Solubilization of Iron in Cereals by Milk and Milk Fractions[1]

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ABSTRACT

Whole milk alone and several of its fractions were evaluated for their effect on the total chemical iron profiles of both added elemental and endogenous iron in a corn-, a three-grain-, and a wheat-based cereal through a sequential pH treatment from the pH of the cereal, which was approximately 6.0 to 2.0 to 6.0. Whole milk, lactose-free milk, and nonfat dry milk generated more total soluble iron than a water control of each pH in the sequence. When deproteinized milk was used, the solubilization effect largely disappeared in the wheat and three-grain cereals, but remained to some degree in the corn cereal. The corn-based cereal also contained a larger amount of ionic iron than the other cereals, at all pH levels, with both the milk fractions and water.

Iron absorption depends on several factors, including the types of iron or food consumed, the iron nutriture of the subject, and the amount of food consumed. In the United States, the most important factors are probably the type of iron and its physicochemical characteristics in food. One of the most important of these characteristics is solubility, as shown by the fact that most of the in vitro techniques for predicting the availability of dietary iron have focused on solubility in one form or another (Motzok et al 1978, Rao and Probhavthi 1978, Miller et al 1981). Also, the widely used extrinsic tag technique depends on the formation of a common pool of nonheme food iron (Bothwell et al 1982).

Inorganic iron salts have characteristic solubilities under standard conditions. However, these solubility characteristics cannot be extrapolated to nonstandard conditions such as those in food. The possibility of decreased iron absorption due to such conditions in cereals and legumes led to the publication of a monograph by INACG (Bothwell et al 1982). These effects could be due to a change in the chemical environment such as pH and reduction potential or to the presence of certain chemical constituents, including phosphates, proteins, phytates, carboxylic acids, and dietary fibers (Cydesdale 1982). With differing degrees of success, several researchers have performed in vitro studies to evaluate various ligands in an attempt to overcome iron insolubility caused by grains, legumes, and some of their components (Kojima et al 1981, Reinhold et al 1981, Camire and Clydesdale 1982, Fernandez and Phillips 1982, Platt and Clydesdale 1983, Rizk and Clydesdale 1983).

Any food or compound that can increase the iron solubility of a meal before consumption has the potential to increase absorption. One such food and its components—which have shown conflicting results in terms of iron absorption—is milk. The lactoferrins comprise a class of transferrinlike proteins originally isolated from milk but since identified in a wide variety of physiological fluids. However, direct evidence for their physiological role in iron absorption is unavailable (Aisen and Leibman 1972). A ferripolyphosphate-whey protein powder has been suggested as a bioavailable source of iron by Jones et al (1975) but has not achieved widespread use. Casein-iron complexes have been identified, and the strong affinity noted has been attributed to the presence of clustered phosphoseryl residues in casein (Osterberg 1961). However, Nelson and Potter (1980) found that a casein-ferrous complex was not as available to rats as either wheat or soy protein-ferrous complexes, and casein-ferric complexes were very poorly absorbed.

Many investigators have studied the possibility of fortifying milk itself. For instance, Rivera et al (1982) recently evaluated ferric lactobionate, with limited success. Little work has been done to evaluate the solubilizing effect of milk or its fractions on the iron in other foods, however. Therefore, this study was initiated to investigate the effect of milk on iron solubilization in cereal. Since iron availability is a function of the food environment, it seems logical to evaluate foods as eaten, such as milk and cereal, rather than as sold. Furthermore, various fractions of milk were also evaluated in the cereals in an attempt to isolate the fraction that contained the solubilizing factor.

MATERIALS AND METHODS

Cereal Samples

Experimental batches of noncommercial cereals were obtained from General Mills, Inc. These cereals were formulated without vitamin C or sugar coatings to reduce the variables that might affect iron solubilization. They included a wheat base, a corn base, and a three-grain (corn, wheat, and oats) base cereal to which was added hydrogen-reduced elemental iron at levels of 35, 43, and 28 mg/100 g, respectively, during manufacture and before drying. Endogenous iron was determined by difference from the values found for total iron.

Procedure

One hundred fifty milliliters of double-distilled deionized (DDD) water at 20° C was blended with each of three 4.7-g samples of each cereal for 2 min in 200-ml glass mixing jars with stainless steel blades to form a slurry. All glassware was acid-washed in concentrated HCl and rinsed in DDD water to remove contaminant iron. One of the three slurries of each cereal was analyzed at its endogenous pH (approximately 6.0). The other two slurries were incubated at room temperature for 20 min after the pH was adjusted to 2.0 with 6*N* HCl to simulate gastric pH conditions. One of these slurries was used for analysis, and sufficient Prep Tyrode buffer was added to the other to produce 10% of the total volume and to approximate the chemical environment in the duodenum. The Prep Tyrode buffer contained the following ingredients in grams made up to 2 L with DDD water: NaCl (16.0); KCl (0.4); $MgSO_4 \cdot 7H_2O$ (0.52); $NaH_2PO_4 \cdot 2H_2O$ (0.13); glucose (2.0); $NaHCO_3$ (2.0); $CaCl_2$ (0.4); NaN_3 (0.4), or chloramphenicol (0.02). NaOH (9*N*) was then added by drops to bring the pH to 6.0, and the sample was analyzed. Preliminary analyses with 10, 20, and 50% NaOH indicated that reagent concentration did not affect solubilization.

