Effect of Sucrose, Fructose and Aspartame on Fortificant Iron Solubility in a Wheat Flake Cereal

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ABSTRACT

The iron solubilizing effect of three sweeteners (sucrose, fructose and aspartame) in a processed wheat flake cereal fortified with either ferric orthophosphate, hydrogen-reduced or electrolytically-reduced elemental iron was evaluated at various stages during a simulated in vitro gastrointestinal digestion. Added sweetener had little influence on soluble iron over controls, regardless of pH, iron or sweetener source, although effects may have been masked by various cereal components known to complex iron.

Many investigators have proposed that the major determinants for the potential mucosal uptake of dietary non-heme iron include the inherent solubility characteristics of the iron source and the resultant chemical interactions with meal constituents in vivo (3,5,10). Consequently, iron deficiency anemia often results not from an iron deficient diet, but rather from the mineral being or becoming unabsorbable in the buffered environment of the duodenum.

In vitro studies have attempted to elucidate this process by tracing the chemical status of various iron sources subjected to simulated in vivo conditions involving pH, temperature, time, intestinal buffers and degradative enzymes with meal components of variable ligand character. To date, considerable attention has been paid to both the deleterious effect of dietary fiber and the enhancing nature of several organic acids, such as ascorbate, citrate, malate and EDTA, on iron absorption. Several studies have implicated fructose as an effective enhancer of iron absorption by virtue of its iron chelating properties and the apparent ability of the ferric-fructose complex to cross the intestinal mucosa and be absorbed (1,16). While sucrose has not shown appreciable iron enhancement properties, it has been successfully evaluated as an iron fortificant vehicle (7). Similarly, the amino acids cysteine, histidine and lysine, have promoted iron absorption theoretically via soluble tridentate chelation (17) and/or contributions to lower solution redox potentials, thereby delaying irreversible ferric hydroxide polymerization and inhibiting less soluble complexes to form at intestinal pH.

The fact that a range of α-amino acids can form stable 1:1 complexes with ferric ion in acid solution has been known for many years (13). In most cases, solution redox potentials provided stability constants linearly dependent on the basic ionization constants of each amino acid. Some, such as aspartic acid, showed increased stability, possibly via terdentate chelation, involving the additional carboxyl group.

In the present study, the iron solubilizing effects of three major commercial sweeteners (sucrose, fructose and aspartame) in a processed wheat flake cereal fortified with either ferric orthophosphate, hydrogen-reduced or electrolytically-reduced elemental iron were evaluated at various stages during a simulated in vitro gastrointestinal digestion. We speculate that the methyl ester of the dipeptide aspartylphenylalanine (i.e., aspartame) may possess a metal cation-binding potential under the given conditions.

MATERIALS AND METHODS

Cereal samples

Three experimental batches of a non-commercial wheat flake cereal were obtained courtesy of General Mills, Inc., Minneapolis. Samples were stored in sealed plastic containers at 1.7°C (35°F), with each containing one of the following iron sources: ferric orthophosphate (FOP), hydrogen-reduced elemental iron (HRI) and electrolytically-reduced elemental iron (ERI) at levels of 22.8, 21.2 and 17.8 mg Fe/100 g. The cereal flakes were formulated with the following ingredients: whole wheat, sugar, salt, malt syrup, calcium carbonate, trisodium phosphate, annatto extract color, niacinamide, pyridoxine HCl, thiamin mononitrate, riboflavin and vitamin B12.
Sweeteners

The level of sweeteners added was calculated on an equivalent sweetness basis as follows:

Sucrose (Aldrich Chemical Co., Milwaukee, WI): 1.88 g added per 4.7 g of cereal sample to simulate a cereal with 40% sucrose by weight.

Fructose (Eastman Kodak Co., NY): 1.71 g per sample, given that fructose is approximately 1.1 times as sweet as sucrose (6).

Aspartame: 18.8 mg per sample. Aspartame has 100 to 200 times the perceived sweetness of sucrose (6); the 100× factor was chosen to provide the larger addition level.

Sequential pH treatment

All glassware and Teflon magnetic stir bars were washed in concentrated HCl and repeatedly rinsed with double distilled deionized (DDD) water to remove contaminant iron.

In all experiments, 150 ml of DDD water was either added alone (control) or with a sweetener (1.88 g sucrose, 1.71 g fructose or 18.8 mg aspartame), to each of three 4.7-g samples of cereal. Following blending, the samples were either analyzed at their endogenous pH (stage E), following a 20-min incubation at pH 2.0 (stage 2) or after a 20-min incubation at pH 2 and pH neutralization to 5.0 (by drop wise addition of 0.1N NaOH in the presence of 10% Preptyrode buffer) to complete the final sequential pH treatment (stage 5) (4).

Iron profile analyses

All samples were analyzed in duplicate for total iron, elemental iron, total nonelemental, total soluble, insoluble nonelemental, soluble complexed, total ionic, ferrous and ferric iron using procedures described previously (8,14). It should be noted that while the term “ionic iron” is used throughout the paper, “bathophenanthroline reactive iron” is implied, because some complexed iron may be preferentially bound by the bathophenanthroline before extraction by CHCl₃ (4).

Statistics

Data were analyzed using analysis of variance (15), with significance at the 95% confidence level (P<0.05).

RESULTS AND DISCUSSION

The effect of added sweetener on the percentage of ferrous, ferric and soluble complexed iron (SCI) formed in each fortified cereal sample is shown in Table 1. The control (i.e., no sweetener) represents the iron fortificant’s inherent solubility at each sequential pH stage (E, 2, 5) in the wheat flake cereal. (It should be noted that percent soluble iron values include the influence of endogenous wheat iron, at a level of 6.2 mg Fe/100 g cereal).

