Effectiveness of Organic Acids to Solubilize Iron From a Wheat-Soy Drink

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ABSTRACT

Changes in the chemical iron profile of a wheat protein concentrate-soy (WPC-Soy) drink resulting from organic acid (ligand) and iron fortification were examined. The ability of each ligand to enhance iron solubility in a WPC-Soy blend was dependent upon both pH and iron source added. Using an 8:1 molar ratio of ligand:iron, the greatest increases in percent soluble iron occurred with ascorbic acid and cysteine at pH 2, ascorbic acid at pH 4, and citric acid at pH 6. Electrolytic reduced iron (ERFe) was considered to be the most suitable iron fortificant (1:1 ratio of exogenous:endogenous iron) for this food on the basis of both iron solubility and technological feasibility. Supplementation of the WPC-Soy blend with ERFe and ascorbic or citric acid was shown to provide the greatest potential for improving iron availability in man as measured by chemical means.

Public Law 480 gave responsibility to the U.S. Department of Agriculture for purchase of commodities for distribution in the foreign donation program (28). The objectives of this law were to promote international trade in agricultural commodities, to combat hunger and malnutrition, and to further economic development (3).

In many developing countries, populations subsist predominantly on cereal and legume diets. Unfortunately, due to the low availability of iron from these diets, those same populations have shown the greatest prevalence and severity of iron deficiency (4). Donated foods, such as corn-soy milk, wheat-soy, and protein concentrate-soy (WPC-Soy) blends, have been distributed to more than 100 million people in 90 developing countries. WPC-Soy is one of the most protein- and calorie-dense foods available for weaning infants, preschool children and lactating mothers (16).

To date, the most reliable means of improving iron availability from cereals and legumes is via the addition of ascorbic acid or meat (4). Ascorbic acid has demonstrated an ability to reverse the inhibitory effects of soy protein products (22), and this reversal has been attributed to ascorbate’s solubilizing capacity in vitro (26).

However, use of ascorbic acid as an enhancer has been limited due to its poor stability under conditions of processing and storage (4).

The purpose of the present study was to evaluate the effectiveness of selected ligands as potential promoters of iron availability in a wheat-soy food blend as estimated by chemical analyses. It was hoped that this investigation would provide further understanding of the chemical interactions between iron and its enhancers and inhibitors.

MATERIALS AND METHODS

Chemical iron analysis

A modification of the procedure of Lee and Clydesdale (18) was used in this study and has been outlined in detail elsewhere (26).

WPC-Soy food blend

The WPC-Soy dry mix was received courtesy of ADM Milling Company, Shawnee Mission, KS. The contents of this food source are listed in Table 1.

Iron sources

Four exogenous iron sources were used for this study. Ferrous sulfate heptahydrate (FeSO₄) contained 20.1% iron and was obtained from Fisher Scientific Company, MA. 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 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

Based on thermodynamic and kinetic stability constant data previously determined in this laboratory (9, 11, 12), five ligands were chosen for evaluation. These included: L-ascorbic acid (ASC), citric acid monohydrate (CIT), and malic acid (MAL) 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 an 8:1 molar ratio (ligand:iron) because Morck et al. (22) found that this level increased iron absorption in humans five-to six-fold.

Sample preparation

In keeping with the manufacturer’s recommended mix for a beverage, 175 ml of double distilled deionized water were blended with 32 g of WPC-Soy dry mix (6.6 mg endogenous iron). Appropriate amounts of ligands and iron sources were added to this wheat-soy slurry in a 250-ml beaker.