Whole Milk

Pasteurized, homogenized whole milk was obtained from local markets. Twenty-milliliter aliquots were added to each 4.7-g cereal sample in accordance with the ratio described on the nutritional label of a typical commercial cereal. The milk-cereal mixture was incubated for 5 min at 20° C to simulate an average eating situation.

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One hundred fifty milliliters of DDD water was added and the solution blended for 2 min in 200-ml glass mixing jars. The procedure described in the last section was then followed.

Nonfat Dry Milk

A commercial nonfat dry milk powder was purchased at a local market and prepared according to label directions with DDD water. Cereal samples were prepared in the same way as described previously.

Deproteinized Milk

Homogenized, pasteurized whole milk was deproteinized according to a procedure adapted from Larson and Rolleri (1955). Five hundred milliliters of milk was placed in a 1,000-ml Erlenmeyer flask and heated to 20° C on a Fisher Thermix Stirring hot plate. The pH was lowered to 4.6 with 10% HCl and the sample incubated for 15 min at 20° C with gentle stirring to precipitate casein. After centrifuging for 15 min at a relative centrifugal force of 2,335 (model K centrifuge, International Equipment Co.), the supernatant was filtered through Whatman No. 1 filter paper into a 1,000-ml Erlenmeyer flask and the pH readjusted to 4.6 with 10% HCl. The whey proteins, excluding the proteose-peptone fraction, were then heat-denatured via a 30-min incubation at 96° C in a constant-temperature water bath. The flask was cooled in an ice bath and the contents centrifuged as before. The supernatant was filtered through a 2-μm millipore filter with vacuum and the pH raised back to the initial value of 6.7 with 10% NaOH. The pale green liquid was reheated to 90° C on the hot plate with stirring and then immediately cooled in an ice bath. The contents were again centrifuged and filtered in the same manner and the pH readjusted to 6.7. A 20-ml aliquot was added to each of the cereal samples, and the procedure described above was followed.

Lactose-Free Milk

Lactaid, a commercial retail enzyme preparation, was added according to package directions to achieve a stated 100% conversion of lactose to glucose and galactose (Sugarlo Co., Inc., Pleasantville, NJ). These "lactose-free" samples were then added to the cereals and treated in the same manner as described above.

Analyses

All cereal samples with water, whole milk, and the modified milk samples described above were analyzed in duplicate for total, elemental, total nonelemental, total soluble, insoluble nonelemental, soluble complexed, total ionic, ferrous, and ferric iron, according to a modification of the method of Lee and Clydesdale (1979).

Elemental Iron

Duplicate samples of elemental iron were abstracted from each slurry by a series of six teflon-coated stirring bars, each of which was placed in the slurry sequentially for 5 min. After stirring, each bar was removed, rinsed with DDD water, and placed in a 250-ml Erlenmeyer flask containing 40 ml of concentrated HCl. All six bars were allowed to stand overnight in this solution, which was then diluted to 1,000 ml with DDD water and aspirated directly into the Atomic Absorption Spectrophotometer (AAS) (Perkin-Elmer model 372 Atomic Absorption Spectrophotometer). Standard iron solutions were prepared from Fisher AAS certified 1,000-ppm stock solutions with HCl concentrations equal to those in the sample to reduce viscosity effects.

Nonelemental Iron

Following the abstraction of elemental iron, duplicate 10-ml aliquots of sample slurry were pipetted into separate 100-ml digestion flasks containing 20 ml of concentrated HCl and three boiling chips. After boiling 15 min, the samples were filtered through Whatman No. 1 filter paper into 50-ml volumetric flasks and made to volume with DDD water. The iron was measured by AAS. A blank containing 10 ml of DDD water in place of the 10-ml slurry was digested for the same amount of time, cooled, and partially diluted with DDD water. One hundred twenty-five microliters of a 1,000-ppm Fisher A.A. certified iron standard was added to this blank to produce a 2.5-ppm iron solution. This digest blank then served as the iron standard for the nonelemental iron samples.

Total Iron

Total iron is the sum of elemental and nonelemental iron and is verifiable by analysis.

Total Soluble Iron

Duplicate 50-ml samples of slurry were placed in 50-ml plastic tubes and centrifuged at a relative centrifugal force of 2,335 $\times g$ for 30 min. Immediately after centrifugation, the supernatant was decanted, and 10-ml aliquots taken by pipette for acid digestion and subsequent AAS analysis as in the determination of nonelemental iron.

Insoluble Nonelemental Iron

Insoluble nonelemental iron is equal to the difference between nonelemental iron and total soluble iron.

