Effect of Pepsin Treatment on the Chemical Iron Profile of Soy-Based Foods Supplemented with Selected Iron Sources and Enhancers

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ABSTRACT

Changes in chemical iron profile occurring from pH 2 to 6.5 in a wheat-soy blend, a corn-soy-milk mix, and a soy-extended beef patty were investigated. Iron solubility in these products, as affected by in vitro digestion with pepsin, was dependent on a combination of ligand, iron source, pH and food. The greatest solubilizing capacity of the ligands added was provided by ascorbic acid at pH 2 and 4, and by citric acid at pH 6. Improvements in percent soluble iron were related to pepsin digestion and the presumed appearance of protein degradation products.

Soy protein products are providing an ever-increasing proportion of the diets in many parts of the world. Substantial tonnages of soy-fortified processed foods and blended food supplements, such as wheat protein concentrate-soy and corn-soy-milk, have been donated by the United States to other countries through Public Law 480 (2). Domestically, the use of soy protein products as meat extenders has become increasingly popular in both the school lunch program and the U.S. military, where up to 30% of the meat requirement may be substituted with hydrated soy (17). Unfortunately, recent evidence has suggested a potential inhibitory effect of soy protein on the bioavailability of iron to man (4, 19).

To date, ascorbic acid and meat appear to be the only reliable means of promoting iron availability from soy products (3). While the affect of ascorbic acid on soy has been attributed to its ability to solubilize iron in vitro (15), the precise mechanism(s) responsible for the ability of meat to improve nonheme iron absorption has not been identified. Many researchers have postulated that the high availability of iron from both animal and vegetable products may actually be due to the nature of the proteinaceous degradation products formed during the digestion process (1, 6, 7, 9, 12).

Previous investigations in this laboratory have examined the effects of selected organic acids, exogenous iron sources and heat processes on the iron solubility of a wheat protein concentrate-soy blend, a corn-soy-milk blend, and a soy-extended beef patty (14, 16). From these studies we were able to provide specific combinations of numbers which exhibited the greatest potential for improving iron availability in man as measured by chemical means.

It was the intent of the present study to investigate the effects of a pepsin-HCl treatment on the chemical iron profiles of the previously described soy-based products.

MATERIALS AND METHODS

Chemical iron analysis

A modification of the Lee and Clydesdale (10) procedure was used in this study and has been outlined in detail (15).

Soy-based food sources

A wheat protein concentrate-soy (WPC-Soy) dry mix, a corn-soy-milk (CSM) blend, and an Ardex-DHV soy protein isolate were obtained courtesy of the ADM Milling Company, Shawnee Mission, KS.

Iron sources

Three exogenous iron sources were used in this study. Both electrolytic reduced iron (ERFe), consisting of 98.3% iron with 98% less than 44 microns, and hydrogen-reduced iron (HRFe), consisting of 96.9% iron with 97% less than 44 microns, were obtained courtesy of Glidden-Durkee, NY. Ferric orthophosphate (FOP), containing 29.1% iron, was obtained courtesy of the Joseph Turner & Company, NJ.

All iron sources were added in sufficient amounts to each sample to provide a 1:1 ratio of exogenous (iron source): endogenous (food) iron.

Ligands

Ligands chosen for evaluation included: L-ascorbic acid (ASC) and citric acid monohydrate (CIT) which were purchased from Fisher Scientific Company, MA; L-cysteine hydrochloride monohydrate (CYS) purchased from J.T. Baker Chemical Company, NJ; and calcium lactate pentahydrate (LAC) purchased from Eastman Kodak Company, NY.
Ligands were added in amounts which provided an 8:1 molar ratio of ligand to iron to conform with previous studies (4, 14, 15).

Sample preparation
The specific iron-organic acid combinations employed in the following sections were chosen for their ability to significantly improve iron solubility in these foods.

WPC-Soy drink
Thirty-two g of WPC-Soy dry mix (6.6 mg endogenous iron) were blended with 175 ml of double distilled deionized water in a 250-ml beaker. Each sample was fortified with HRFe and either ASC or CIT.

Boiled CSM
Twenty g of CSM (3.85 mg endogenous iron) were mixed with 200 ml of double distilled deionized water. The sample was boiled for 5 min, cooled to room temperature (20°C), and brought back to volume with double distilled deionized water before analysis. Appropriate amounts of FOP and ASC were added to the CSM before boiling.

Baked CSM
Combinations of HRFe with either ASC, CIT or CYS were added to 20 g of CSM and 8 ml of double distilled deionized water and mixed to a pasty consistency. Each sample was baked at 218°C (425°F) for 20 min, cooled to room temperature, and blended with 200 ml of double distilled deionized water as described previously (14).

