Iron Fortification Technology Development: New Approaches

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ABSTRACT The objective of our fortification technology development has been to deliver meaningful levels of bioavailable iron via commonly consumed foods and beverages without compromising taste, appearance, and stability. However, fortification of foods is accompanied with unsolved problems such as unacceptable taste, color, stability, and bioavailability. To solve these problems, we developed a fortification technology that prevents the iron-mediated undesirable taste and appearance of the final product while preserving stability and bioavailability. Iron was stabilized by applying principles of colloid chemistry (encapsulation), chelation, and electrochemical chemistry (redox modulation). Results from color and sensory evaluations showed that formulation of products using the new fortification technology known as “GrowthPlus” eliminated detrimental effects on taste, appearance, and product stability. Bioavailability evaluation using animal models and human subjects showed the GrowthPlus technology does not interfere with the bioavailability of iron from either ferrous bis-glycinate or ferrous fumarate. Multiple intervention trials showed that repeated consumption of the redox stabilized iron in the form of a powdered fruit beverage increased iron status indicators (hemoglobin and ferritin) and reduced iron deficiency anemia significantly in school children, adolescent girls, and pregnant women. J. Nutr. 136: 1059–1063, 2006.

KEY WORDS: • iron • fortification • encapsulation • redox modulation

Iron deficiency is one of the most widespread nutritional deficiencies. Globally >2 billion people are suffering from iron-deficiency anemia (1). If not prevented or corrected, iron-deficiency anemia may cause stunted growth, impaired mental development, poor school performance, reduced productivity, increased morbidity and mortality, and lower self-esteem (2). Food fortification with iron has been recommended as one of the preferred approaches for preventing and eradicating iron deficiency. However, fortification with bioavailable iron sources often presents multiple challenges in product acceptance, product shelf life, and effectiveness in improving iron status (3,4). The success of iron fortification is dependent on delivering a meaningful level of bioavailable iron without affecting the taste and appearance of the finally consumed product. Iron fortification may cause 1) metallic aftertaste, 2) unacceptable flavor as a result of the oxidation-mediated rancidity of fats, 3) undesirable color changes resulting from interactions with anthocyanins, flavonoids, and tannins, and 4) degradation of vitamins (e.g., vitamin C and vitamin A, which are important for iron absorption and utilization) and minerals (i.e., iodine from the oxidation of iodide/iodate to free iodine that escapes as a gas). In addition, the bioavailability of iron is dependent not only on the iron source but also the type of food and/or beverage consumed with it (3–8). Many staple foods and commonly consumed beverages (e.g., rice, beans, coffee, tea, and milk) contain components that interfere with iron absorption (3–8).

Currently, there are a number of iron sources available as food fortificants (3,4). Based on bioavailability, these iron fortificants are classified into 2 groups. The highly bioavailable iron sources (e.g., ferrous sulfate and ferrous fumarate), which are soluble in neutral and/or acidic aqueous environments but may cause organoleptic changes such as poor product acceptability and shortened product shelf life (3,4), and those with poor bioavailability (e.g., iron pyrophosphate and reduced iron), which are less soluble in water but are more compatible with the foods used as vehicles (3,4). Developing a fortification technology that makes either the bioavailable iron sources more compatible with the food vehicle or the compatible ones more bioavailable remains a challenge for food scientists and the food industry.

Iron fortification technology development. The success of iron fortification in addressing the prevalence of iron deficiency anemia, particularly in developing countries, has been limited by the lack of a robust, simple, and easy-to-transfer fortification technology (4,5). The challenges are chemistry based. Both the vehicle (food or beverage) and the fortificants (nutrient sources) have reactive functional groups. Most commonly
used vehicles contain moisture and oxidizing agents. Such an environment is conducive to a reaction process that causes undesirable taste (e.g., metallic aftertaste, rancidity), off color, degradation of vitamins, and reduced bioavailability (3–8). In developing an effective iron fortification technology, it is critical that the chemical property of iron that contributes to the development of undesirable organoleptic properties is taken into consideration (5,7,8). Two iron forms that are commonly used in food fortification are ferrous (Fe$^{2+}$) and ferric (Fe$^{3+}$). Because both of these species contain unfilled $d$ orbitals, they readily form complexes with electron-rich components yielding species that influence taste and bioavailability. Also, iron has the ability to undergo oxidation-reduction (redox) reactions that cause many of the unwanted outcomes related to taste, appearance, and bioavailability (5,7,8). The species of iron in a given environment are shown in the Eh–pH diagram (Fig. 1). It is defined by the Nernst equation ($E_h = E_o + 0.059/n \log [\text{Fe}^{3+}]/[\text{Fe}^{2+}]$) (9). $E_o$, $E_h$, and $n$ are system redox potential, standard redox potential, and number of electrons, respectively. The oxidation state of iron is dependent on both pH and redox potential (9). At low pH, iron prefers to stay as [Fe$^{2+}$]. However, as pH increases, it rapidly oxidizes to [Fe$^{3+}$] to form Fe(OH)$_3$, which with time precipitates as rust. Likewise, the oxidative state of iron is dependent on the redox potential ($E_h$) of the environment. At a higher $E_h$, iron prefers to remain as ferric. The electrochemical property of iron shown in Fig. 1 was used in developing our iron fortification technology. It is based on a model called “Lock-Unlock” (7). During the “Lock Stage,” the technology is designed to keep the iron unreactive. Two different strategies, 1) chelation-redox modulation (for products with a pH <5) and 2) encapsulation (for products with a pH >5), were used.