Ionic, Ferric, and Ferrous Iron

References to ionic and ferrous iron are in fact references to any soluble iron that is reactive with bathophenanthroline, with and without added hydroxylamine, respectively. Since ferric iron is calculated by difference, it is also dependent upon the reaction with bathophenanthroline.

A few minor modifications of the original bathophenanthroline procedure (Lee and Clydesdale 1979) were used in this study. In the present study, 1 ml of 1.0 M sodium acetate buffer (pH 4.0) was added to each 60-ml separatory flask. One milliliter of 10% hydroxylamine hydrochloride (a reducing agent) was added to the odd-numbered flasks. The total volume for each flask was then increased to 10 ml with DDD water. Fifteen milliliters of 0.012% bathophenanthroline in 95% ethanol was added to each funnel. The reason for following this order of addition was to avoid interaction between the food and the buffer, which would change the incubation pH before analysis (Platt and Clydesdale 1983).

Five-milliliter aliquots of the cereal slurry supernatant obtained as described previously were pipetted into each of the two separatory flasks described above, with one containing a reducing agent (ionic iron), and the other without (ferrous iron). The amount of ferric iron was calculated by difference (ionic minus ferrous). The solutions were shaken for 5 sec, 10 ml of chloroform was added exactly 30 sec later, and the flasks were reshaken. This precise timing was followed because bathophenanthroline may react with complexed iron, as was noted by Gorman and Clydesdale (1983) in the case of ascorbic acid. Bathophenanthroline was found to have a greater binding constant than ascorbic acid, and its ability to react was time dependent. Therefore, a constant time allows comparison but is not strictly a measure of ionic iron only.

Reagent blanks for ionic and ferrous iron were analyzed simultaneously with the samples. These included two separatory flasks, one with and one without reducing agent, with all reagents added except the sample supernatant. In addition to the reagent blank, an organic blank was produced by extracting the sample (minus the bathophenanthroline) with 10 ml of chloroform. This blank would have accounted for any chemical specie that was both extracted into the organic layer and absorbed at 533 nm. Absorbance readings found in both blanks were subtracted from sample readings.

For each sample, the extracted bathophenanthroline complex in the lower chloroform layer was drained into a 25-ml volumetric and diluted to volume with 95% ethanol. The absorbance of each sample was measured in 1-cm glass cuvettes at 533 nm, using a Hitachi Perkin-Elmer UV-vis spectrophotometer, model 139. In some of the milk-cereal slurries, an additional centrifugation procedure was necessary to remove what appeared to be protein-induced emulsions that formed at the aqueous-chloroform phase interface. This was done by emptying the separatory funnels into 50-ml plastic tubes and centrifuging for 15 min at 2,335 $\times g$. The aqueous phase was removed by pipette, and the organic phase plus any remaining interface emulsion was filtered through Whatman

No. 1 filter paper into 25-ml volumetric flasks and made to volume with 95% ethanol.

Soluble Complexed Iron

The soluble complexed iron was equal to the difference between total soluble iron and ionic iron.

Total Iron in Milk

The endogenous iron in the whole milk and modified milk samples was analyzed by AAS as follows: 10-ml samples were placed in 100-ml Kjeldahl flasks with 20 ml of concentrated HCl and three glass bead boiling chips. After boiling 15 min, the samples were allowed to cool and were filtered through Whatman No. 1 filter paper into 50-ml volumetric flasks and made to volume with DDD water.

Evaluation of the Effect of Iron Concentration on Soluble Iron

Since the amount of iron added to the three cereals differed by as much as 50%, it was decided to evaluate the effect of the initial concentration of added iron on the final chemical profile of the iron in a wheat-based cereal. Hydrogen-reduced iron was added to the control to provide a sample with 60% more iron. Both samples were then subjected to the sequential pH treatment described previously and analyzed for percentage total soluble iron.

RESULTS

The bioavailability of iron depends on its physicochemical state at the site of absorption in the duodenum. Furthermore, this state is in part due to the various environmental pH values to which the iron is subjected before reaching the site of absorption. Therefore, this study incorporated a sequential pH treatment consisting of an endogenous pH of approximately 6.0 (as eaten), pH 2.0 (stomach), pH 6.0 (duodenum), with an analysis of the complete iron profile conducted at each level. This analysis provided data for nine forms of iron for each sample at each pH value. However, for clearer comprehension and comparison it was decided to present the data in the form of figures that show percentage insoluble nonelemental, soluble complexed, and soluble ionic iron. The sum of these three equals total nonelemental iron, and this sum subtracted from 100 equals elemental iron. The sum of ionic and soluble complexed equals total soluble iron and the ionic is made up of ferrous and ferric iron. Thus, all the forms, with the exception of ferrous and ferric, may be obtained from the figures and, where appropriate, the percentage of these two forms are stated in the discussion. The soluble forms presented in the figures are the most meaningful in terms of their potential effect on bioavailability. The ionic iron may consist of both ionic and complexed iron, which reacts with bathophenanthroline in a 30-sec interval under the conditions of this study.