Control values for FOP reveal the inertness of this common iron fortificant. Most notably, FOP produced a slight decrease in SCI from stage 2 to 5, whereas the SCI of HRI and ERI increased approximately 7 and 14%, respectively. Here, the greater solubility of ERI over HRI can be partially explained by their structural difference. Scanning electron micrographs have shown ERI particles to be irregular and dendritic (i.e., “fernlike”) thereby producing a greater active surface area and HRI particles as more rounded and annealed by the high temperature process of H₂ reduction (12).

In all cases, increases in both ionic (largely Fe⁺²) and SCI following pH reduction from endogenous to pH 2 presumably reflect the enhanced solubility of each iron source at low pH. Samples with added sweetener offered no appreciable gain in total iron (i.e., ionic and SCI) over controls, regardless of pH, iron or sweetener source. For stages E and 2, this finding was somewhat expected, as soluble iron-carbohydrate complexes most favorably form in acid solution upon addition of base (2). In other words, had the sweeteners chelated iron at pH 2, enhancement of SCI at pH 5 would occur. While such an effect was seen in most analyses involving added sweetener, this effect was also seen in the controls, indicating that cereal components were capable of forming soluble iron complexes during the pH shift from 2 to 5 and in turn, inhibiting insoluble iron hydroxide polymerization.

Considering the relatively small simultaneous losses in ionic iron (largely Fe⁺²) from pH 2 to 5, increased SCI values at pH 5 do not occur entirely at the expense of ionic iron. Assuming that little iron can be solubilized additionally from either FOP, HRI or ERI during the neutralization procedure, it appears that insoluble complexed iron at pH 2 becomes solubilized upon the addition of base. These complexes may result due to interactions between fortificant iron and cereal components and/or reflect the influence of the endogenous wheat iron. Nelson and Potter (11) have shown that as the pH of an in vitro wheat gluten system was raised from pH 4 to 10, Fe⁺³ uptake by the insoluble fraction was inhibited, whereas the soluble protein fraction steadily increased its Fe⁺² uptake, with maximal Fe⁺² uptake at pH 5. Similarly, endogenous wheat iron, which predominately exists as monoferric phytate, exhibits greater aqueous solubility at neutral vs. acid pH (9). The influence of added sweeteners on iron solubility may have also been tempered by the presence of both sugar and malt syrup as wheat flake ingredients.

In conclusion, sucrose, fructose and aspartame offered no appreciable change in either ionic or soluble complexed iron in a fortified wheat flake cereal under the conditions of this experiment. Control values indicate the following order of fortificant solubility: ERI > HRI > FOP. However, cereal components may have influenced the effect of added sweeteners on iron solubility.

REFERENCES

TABLE 1. Percent ionic (Fe³⁺ and Fe²⁺) and soluble complexed iron in cereal samples subjected to a sequential pH treatment from pH E (endogenous) to 2 to 5.

<table>
<thead>
<tr>
<th>Sweetener</th>
<th>pH² stage</th>
<th>Ionic Fe²⁺</th>
<th>Fe³⁺</th>
<th>Soluble complexed</th>
<th>pH³</th>
<th>Ionic Fe²⁺</th>
<th>Fe³⁺</th>
<th>Soluble complexed</th>
<th>pH³</th>
<th>Ionic Fe²⁺</th>
<th>Fe³⁺</th>
<th>Soluble complexed</th>
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<tr>
<td>None (control)</td>
<td>E</td>
<td>0.0</td>
<td>0.0</td>
<td>8.0</td>
<td>6.2</td>
<td>0.0</td>
<td>0.1</td>
<td>8.7</td>
<td>6.1</td>
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<td>0.0</td>
<td>8.5</td>
<td>6.2</td>
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<td></td>
<td>2</td>
<td>0.4</td>
<td>0.0</td>
<td>15.5</td>
<td></td>
<td>0.5</td>
<td>0.4</td>
<td>17.5</td>
<td></td>
<td>1.9</td>
<td>0.9</td>
<td>19.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.0</td>
<td>0.0</td>
<td>13.9</td>
<td></td>
<td>0.2</td>
<td>0.8</td>
<td>24.8</td>
<td></td>
<td>0.0</td>
<td>1.3</td>
<td>33.8</td>
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<td>Sucrose (1.88g/sample)</td>
<td>E</td>
<td>0.0</td>
<td>0.0</td>
<td>10.3</td>
<td>6.1</td>
<td>0.0</td>
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<td>10.8</td>
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<td>0.2</td>
<td>16.3</td>
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<td>0.6</td>
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<td>0.3</td>
<td>18.0</td>
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<td>0.8</td>
<td>23.6</td>
<td></td>
<td>0.0</td>
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<td>24.3</td>
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<tr>
<td>Fructose (1.71g/sample)</td>
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<td>0.0</td>
<td>9.9</td>
<td>6.1</td>
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<td>0.9</td>
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<td>0.0</td>
<td>0.8</td>
<td>17.5</td>
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<tr>
<td>Aspartame (18.8mg/sample)</td>
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<td>10.0</td>
<td>6.2</td>
<td>0.0</td>
<td>0.2</td>
<td>10.0</td>
<td>6.0</td>
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<td>0.0</td>
<td>12.6</td>
<td>6.1</td>
</tr>
<tr>
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*pH E, 2 and 5 represent the three pH stages studied.

bCereal slurry's endogenous pH following blending.

cDenotes a significant difference (P<0.05) between the value and its control.