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TABLE 1. Composition of the WPC-Soy food blend.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full fat soy flour</td>
<td>35.85</td>
</tr>
<tr>
<td>Wheat protein conc.</td>
<td>40.55</td>
</tr>
<tr>
<td>Corn syrup solids</td>
<td>8.95</td>
</tr>
<tr>
<td>Soy oil</td>
<td>12.00</td>
</tr>
<tr>
<td>Vitamin premix</td>
<td>0.10</td>
</tr>
<tr>
<td>Mineral premix</td>
<td>2.55</td>
</tr>
<tr>
<td>TOTAL</td>
<td>100.00</td>
</tr>
</tbody>
</table>

\(^a\) Vitamin premix (per 100 kcal) includes: vitamin A (395.9 I.U.), vitamin D (47.8 I.U.), vitamin E (2.3 I.U.), ascorbic acid (9.6 mg), thiamin (0.36 mg), riboflavin (0.14 mg), pyridoxine (0.12 mg), folacin (79.0 µg), pantothenic acid (0.88 mg) and vitamin B12 (0.95 µg).

\(^b\) Mineral premix (per 100 kcal) includes: calcium (164 mg), phosphorus (134 mg), magnesium (48 mg), iron (4.9 mg), iodine (11.9 µg), zinc (1.1 mg), sodium (70.7 mg) and potassium (149.0 mg).

Dual pH incubation procedure
A Teflon-coated magnetic stirring bar was added to each food slurry and placed on a Fisher Thermix stirring plate. Each sample was stirred, i.e., allowed to equilibrate for 30 min at ambient temperature (ca. 20°C) during which time the solution pH (endogenous pH; pH E) was determined. Immediately following this incubation period, an iron analysis was performed. The pH of this blend was then adjusted to 2.0 using concentrated HCl and stirred for another 30 min, at which time another iron analysis was performed.

Selection of best iron-ligand combinations
The exogenous iron source which provided both the largest enhancing factor, (defined as the ratio of the percent soluble iron in a treated sample to the percent soluble iron in an untreated sample) and percent soluble iron values in the presence of each ligand-WPC-Soy blend was examined further. Those iron-ligand combinations which showed the greatest potential were reevaluated using an in vitro method developed in this laboratory. This method simulated the pH, time and temperature conditions of the gastrointestinal tract and has been described in detail elsewhere (26).

Statistical analysis
Data were analyzed using Student t-tests to determine significant differences between two means (29).

TABLE 2. Average percent soluble iron in the WPC-Soy drink.

<table>
<thead>
<tr>
<th>Iron added</th>
<th>pH</th>
<th>Control</th>
<th>ASC</th>
<th>LAC</th>
<th>CIT</th>
<th>MAL</th>
<th>CYS</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>E</td>
<td>6.5</td>
<td>5.9</td>
<td>5.7</td>
<td>9.5*</td>
<td>5.5</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>31.9</td>
<td>66.1**d</td>
<td>32.8</td>
<td>42.5*</td>
<td>45.2*</td>
<td>33.6</td>
</tr>
<tr>
<td>FeSO₄</td>
<td>E</td>
<td>19.6</td>
<td>15.4</td>
<td>19.6</td>
<td>19.0</td>
<td>10.3</td>
<td>13.4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>28.6</td>
<td>70.5**</td>
<td>28.6</td>
<td>34.1</td>
<td>28.0</td>
<td>42.2*</td>
</tr>
<tr>
<td>HRFe</td>
<td>E</td>
<td>5.5</td>
<td>4.3</td>
<td>2.5</td>
<td>8.1</td>
<td>4.4</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>22.1</td>
<td>32.0**</td>
<td>20.2</td>
<td>25.3</td>
<td>21.7</td>
<td>25.6*</td>
</tr>
<tr>
<td>ERFFe</td>
<td>E</td>
<td>3.5</td>
<td>4.8</td>
<td>3.4</td>
<td>9.3*</td>
<td>4.2</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>22.7</td>
<td>47.8**</td>
<td>31.6*</td>
<td>29.1*</td>
<td>26.7*</td>
<td>37.7*</td>
</tr>
<tr>
<td>FOP</td>
<td>E</td>
<td>4.6</td>
<td>4.1</td>
<td>2.6</td>
<td>5.3</td>
<td>3.3</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>23.3</td>
<td>35.2**</td>
<td>22.2</td>
<td>25.8</td>
<td>21.4</td>
<td>28.1*</td>
</tr>
</tbody>
</table>

\(^a\) ASC, ascorbic acid; LAC, lactic acid; CIT, citric acid; MAL, malic acid; and CYS, cysteine.