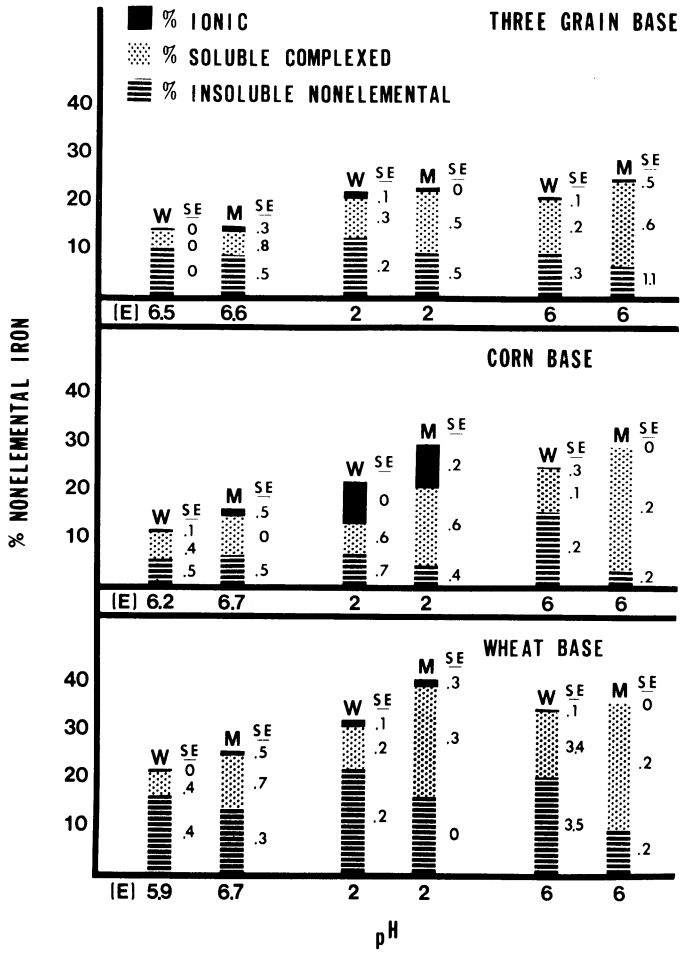

Fig. 1. The effect of whole milk versus double-distilled deionized water on the percentage of ionic, soluble, and insoluble-complexed iron in three-grain, corn, and wheat cereals over a sequential pH treatment from endogenous to 2.0 to 6.0. E = endogenous pH of cereal, W = sample with water, M = sample with milk, SE = standard error expressed as ± percent.

Cereal and Whole Milk Slurries

A comparison of the effect of whole milk on the iron profile of the three cereals versus the effect of DDD water at each sequential pH level tested is shown in Fig. 1. It should be noted that the pH values, in the first step of the sequential treatment marked (E) in Fig. 1, are different both between cereals and within cereal samples. It was decided not to control pH in this step because it represents pH that would occur if the water and milk were added before consumption.

Upon addition of whole milk to the three-grain water slurry (Fig. 1) at endogenous pH there was an increase in total soluble iron from 3.94 to 6.83% and an increase in ionic iron from 0.25 to 1.25% in the ferric form only. When the endogenous pH was lowered to 2.0, soluble iron in both cases increased, with the milk sample still showing the greatest effect. At this pH some ferrous iron was formed, but the total percentage of ionic iron was still low. As the pH was raised from 2.0 to 6.0, there was a further increase in soluble iron, with the milk sample continuing to be most affected and ionic iron remaining at approximately the 1% level.

Rizk and Clydesdale (1983) suggested the use of an "enhancing factor" to remove effects due to absolute changes in iron concentration at various pH values and focus on comparisons between pH levels. They defined this factor as the ratio of the percentage of soluble iron in a treated sample to the percentage of soluble iron in an untreated sample. This technique was also used in this study to compare effects of different pH levels and treatments. Thus, Table I shows that the relative effect of milk, as expressed by the enhancing factor, on the three-grain base was greatest at

TABLE I
The Relative Effect of Milk versus Water on Total Soluble Iron in Cereals as Expressed by the Enhancing Factor[a]

Comparative Treatment and Sample	Endogenous[b]	2.0	6.0
Whole milk			
Three-grain	1.73	1.40	1.48
Corn	1.68	1.73	2.68
Wheat	2.17	2.56	1.93
Nonfat dry milk			
Three-grain	1.66	1.42	1.57
Corn	1.56	1.76	2.54
Wheat	1.39	2.57	2.17
Lactose-free milk			
Three-grain	1.60	1.19	1.55
Corn	1.71	1.80	2.45
Wheat	1.91	2.69	2.26
Deproteinized milk			
Three-grain	1.57	0.74	0.89
Corn	2.00	1.11	2.48
Wheat	1.28	1.01	0.63

[a]Enhancing factor = percent total soluble iron with milk/percent total soluble iron without milk.
[b]Endogenous pH as shown in Figs. 1–4.

endogenous pH and slightly lower at both pH 2.0 and 6.0. This and the percentage changes in Fig. 1 allow more direct comparisons and avoid the influence of different values at the endogenous pH.

