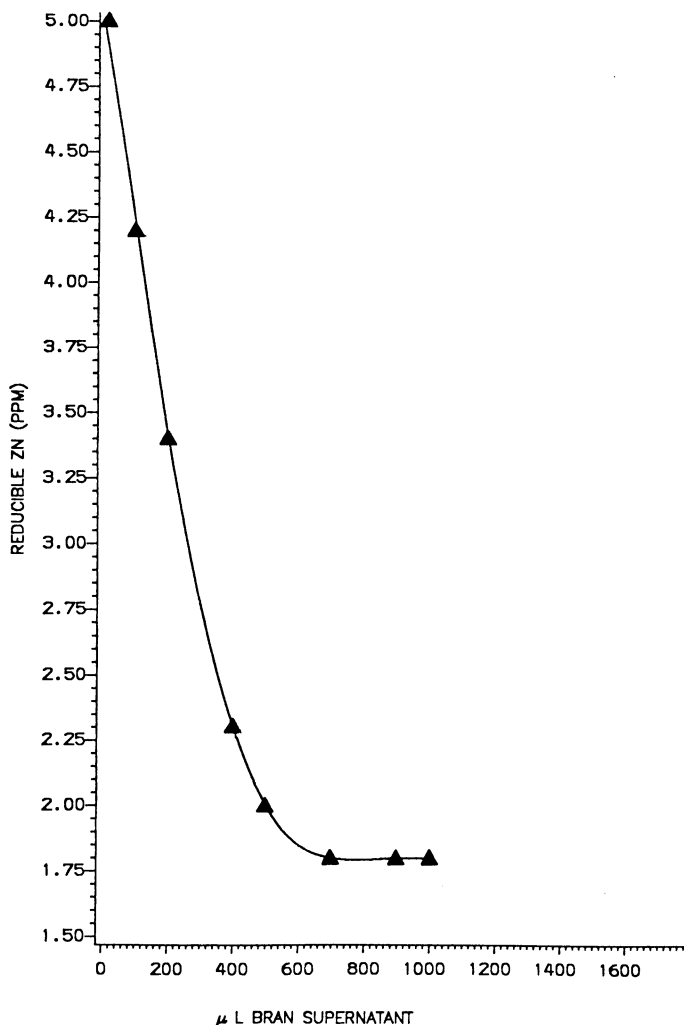

Fig. 11. Titrating rice bran supernatant (100 μl–2 ml) into 20 ml electrolyte spiked with 5 ppm of Zn(II). System: 0.125 g bran/ml into 0.1 N NaOAc.
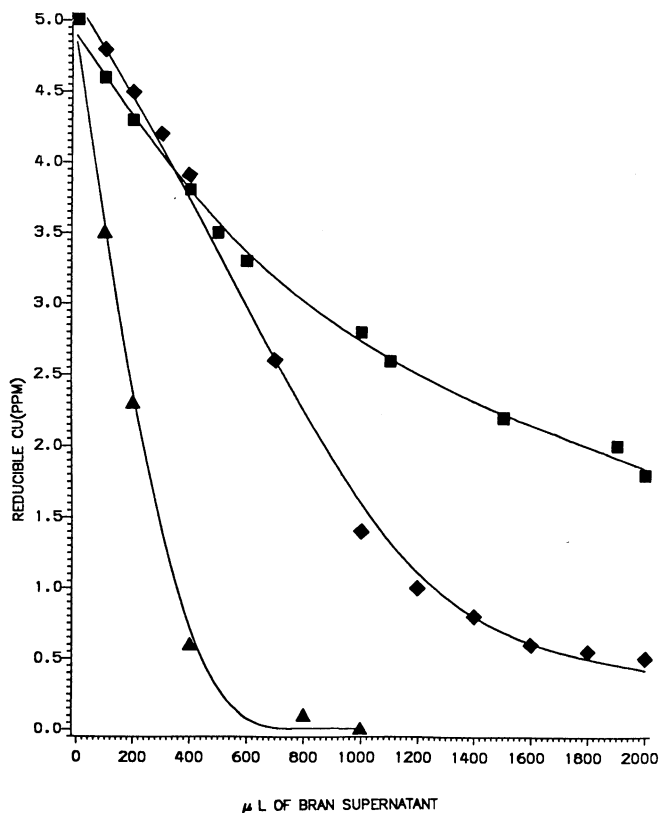

Fig. 12. Titrating rice bran supernatant (100 μl–2 ml) into 20 ml electrolyte spiked with 5 ppm of Cu(II). Systems: 0.125 g bran/ml into 0.1 N NaOAc (▲); 0.25 g bran/ml into 0.1 N NH$_4$citrate (◆); 0.10 g bran/ml into 0.1 N EDTA/0.8 N NaOAc (■).