\(^b\) None, endogenous; FeSO₄, ferrous sulfate heptahydrate; HRFe, hydrogen reduced elemental iron; ERFFe, electrolytic reduced elemental iron; and FOP, ferric orthophosphate.

\(^c\) E, endogenous pH.

\(^d\) Values marked with an asterisk are significantly greater than the control (without organic acid) at either the 5% or 1% level.

RESULTS AND DISCUSSION

The effects of selected organic acids, both with and without added iron sources, on the chemical iron profile of the WPC-Soy drink at pH E (endogenous) and pH 2 are shown in Table 2. It was decided to examine all samples at both the endogenous pH (range of pH 5.3 to 6.5) and at the pH of the stomach (pH 2.0) in order to cover a range of pH values where ligands could have a potential effect on iron solubility. These tests were performed in an attempt to screen for those iron-ligand combinations which possessed the greatest potential for enhancing iron availability in man as measured by chemical means.

The endogenous pH (E) was directly related to the ligand added and ranged from pH 6.5 (without added ligand) to pH 5.3 (with added citric acid). In a separate experiment, controls were developed to replace each organic acid with sufficient concentrated HCl to produce equivalent pH values. Since the HCl controls produced no significant differences in chemical iron profile at either pH E or 2, then any difference noted upon addition of a ligand were considered a result of the ligand alone.

The chemical iron profiles of the WPC-Soy food systems reflect a dependency on pH, ligand, and/or iron source added. Almost all soluble iron values at the endogenous pH were 6% or less with the exception of CIT- and FeSO₄-added samples. When the pH of each system was lowered to 2.0, significant increases in percent soluble iron were noted over the pH E profiles. Based on previous analyses performed on a soy protein isolate (26), where iron solubility actually decreased at pH 2 (without added ligands), the chemical iron profiles observed in the WPC-Soy drink would appear to have been influenced more by the other soy components than protein. This observation is certainly noteworthy in light of recent studies (7, 22, 30), which have suggested an overwhelming con-
trIBUTION OF SOY PROTEIN TO THE INHIBITION OF NONHEME IRON ABSORPTION FROM SEVERAL FOOD STUFFS.

**Effect of ligands on WPC-Soy iron profile**

Statistically significant increases in percent soluble iron at the endogenous pH (E) were observed in the WPC-Soy blends only when CIT was added to either the endogenous iron or ERFe systems. Since the endogenous pH (pH 5.3 to 6.5) was in the range of that observed in the intestinal lumen where most iron absorption occurs, these improvements in chemical iron profile due to CIT may offer a partial explanation for citrate’s ability to promote iron absorption in man (10). At pH 2, ASC was the only ligand to provide significant enhancement of iron solubility in all iron systems tested. CYS was the next most beneficial ligand, with significant increases in the ERFe, HRFe, FOP, and FeSO₄ systems. CYS was followed by MAL and CIT, with significant increases in the endogenous and ERFe systems. LAC was the least effective ligand at pH 2, significantly increasing the percent soluble iron only in the ERFe system.

In general, CIT provided the best ability to solubilize iron from the WPC-Soy blend at the endogenous pH, whereas ASC was the best enhancer at pH 2. This is in agreement with the work of Kojima et al. (14) who determined that the ability to solubilize iron from a bean suspension was optimal for ASC under acidic conditions, whereas CIT was maximally effective near pH 6.

CYS provided the second most effective solubilizing capacity at pH 2. To date, CYS appears to be the only amino acid capable of enhancing nonheme iron absorption in humans (17, 19, 20). However, any attempt at fortifying the WPC-Soy blend with similar levels of CYS:iron (48:1) as was used by Layrisse et al. (17), would be prohibitive because sensory problems became evident even at molar ratios used in this study (8:1).