As Fig. 1 shows, the same solubilizing trends with pH are observed for the corn- and wheat-base cereals as were noted with the three-grain base cereals. In all cases, more iron was solubilized with whole milk than with water. However, the relative effects were different for each cereal, as shown by examination of the enhancing factors (EF) in Table I. The three-grain cereal showed a maximum solubilizing effect of milk and therefore a maximum EF (1.73) at endogenous pH, whereas corn showed a maximum EF of 2.68 after sequential treatment at pH 6.0. Wheat, on the other hand, had a maximum at pH 2.0 (EF = 2.56). Interestingly, all three cereals, in both water and milk, had ionic iron formed at pH 2.0, with the corn-based cereal having the greatest amount (9% versus 1%) mainly in the ferrous form. After the pH was raised to 6.0, the ionic iron almost disappeared.

The absolute amount of iron solubilized by whole milk after sequential pH treatments was not as great in the three-grain-base cereal (12% with water versus 18% with milk) as it was in the corn (9% with water versus 24% with milk) and wheat (13% with water versus 26% with milk). However, in all cases, the potential for improved bioavailability was present. Therefore, it was decided to evaluate several fractions of milk in the same manner to attempt to identify the particular fraction that contained the solubilizing factor.

Cereal and Nonfat Dry Milk Slurries

The effect of nonfat dry milk in the three cereals, versus DDD water, on the iron profiles at each sequential pH level is shown in Fig. 2. Examination shows that nonfat milk produced the same trends as whole milk and similar iron profiles in every case. Furthermore, a comparison of the enhancing factors in Table I shows similar effects, with the exception of wheat at endogenous pH where the EF changes from 2.17 to 1.39 with the change from whole to skim milk. However, this may be due to the lower pH (5.9) of the endogenous wheat-water sample as compared to the corn and three-grain slurries at pH 6.2 and 6.5, respectively. Again, the greatest amount of ionic iron was formed at pH 2.0 in all three cases. However, with the nonfat milk fraction, the ratio of ferrous to ferric was different than for whole milk with 64% of the ionic iron being ferric in the corn and 60% in the wheat, respectively.

Cereal and Lactose-Free Milk Slurries

The results of the study utilizing lactose-free milk are shown in Fig. 3. Again, the results are strikingly similar to those found with both whole and nonfat milk. Confirmation of this similarity is shown in Table I, where the EF values are shown. In ionic iron, the three-grain slurry was all ferrous, the corn 75% ferrous, and the wheat mostly ferric at pH 2.0, although the total ionic in both three-grain and wheat was low. The ionic iron again almost disappeared when the pH was raised to 6.0.

Cereal and Deproteinized Milk Slurries

The effect of deproteinized milk on the solubilization of iron, unlike the other milk fractions, was quite different from whole milk, as shown in Fig. 4. At the endogenous pH, total soluble iron from the water slurry increased because of the deproteinized milk of 4–6% in the three-grain cereal, 6–11% in corn, and 5–7% in wheat. In the three-grain and the corn, the effect was similar to whole milk but was less in the wheat-base cereal slurry, as is also