obtained by comparing differential pulse polarography and ICP results. It is well known that the limiting polarographic current caused by the reduction of a metal ion may be considerably depressed by the presence of protein (Kolthoff and Lingane 1941), because complex formation between the metal ion and the protein results in a smaller diffusion coefficient (Tanford 1951). In these experiments, there was no statistical difference between the mineral concentrations of the rice bran supernatants containing exogenous iron, zinc, or copper as determined by differential pulse polarography and the concentrations of these minerals as determined by ICP on HNO_3-$HClO_4$ digests of the supernatants. This implied that all of the iron, zinc, or copper content of the supernatants was in a form or forms that could be reduced in the 0 to −1.5 V range, and that, in the supernatants, the protein (albumin) and the iron, zinc, and copper species were not associated.

All of the exogenous iron in the rice bran supernatants was found as Fe(III), regardless of whether it was added as Fe(II) or Fe(III) ions to the bran slurries. Thus, the Fe(II) ions that were not rendered insoluble (bound) were oxidized to the higher oxidation state by the rice slurry medium.

Solubility Behavior of Phosphorus

Whereas the protein solubility curves obtained from the addition of Fe(II) (Fig. 1), Fe(III) (Fig. 1), Zn(II) (Fig. 4), or Cu(II) (Fig. 6) ions were similar, the phosphorus solubility curves differed (Figs. 2, 3, 5, and 7). The phosphorus solubility curve resulting from the addition of iron as Fe(II) ions followed the protein, copper, and zinc solubility curves, suggesting an association with these endogenous minerals and proteins (Figs. 1 and 2). The phosphorus solubility curves resulting from the addition of Fe(III) and Cu(II) ions (Figs. 3 and 7) followed the solubility of phosphorus as a function of pH (Champagne et al 1984). Adjustment to pH 7.1, after the addition of Fe(III) and Cu(II) ions, resulted in phosphorus solubility curves that showed a gradual decrease in phosphorus solubility (Figs. 3 and 7). There was no indication from these phosphorus solubility curves of association of phosphorus with the endogenous zinc, copper, and protein components. The phosphorus solubility curve resulting from the addition of Zn(II) ions (Fig. 5) showed no relationship to the iron and protein solubility curves (Fig. 4) and did not follow the solubility curve for phosphorus as a function of pH. This phosphorus solubility curve, however, was followed by the copper solubility curve obtained from the addition of Zn(II) ions (Fig. 4).

Calcium, Magnesium, and Potassium Solubilities

In general, the solubility curves of calcium, magnesium, and potassium resembled those of phosphorus for the addition of Fe(II), Fe(III), Zn(II), or Cu(II) ions. However, pH effects also appeared to be involved. For example, when Fe(II) ions were added, the magnesium and calcium solubility curves followed the phosphorus curve for additions up to approximately 2 mg of Fe(II) ions per gram of bran. For additions of 2–10 mg of Fe(II) ions per gram of bran (which gave rice bran slurries with pH values of 6.7–5.4) magnesium and calcium solubilities no longer followed phosphorus solubility. The solubility of magnesium increased and calcium solubility remained constant. When Fe(II) ions were added, the solubility of potassium followed that of potassium as a function of pH (Champagne et al 1985) rather than the solubility of phosphorus.

CONCLUSIONS

The addition of increasing amounts of Fe(II), Fe(III), Zn(II), or Cu(II) ions as the sulfates to rice bran slurries affected the solubilities of the endogenous minerals, proteins, and phytates. From these data and results of SDS gel electrophoresis, pH, and differential pulse polarography experiments, it is postulated that: 1) The addition of Fe(II), Fe(III), and Cu(II) ions led to the formation of insoluble iron-copper-zinc-albumin complexes. The insoluble complexes which resulted from the addition of Fe(II) ions also may have had a phytate component—the phosphorus

solubility curve paralleled that of protein, zinc, and copper. 2) Insoluble zinc-albumin complexes formed as the result of the addition of Zn(II) ions. 3) The albumin was not associated with the iron, zinc, and copper species in the supernatants. 4) The potassium, magnesium, and calcium species were present as phytates; their solubilities were affected by pH but not by the addition of mineral ions. The results of this investigation contribute to an understanding of the effects of the chemical environment on the interactions of the minerals, proteins, and phytic acid in rice bran.

LITERATURE CITED

BRESLOW, E. 1973. Metal-protein complexes. Page 227 in: Inorganic Biochemistry. Vol. 1. G. L. Eichorn, ed. Elsevier Scientific Publishing Co.: New York.