SH patty
In accordance with the regulations of the Interim Federal Specification publication PP-B-2120 (July, 1980), the Ardex-DHV was blended with double distilled deionized water and beef hamburger to produce a soy hamburger (SH) patty as previously described (14). Combinations of HRFe and either ASC, LAC or CIT were mixed with a 50-g SH patty (1.0 mg endogenous iron) before processing. Each sample was then blended with 150 ml of double distilled deionized water to produce a slurry for chemical iron analysis.

Use of pepsin in a simulated gastrointestinal procedure
The temperature of each sample slurry was maintained at 37 ± 2°C for the entire time of each incubation period. Each food slurry was incubated at the endogenous pH (pH 5.0 to 6.5) for 30 min. After sampling, the pH of the slurry was adjusted to pH 2.5 using concentrated HCl, the pepsin enzyme added, and the slurry pH further lowered to pH 2.0. The sample was then sequentially incubated at pH 2 (30 min), pH 4 (10 min) and pH 6 (10 min). Sample pH was adjusted to pH 4 and 6 using 50% (wt/wt) NaOH. A chemical iron analysis was performed on the samples after each incubation period.

Porcine pepsin enzyme (600 units activity/mg solid) was purchased from the Sigma Chemical Company (St. Louis, MO). Sufficient amounts of this enzyme were added to each food slurry at pH 2.5, to produce 6000 units of pepsin activity/g food (wt/wt). These quantities were calculated from pepsin activity levels as measured by several other investigators (5, 6, 11-13).

Statistical analysis
Data were analyzed using T-tests to determine significant differences between two means (18).

RESULTS AND DISCUSSION

The term “enhancing factor” has been defined as the ratio of the percent soluble iron in a treated sample to the percent soluble iron in an untreated sample (15). This factor removes the effects due to absolute changes in iron solubility at various pH values and concentrates on the effect of the treatment at a given pH, thus allowing for more valid comparisons between pH values. The treated and untreated samples refer to those with and without pepsin, respectively. A value greater than one indicated an enhancing or solubilizing effect of pepsin on iron. The calculated enhancing factors are presented in Table 1.

The pepsin treatment caused no significant difference in percent soluble iron at any pH when the WPC-Soy blend was supplemented with ASC. However, supplementation with CIT caused a significant improvement in percent soluble and soluble complexed iron at pH 4. Similar results were observed for the fried SH patty system where iron solubility was significantly enhanced by pepsin treatment when this food was supplemented with CIT at pH 2, 4 and 6, but not with ASC.

Kane and Miller (7) recently investigated the effects of an in vitro digestion on the dialyzable iron of several protein sources. Iron availability, as estimated by dialyzable iron, was high for beef protein, but low for casein, gluten and soy protein isolate. While direct comparison to the results of Kane and Miller (7) was not possible, it was interesting to note that a greater dialysis of iron was observed when iron-citrate complexes were added as compared to iron-ascorbate complexes. Supplementation of CIT to the soy-based products in the present study provided less soluble iron (soluble = ionic plus soluble complexed) than ASC supplementation at pH 2 and 4, but significantly more at pH 6 (Figs. 1 to 3). The results at pH 6 compare favorably with those of Kane and Miller and may be attributed to measurement of dialyzable iron at a more neutral pH (7.5).

A large, but nonsignificant, improvement in enhancing factor (1.70; Table 1) was calculated for the ASC-supplemented SH patty at the endogenous pH (pH E). This large value was actually the result of a small increase in percent soluble iron from 4.4 to 7.5%. However, when such low absolute values are being considered, greater relative errors and less reliable enhancing factors may result. Therefore, it was essential to examine both the enhancing factor (Table 1) and the total percent soluble iron at each pH (Figs. 1 to 3) in order to properly evaluate the effect of a pepsin digest of these food systems.

The solubilizing capacity of the organic acids on iron in any food was greatest for ASC at pH 2 (49 to 61% soluble) and pH 4 (38 to 49% soluble), and for CIT at pH 6 (21 to 29% soluble). These results agree with those of Kojima et al. (8) who showed that the ability to solubilize iron from a pinto bean suspension was maximal for ASC under acidic conditions, whereas CIT was maximally effective near pH 6.

Of the baked CSM samples (Fig. 2), the CYS-supplemented mix provided percent soluble iron levels at pH 4 and 6 which were similar to those caused by CIT supplementation. However, in the case of CYS, a substantial portion of this soluble iron was provided only after pepsin digestion as suggested by the enhancing values greater than 1.0 (Table 1). This implies that more of the increase in soluble iron may be accounted for by increases in binding to protein digestion products such as amino acids and polypeptides.