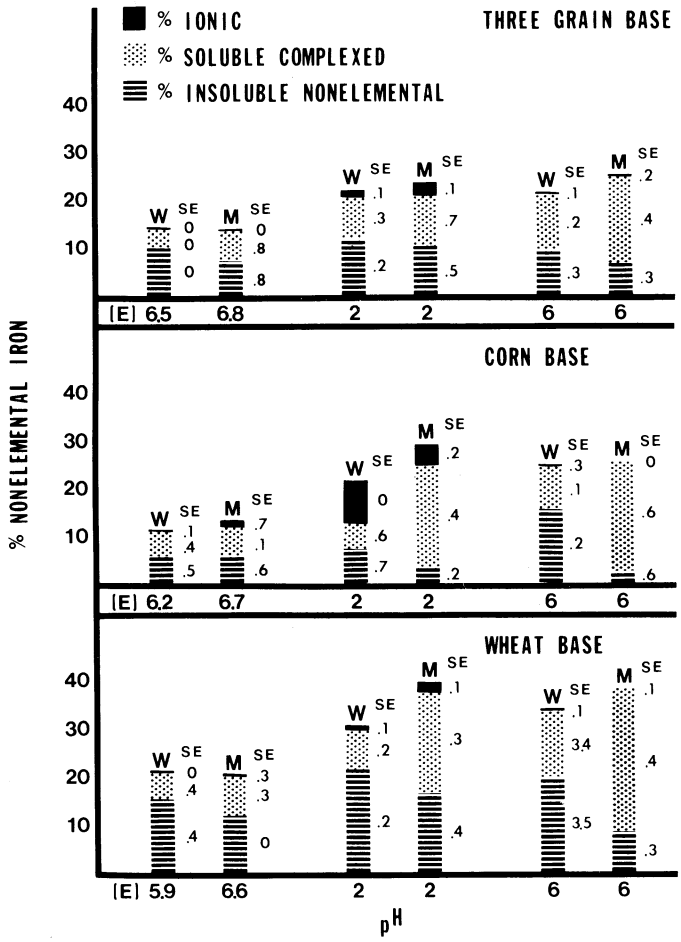

Fig. 2. The effect of nonfat dry milk versus double-distilled deionized water on the percentage of ionic, soluble, and insoluble-complexed iron in three-grain, corn, and wheat cereals over a sequential pH treatment from endogenous to 2.0 to 6.0. E = endogenous pH of cereal, W = sample with water, M = sample with milk, SE = standard error expressed as ± percent.

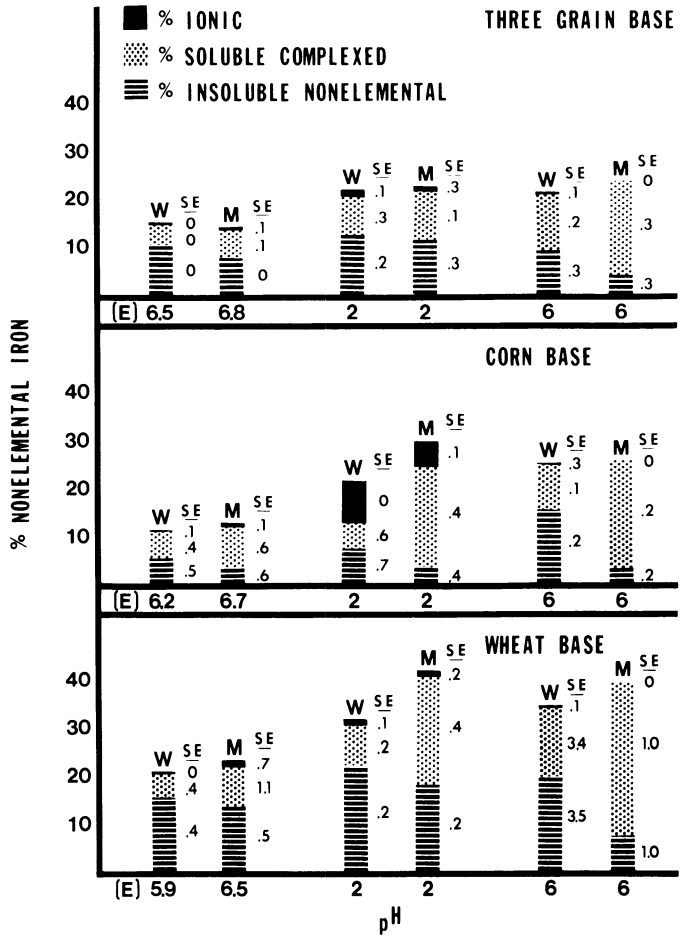

Fig. 3. The effect of lactose-free milk versus double-distilled deionized water on the percentage ionic, soluble, and insoluble complexed iron in three-grain, corn, and wheat cereals over a sequential pH treatment from endogenous to 2.0 to 6.0. E = endogenous pH of cereal, W = sample with water, M = sample with milk, and SE = standard error expressed as ± percent.

evident from the EF values in Table I. At pH 2.0, the comparison to whole milk is much more dramatic in that the corn and wheat base slurries with deproteinized milk had approximately the same total soluble iron as the water slurry and the three-grain slurry even less. This is also shown in Table I, where the EF values for the three-grain, corn, and wheat slurries are 0.74, 1.11, and 1.01, respectively, as compared to 1.40, 1.73, and 2.56 for whole milk. After sequential pH treatment at pH 6.0, this same trend for the three-grain and wheat slurries continued with EF values equal to 0.89 and 0.63, respectively, compared with whole milk at 1.48 and 1.93 (Table I). However, in corn, the deproteinized milk solubilized as much iron as whole milk, resulting in an EF of 2.48 as compared to 2.68 for the latter. The three-grain and wheat slurries had less than 1% ionic iron at all pH levels, and the corn had 4.7% ionic at pH 2.0, of which 78% was ferrous. At pH 6.0, the ferrous was converted to ferric.

DISCUSSION

The results obtained in this study indicate that the factor in milk that solubilizes both added and endogenous iron in the cereals under study is contained in the protein fraction. It is tempting to attribute this effect to the iron-casein complexes studied by Osterberg (1961) and Nelson and Potter (1979, 1980), but unfortunately the evidence is not completely clear-cut. This fraction may be defined as that obtained by the procedure of Larson and Rolleri (1955). In the deproteinized milk, the corn cereal samples showed a reduced solubilization effect at both pH 2.0 and 6.0 in comparison to whole milk samples but not at the endogenous pH. Although the reason for this difference is not immediately apparent, a few possible factors may be considered.