In a recent investigation (5), the percent dialyzable iron, which is used as a predictor of human iron availability, was estimated in an unfortified cereal and indicated only slight improvements in iron availability due to addition of citric or ascorbic acid, as compared to addition of malic or tartaric acids, or the cereal meal alone. However, addition of citric acid to the reduced iron-fortified wheat cereal resulted in a 1.5-fold increase in iron availability over addition of either ascorbic, malic or tartaric acids.

Feeding a basic rice meal enriched with several organic acids, Gillooly et al. (10) demonstrated that ascorbic acid was the most potent enhancer of nonheme iron absorption, and 1 g of either citric or malic acid was required to produce similar improvements in iron absorption as that produced by only 15 mg of ascorbic acid.

Comparison of these observations with the results of the present investigation suggests that iron availability, as measured by in vivo or in vitro means, is dependent on the form of iron (endogenous vs. exogenous), the type and amount of organic acid present, and the foodstuff under study. Therefore, extrapolation of the effects of various organic acids from one food system to another would be a questionable practice and may lead to erroneous and costly fortification policies.

**Effect of exogenous iron on WPC-Soy iron profile**

No significant difference in percent soluble iron was noted at pH E between the endogenous iron (6% soluble) sample and those with added iron (range of 4 to 6%), with the exception of the FeSO₄ system (20% soluble) which contained significantly greater percentages of percent soluble iron.

When the sample pH was decreased to pH 2.0, significantly less percent soluble iron was observed in the exogenous iron systems as compared to the endogenous iron control.

Since the total iron in the exogenous iron systems was twice that analyzed in the WPC-Soy sample alone, then the actual amount of soluble iron was greater in the former systems. This is in contrast to results from a previous investigation using a soy protein isolate, which suggested that all added iron became insolubilized or bound to the protein at pH 2 or 4 (26).

It was already realized that FeSO₄ is a very soluble iron source which is quite acceptable for use in cereal products, such as bread, having a short shelf life. Unfortunately, its usefulness as a fortificant in a cereal-soy blend may be limited due to its tendency to catalyze oxidative reactions resulting in undesirable odors and flavors (8). In addition, it has been demonstrated that the more reactive and bioavailable iron sources, such as FeSO₄, may be converted to insoluble hydroxides when stored at the pH of cereals and remain insoluble even if the pH is lowered to 2.0 (6).

In contrast, elemental iron powders have exhibited a much lower potential for rancidity effects, even when stored at elevated temperatures for extended periods of time (1, 13, 24). This is especially significant since supplemental foods, such as WPC-Soy blend, may be stored for an extended time and under adverse conditions.

Of the three remaining exogenous iron sources, ERFe was the only fortificant that resulted in significant increases in percent soluble iron at pH 2, over the control, for each ligand added. Also, addition of ERFe to the WPC-Soy blend produced the second greatest amount of soluble iron, and at pH 2, was 90% (range of 68 to 111%) as soluble as FeSO₄. Therefore, these data would support selection of ERFe as the most suitable iron source for the WPC-Soy drink.

**Effects of the simulated gastrointestinal test on the selected iron-ligand combinations**

Depending upon the meal, the pH of the human intestinal lumen is actually as low as pH 4.5 to 6.5, and only gradually rises to 7.3 in the ileum (2, 15). Since the majority of iron absorption occurs in the duodenum, this investigation attempted to simulate the approximate temperature and pH of this intestinal segment.

The simulated gastrointestinal (G.I.) test was performed on the WPC-Soy fortified with this iron source in combination with each ligand and involved sequen-
tially incubating each sample at pH E, 2, 4 and 6, for 30, 30, 10 and 10 min, respectively, at 37°C.

The use of only two pH levels in the dual pH incubation method (pH E to pH 2) as a screening test for predicting the relative efficacy of ligands to solubilize iron was demonstrated to be as effective as the more involved G.I. test with four pH levels. No significant difference was observed between the two methods in percent soluble iron, except with ASC and CIT at pH 2. However, even these differences did not alter the relative ability of each ligand to increase the soluble iron at pH 2 as established by the enhancing factors. These results were not surprising since the only difference between the two methods at pH E and pH 2 was the incubation temperatures (20 vs. 37°C).