Iron solubilization from the fried SH patty was greatest at pH 2 regardless of the organic acids added. Providing
TABLE 1. A comparison of enhancing factorsa resulting from pepsin digestion of several soy-based food products supplemented with selected organic acids and exogenous iron sources.

<table>
<thead>
<tr>
<th>Foodb (Iron sourcec)</th>
<th>pH</th>
<th>ASC</th>
<th>CIT</th>
<th>LAC</th>
<th>CYS</th>
</tr>
</thead>
<tbody>
<tr>
<td>WPC-Soy (ERFe)</td>
<td>E</td>
<td>0.85</td>
<td>0.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.97</td>
<td>1.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.99</td>
<td>2.15f</td>
<td>-</td>
<td>-</td>
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<td></td>
<td>6</td>
<td>0.92</td>
<td>0.90</td>
<td></td>
<td></td>
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<tr>
<td>Boiled CSM (FOP)</td>
<td>E</td>
<td>0.97</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.07</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.32*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>2.07*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baked CSM (HRFe)</td>
<td>E</td>
<td>1.02</td>
<td>1.00</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.01</td>
<td>0.45*</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.31*</td>
<td>1.09</td>
<td>-</td>
<td>1.60*</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>2.34*</td>
<td>0.97</td>
<td></td>
<td>2.77*</td>
</tr>
<tr>
<td>Fried SH (HRFe)</td>
<td>E</td>
<td>1.70</td>
<td>1.41</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.07</td>
<td>1.41*</td>
<td>1.32*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.91</td>
<td>1.80*</td>
<td>0.69</td>
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<tr>
<td></td>
<td>6</td>
<td>1.05</td>
<td>1.48*</td>
<td>0.88</td>
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</table>

aEnhancing factor is the ratio of the percent soluble iron detected with pepsin present to the percent soluble iron without pepsin present.
bASC, ascorbic acid; CIT, citric acid; LAC, lactic acid; and CYS, cysteine.
cWPC, wheat-protein-concentrate; CSM, corn-soy-milk; and SH, soy-hamburger.
dERFe, electrolytic reduced iron; HRFe, hydrogen reduced iron; and FOP, ferric orthophosphate.
E, endogenous pH.
fValues marked with an asterisk show significant differences from the control (without pepsin) at the 5% level.

In a more neutral environment (pH 4 and 6) caused reductions in percent soluble iron for all ligands, but was greatest for the LAC-supplemented sample. This indicates that neither LAC nor the protein degradation products present were capable of providing a suitable environment for iron absorption as estimated by iron solubility.

Nelson and Potter (12) demonstrated that iron solubilization from wheat gluten, casein or soy isolate proteins was significantly greater when treated by HCl-pepsin than by HCl alone, and parallelled protein digestion. The extent to which bound iron was released was dependent upon the form of iron (ferrous or ferric) and the protein source evaluated. The present data are in agreement with those of Nelson and Potter (12) and also indicate that organic acid supplementation dramatically influences the chemical iron profiles of soy-based food products when treated with pepsin.

In summary, clinical studies have demonstrated that the iron in cereal-soy blends and SH patties is poorly absorbed (3). Supplementation of these products with specific iron-ligand combinations has provided us with a means of improving soluble iron (14, 16). The further improvements in iron solubility in these systems noted after a pepsin digestion indicate that previous studies may have underestimated the full potential of these enhancers. While the efficacy of such treatments on human iron absorption remains to be determined, understanding the chemical behavior of competing ligands, such as soy protein and organic acids, a greater knowledge of the conditions influencing iron availability in man will ultimately be acquired.

**ACKNOWLEDGMENTS**

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**REFERENCES**

Figure 1. Percent of ionic, soluble complexed and insoluble iron in a wheat-protein-concentrate-soy (WPC-Soy) supplemented with electrolytic reduced iron (ERFe) and ascorbic or citric acids in a boiled corn-soy-milk (CSM) blend supplemented with ferric orthophosphate (FOP) and ascorbic acid.

Figure 2. Percent ionic, soluble complexed and insoluble iron in a baked corn-soy-milk (CSM) blend supplemented with hydrogen-reduced iron and either ascorbic, citric acid or cysteine.

Figure 3. Percent ionic, soluble complexed and insoluble iron in a fried soy-hamburger (SH) patty supplemented with hydrogen-reduced iron and either ascorbic, lactic or citric acid.

of the elemental, ferrous, ferric, soluble, and complexed iron in foods. J. Food Sci. 44:549-554.