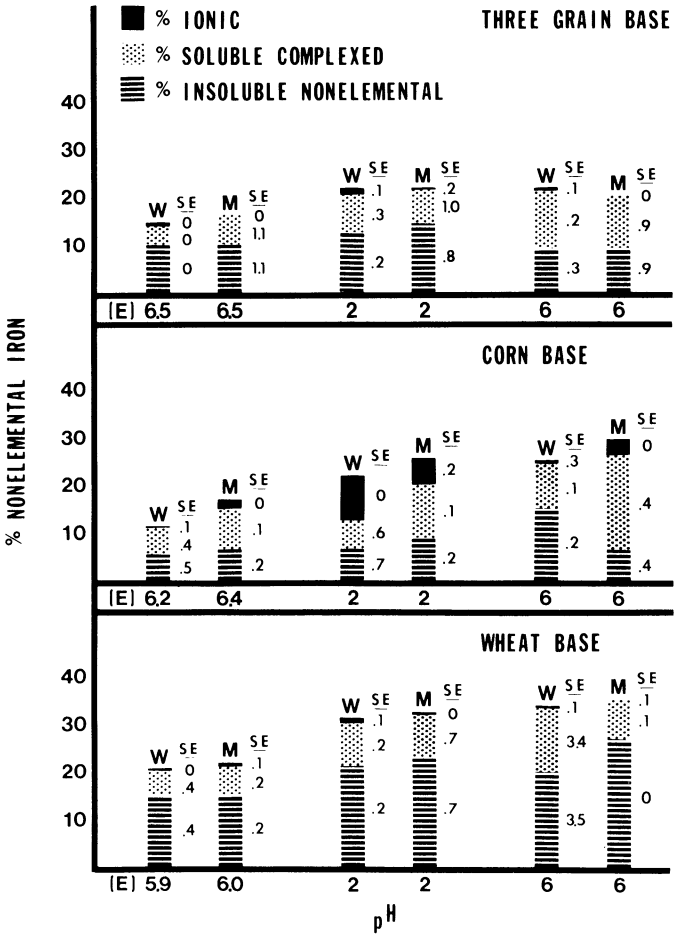

Fig. 4. The effect of deproteinized milk versus double-distilled deionized water on the percentage ionic, soluble, and insoluble complexed iron in three-grain, corn, and wheat cereals over a sequential pH treatment from endogenous to 2.0 to 6.0. E = endogenous pH of cereal, W = sample with water, M = sample with milk, and SE = standard error expressed as ± percent.

The first of these is the potential effects of differences in initial iron concentrations since the corn cereal had the greatest initial concentration of iron. As stated previously, hydrogen-reduced elemental iron was added at levels of 35, 43, and 28 mg/100 g to the wheat, corn, and three-grain cereals, respectively. Total iron was found at levels of 43, 52, and 36 mg/100 g, providing values of 8, 9, and 8 mg/100 g of endogenous iron, by difference, for the wheat, corn, and three-grain cereals, respectively. Since the endogenous levels are very similar, any concentration effects might be due to added iron. However, when two samples of another wheat-based cereal in water containing 26.9 mg of iron/100 g and 42.8 mg/100 g were subjected to the sequential pH treatment, the formation of soluble iron at the different pH levels was similar in both samples. From endogenous pH to 2.0 to 6.0, one sample went from 1.97 to 4.54 to 2.30 mg/100 g, and the other from 2.63 to 5.53 to 2.93. Therefore, concentration differences alone could not explain the unique effect the corn cereal showed with the deproteinized milk samples.

The second factor that should be considered is the composition of the cereals. The grains involved are corn, wheat, and a combination of corn, wheat, and oats in the three-grain cereal. Anderson and Clydesdale (1980a, 1980b) have reported similar values for the dietary fiber content of wheat and corn bran. However, Chen and Anderson (1980) point out that oat bran has a lower water-soluble fiber and is poor in cellulose, whereas wheat bran has a lower water-soluble fiber content and a higher cellulose content. Since similar results were achieved for three-grain and wheat and dissimilar results between corn and both of these, it would seem that differences noted in dietary fiber are not responsible for differences noted in iron solubilization. If they were responsible, the corn and the wheat would react in a similar manner, with the three-grain showing a different trend based on fiber differences. This indicates that differences noted are probably due to the proteins of the cereals. The protein content of wheat is approximately 12%, consisting primarily of gliadin and glutenin, which together constitute gluten. Corn kernels are about 10% protein, with most of the protein in the endosperm in the form of zein (40–50%) and glutenin (30–40%). However, large varietal differences have been noted. The corn embryo consists of albumins and globulins, which are less than 10% of the protein in the endosperm. Oats have a protein content of 11–15%, but their nature has not been as thoroughly investigated as that of corn and wheat (Inglett 1977).