CAMON, L. 1956. Iron-serum albumin interreactions. Zaragoza Ser. 2. 11:35.

CHAMPAGNE, E. T., RAO, R. M., LIUZZO, J. A., ROBINSON, J. W., GALE, R. J., and MILLER, F. 1985. Solubility behaviors of the minerals, proteins, and phytic acid in rice bran with time, temperature, and pH. Cereal Chem. 62:218.

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.

CODDINGTON, A., and PERKINS, D. J. 1960. The binding of Fe^{+++} to native and chemically modified human serum albumin in the presence of sodium citrate. Biochim. Biophys. Acta 44:361.

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

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

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.

GURD, F. R. N. 1954. Effect of temperature on the interaction of human serum mercaptalbumin with zinc ions. J. Phys. Chem. 58:788.

GURD, F. R. N., and GOODMAN, D. S. 1952. Preparation and properties of serum and plasma proteins. XXXII. The interactions of human serum albumin with zinc ions. J. Am. Chem. Soc. 74:670.

GURD, F. R. N., and WILCOX, P. E. 1956. Complex formation between metallic cations and proteins, peptides, and amino acids. Page 312 in: Advances in Protein Chemistry. Vol. 11. M. L. Anson, K. Bailey, and J. T. Edsall, eds. Academic Press: New York.

JULIANO, B. O. 1972. The rice caryopsis and its composition. Page 16 in: Rice Chemistry and Technology. D. F. Houston, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.

KLOTZ, I. M., and CURME, H. G. 1948. The thermodynamics of metallo-protein combinations. Copper with bovine serum albumin. J. Am. Chem. Soc. 70:939.

KLOTZ, I. M., and FIESS, H. A. 1951. The thermodynamics of metalloprotein combinations. J. Phys. Colloid Chem. 55:101.

KLOTZ, I. M., TALLER, J. D., and URQUHART, J. M. 1950. Spectra of copper complexes with some proteins, amino acids, and related substances. J. Phys. Colloid Chem. 54:18.

KLOTZ, I. M., URQUHART, J. M., KLOTZ, T. A., and AYERS, J. 1955. Slow intramolecular changes in copper complexes of serum albumin. The role of neighboring groups in protein reactions. J. Am. Chem. Soc. 77:1919.

KOLTHOFF, I. M., and LINGANE, J. J. 1941. Page 121 in: Polarography. Interscience Publishers: New York.

MARTELL, A. E., and SILLEN, L. G. 1964. Stability Constants. Special Publication No. 17. The Chemical Society: London.

MARTELL, A. E., and SMITH, R. M. 1982. Critical Stability Constants. Vol. 5. First Supplement. Plenum Press: New York.

McMENAMY, R. H. 1977. Albumin binding sites. Page 143 in: Albumin Structure, Function, and Uses. V. M. Rosenoer, M. Oratz, and M. A. Rothschild, eds. Pergamon Press: New York.

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

PADHYE, V. W., and SALUNKHE, D. K. 1979. Extraction and characterization of rice proteins. Cereal Chem. 56:389.

RAO, M. S. N., and LAL, H. 1958a. Metal protein interactions in buffer solutions. II. A polarographic study of the interaction of Zn(II) and Cd(II) with bovine serum albumin. J. Am. Chem. Soc. 80:3222.

RAO, M. S. N., and Lal, H. 1958b. Metal protein interactions in buffer solutions. III. Interaction of Cu(II), Zn(II), Cd(II), Co(II), and Ni(II) with native and modified bovine serum albumins. J. Am. Chem. Soc. 80:3226.

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

SAROFF, H. A., and MARK, H. J. 1953. Polarographic analysis of the serum albumin-mercury and zinc complexes. J. Am. Chem. Soc. 75:1420.

SHEARER, W. T., BRADSHAW, R. A., and GURD, F. R. N. 1967. The amino acid sequence and copper(II)-binding properties of peptide 1-24 of bovine serum albumin. J. Biol. Chem. 242:5451.

TANFORD, C. 1951. The effect of serum albumin on the polarographic diffusion current of cadmium. J. Am. Chem. Soc. 73:2066.

TANFORD, C. 1952. The effect of pH on the combination of serum albumin with metals. J. Am. Chem. Soc. 74:211.

WEBER, K., and OSBORN, M. 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. 244:4406.

WHITE, A., HANDLER, P., SMITH, E. L., HILL, R. L., and LEHMAN, I. R., eds. 1978. Page 107 in: Principles of Biochemistry. 6th ed. McGraw-Hill Book Co.: New York.