At every pH, the majority of iron was either insoluble or in an elemental form, with the exception of the ASC-added system. The percent soluble iron was inversely related to the pH of the solution. Complexed iron accounted for most of the soluble iron at pH E, 4 and 6, whereas formation of ionic iron, particularly ferrous iron, was favored at pH 2.

However, the quantitation of ionic iron in this study using the bathophenanthrolone compound must be interpreted with caution since it has been demonstrated that bathophenanthrolone has a larger binding constant for iron than the organic acids employed in this investigation (9, 11, 12). In fact, it was possible that the ionic iron was in an iron-organic acid complex. Therefore, it is more appropriate to think of the ionic iron as bathophenanthrolone-reactive iron.

One way of expressing the effects of a ligand on the exogenous or endogenous iron in the WPC-Soy drink was to calculate an enhancing factor for the percent soluble iron (Table 3). The enhancing factor has been defined as the ratio of the percent soluble iron in a treated sample, i.e., with added ligand, to the percent soluble iron in an untreated sample, i.e., without ligand present (26). Therefore, a value greater than one would suggest an enhancing effect on iron due to the ligand, whereas a value less than one would indicate binding or insolubilization of iron.

ASC and CYS produced the greatest increases in percent soluble, ionic and ferrous iron at pH 2 of all ligands tested (Table 3). At this pH, CYS and ASC were apparently acting as reducing agents. Rizk and Clydesdale (27) have recently demonstrated that the solubilizing effect of ASC on a soy protein concentrate was predominantly a function of its reducing and/or complexing ability rather than its effect on pH. However, the reducing and solubilizing capacities of these ligands were either decreased or abolished as the pH was raised to pH 4 or 6.

Addition of ligands to the ERFe-fortified WPC-Soy (Figs. 1 and 2) produced very few deviations in chemical iron profiles as compared to the control (Fig. 1). The only ligands that were effective in significantly improving the percent soluble iron were ASC and LAC at pH 4 and CIT at pH 6.

In summary, the ability of each ligand to enhance iron solubility in a WPC-Soy blend was dependent upon both the solution pH and iron source added. Since iron solubility has been related to iron availability on many occasions (21, 23, 25), the substantial increases in soluble iron observed in this study at intestinal pH values (pH 4 and 6) as a result of ASC or CIT supplementation may offer a viable means for improving iron absorption from a WPC-Soy drink. In addition, ERFe was the iron fortificant of choice for this foodstuff because it provided the greatest potential for improving iron availability as measured by chemical means and would be expected to cause the least technological problems.

TABLE 3. A comparison of enhancing factors at four pH values for WPC-Soy supplemented with selected organic acids and electrolytic reduced elemental iron (ERFe).

<table>
<thead>
<tr>
<th>Slurry pH</th>
<th>Organic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ASC</td>
</tr>
<tr>
<td>E</td>
<td>1.22</td>
</tr>
<tr>
<td>2</td>
<td>2.00**&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>5.33**</td>
</tr>
<tr>
<td>6</td>
<td>1.53</td>
</tr>
</tbody>
</table>

<sup>a</sup>Enhancing factor is the ratio of the percent soluble iron detected with organic acid present to the percent soluble iron without organic acid present.

<sup>b</sup>ASC, ascorbic acid; LAC, lactic acid; CIT, citric acid; MAL, malic acid; and CYS, cysteine.

<sup>c</sup>E, endogenous pH.

<sup>d</sup>Values marked with an asterisk are significantly greater than the control (without organic acid) at either the 5%* or 1%** level.
Figure 1. Percent ionic, soluble complexed and insoluble iron in the WPC-Soy blend fortified with 6.6 mg iron as electrolytic reduced elemental iron (CON) with or without added ascorbic acid (ASC) or lactic acid (LAC) at pH E (endogenous), 2, 4 and 6.


REFERENCES


Rizk and Clydesdale, con't. from p. 652