Although this study was not extensive enough to prove the involvement of any particular component, interactions with zein may be responsible for the binding characteristics that are unique to the corn sample. Nelson and Potter (1979, 1980) have conducted investigations of model systems of various proteins bound to both ferric and ferrous iron. Interestingly, they found striking differences between the binding characteristics of zein and wheat gluten with iron, both in response to pH and to time (Nelson and Potter 1979). Certainly, the present study cannot be compared directly to their study, but it does indicate that future work might examine the interactions between zein and iron in food more closely.

Another interesting phenomenon noted with the cereal samples under study was the formation of bathophenanthroline-reactive iron, which may consist of both ionic and soluble complexed iron, and the valence state at each pH. Clydesdale (1982) has discussed the interrelationship of pH and reduction potential on iron chemistry whereby ionic iron achieves its lowest thermodynamic and therefore most stable state according to the following reactions;

$$Fe^{+3}\ (\text{aqueous}) + e^{-} \leftrightarrow Fe^{+2}\ (\text{aqueous})\ (\text{acid solution})$$

$$Fe(OH)_3\ (\text{solid}) + e^{-} \leftrightarrow Fe(OH)_2\ (\text{solid}) + (OH)^{-}\ (\text{neutral or basic solution})$$

In the first reaction in acid solution, the standard reduction potential is +770 mV, indicating a tendency to occur spontaneously. However, in the second reaction, at a higher pH, the standard reduction potential changes to −560 mV. This means that at acid pH, Fe^{+2} is formed spontaneously while at more basic pH, the formation of Fe^{+3} is favored. In a series of experiments with

different iron sources at different pH values and reduction potentials, in different foods these reductions were shown to hold in nearly all cases (Nojeim and Clydesdale 1981, Nojeim et al 1981).

From Figs. 1–4, it is clear that the greatest amount of ionic iron was formed in the corn cereal samples both with water and with milk fractions. In the other cereals, the total soluble iron existed mainly as complexed iron. This implies that the interrelationship between reduction potential and bond strength of the iron complexes formed in the corn cereal was different from that in the other cereals.

In the whole milk, lactose-free, and deproteinized samples (Figs. 1, 3, 4), the changes in valence state and total ionic iron with pH were as would be expected. That is, at pH 2.0 the most ionic iron formed, as would be expected, according to solubility, and this iron was mainly in the form of Fe^{+2}, as would be expected from the predicted reduction potentials at this pH. At pH 6.0, at the end of the sequential treatment, the amount of ionic iron decreased, as would be expected from known solubility values at this pH, and the ionic iron in solution was mainly in the Fe^{+3} form, consistent with predictions.

However, in the nonfat dry milk samples (Fig. 2), although most ionic iron formed at pH 2.0, there was an unexpectedly large amount of Fe^{+3} in the corn and wheat (64% and 60% Fe^{+3}, respectively) samples. Without further studies, it would be unwise to speculate on the reasons for this occurrence.

As indicated previously, however, the ionic fraction consists of all-soluble iron, which reacts with bathophenanthroline in the presence of hydroxylamine. This may include some iron that is complexed to fiber in the samples as well as true ionic iron. Therefore, the explanations based on reduction potential would only apply to free ionic iron, which is undoubtedly limited in a mixture of cereal and milk. However, several studies by Camire and Clydesdale (1981), Fernandez and Phillips (1982), and Reinhold et al (1981) have indicated that dietary fiber does not bind at pH values below 4.0–5.0, which would explain any increases in free iron from iron complexes at pH 2.0. However, this does not apply to protein complexes as noted by Clydesdale (1983) and therefore cannot explain all the increases in the bathophenanthroline reactive iron.

In conclusion, this investigation clearly indicated an iron solubilizing effect achieved with a combination of milk and cereal that did not occur with cereal and DDD water. Furthermore, in the wheat base and three-grain base cereals, solubilization largely disappeared with the deproteinization procedures used in this study. This effect was unpredictable from a theoretical point of view and is therefore an important observation, particularly because soluble iron has a much greater potential for absorption. With our present state of knowledge, elemental iron is probably the best iron additive in dry cereals because it is nonreactive. It has been shown that more reactive and potentially more bioavailable iron sources are converted to insoluble hydroxides when stored at the pH of cereals and become refractory and, therefore, irreversibly insoluble even when the pH is lowered to 2.0 (Clydesdale 1983). Thus, any process that increases solubilization of the elemental iron just before eating is important. These samples also did not have any ascorbic acid added, which has a dramatic iron-solubilizing effect in both wheat (Camire and Clydesdale 1982) and soy (Rizk and Clydesdale 1983). Therefore, the potential for increased bioavailability could be increased further by other technological intervention techniques.

These results indicate that predictions of iron bioavailability must be made on the basis of food as eaten in a meal and not on isolated iron sources or single foods. Further studies should be undertaken to establish the chemical nature of this phenomenon and to explain some of the apparent discrepancies. Such a chemical understanding is essential to understanding the mechanism of iron absorption from food, which must be clearly defined if the problem of worldwide iron deficiencies is to be resolved.

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