In the chelation-redox modulation-based iron fortification strategy, the iron-mediated metallic aftertaste was prevented through chelation. Ferrous bis-glycinate (Albion Laboratories), an amino acid–chelated iron source, was used. A mole of ferrous iron is chelated by 2 moles of glycine. Similar to the other water-soluble iron compounds, ferrous bis-glycinate is easily oxidized to [Fe$^{3+}$], which then causes off-color development and fat oxidation (5,10,11). Redox modulation was applied to prevent the oxidation of ferrous to ferric. The environment was made reducing rather than oxidizing by lowering the pH and reducing $E_h$ (adding organic acids and reducing agents, respectively). The effect of redox modulation on the rate of ferrous iron oxidation is shown in Table 1. [Fe$^{2+}$] was measured using a modified Ferrozine method, which is specific to ferrous iron (5,12). Lowering pH with citric acid and $E_h$ with ascorbic acid prevented the oxidation of ferrous iron to ferric. In contrast, when the ferrous bis-glycinate was added to water without the redox modulation technology, it rapidly oxidized to the ferric form. However, as indicated by the absorbance at 560 nm and the percentage of ferrous iron recovered, the redox modulation technology is effective in preventing the oxidation of [Fe$^{2+}$] to [Fe$^{3+}$]. It is important to note that this phenomenon of iron oxidation is not unique to ferrous bis-glycinate. Ferrous compounds in general are known to be oxidized to the ferric form when added to foods and beverages (5,8,9,13).

Stabilizing ferrous iron by redox modulation has its own limitation in an environment above pH 5. Higher pH favors the formation of [Fe$^{3+}$] over [Fe$^{2+}$] (Fig. 1). Also, according to Hsieh and Hsieh (13), the reduction rate of ferric iron by reducing agents such as ascorbic acid decreases markedly as the pH increases. Thus, encapsulation-based technology was used to solve the iron-mediated problems in products above pH 5. The iron was made unreactive during formulation and storage by isolating it from the vehicle components (e.g., tannins, fat, and vitamins) and oxidizing agents (e.g., dissolved oxygen and chlorine) using vesicle- or liposome-forming emulsifiers (e.g., lecithin). The technology works only for ferrous fumarate and ferrous succinate. The likely reason is that these are less polar than the other water-soluble iron sources such as ferrous citrate, ferrous tartrate, and ferrous sulfate. Ferrous fumarate is mixed with lecithin, which is a vesicle-forming emulsifier. When the iron–lecithin mixture is exposed to water, the lecithin molecules rapidly form a bilayer. After mixing, they are transformed into vesicles. Results obtained by adding known bilayer stabilizers (e.g., cholesterol and phytosterols) showed that stabilization of ferrous fumarate and ferrous succinate is dependent on the bilayer formation by lecithin (data not shown).

Technology evaluation and product acceptance. For an iron-fortified product to be consumed by target groups, the iron
added should not cause the development of undesirable color or flavor changes. Therefore, iron fortification should not change the appearance of the product. The effectiveness of the “GrowthPlus” technology to keep the iron sources (ferrous bis-glycinate and ferrous fumarate) nonreactive has been tested using different vehicles (e.g., water, chocolate milk, and baby cereal). The prevention of the iron-mediated off-color development by redox modulation technology is shown in Figure 2. Relative to the control samples (no iron), addition of ferrous iron (ferrous bis-glycinate) caused the water, chocolate milk, and baby cereal to become brown, grayish brown, and dark-green, respectively. However, this was prevented when the stabilized ferrous bis-glycinate was used.

The prevention of the ferrous fumarate-mediated off-color development in chocolate milk by lecithin-based encapsulation is shown in Table 2. Hunter colorimetry was used to measure color change (14). A decrease in Hunter “a” value is an indication of off-color development. Fortification with ferrous fumarate caused a decrease of the “a” value from 6.8 (control with no iron) to 3.2. In contrast, the ferrous fumarate-mediated change in “a” value was prevented by encapsulation with lecithin.

In addition to off-color development, iron fortification is also known to cause metallic aftertaste and lipid oxidation. The effect of the redox modulation–stabilized iron (ferrous bis-glycinate) was evaluated by carrying out a home-use test among households in the Philippines (7) and anthropologic study in pregnant women in Tanzania (15). The evaluation was done in the form of an iron-containing, multiple-micronutrient-fortified powder fruit beverage. In addition to the iron, the beverage contained 8 vitamins (vitamin A, vitamin C, vitamin E, niacin, B-6, B-2, B-12, and folate) and 2 minerals (zinc and iodine). The composition of the beverage and the levels of micronutrients are explained in detail elsewhere (7). In the home-use test, each family received either an unfortified (placebo) or fortified powder beverage adequate for 5 d consumption. At the end of the 5 d, they were asked to rate the products for an overall acceptance, color, flavor, and metallic aftertaste. The powdered fruit beverage fortified with the chelation-redox modulation–stabilized ferrous bis-glycinate had no significant effect on the multiple sensory parameters when compared with the same powder beverage but without the iron-containing multiple micronutrients (7). The anthropologic study was based on home visits and interviews to understand what the pregnant women who were participating in the micronutrient fortified field trial in Tanzania (16) thought about the powdered beverage. Overall, the women liked the beverage. In addition, they preferred the beverage over pills as a micronutrients delivery vehicle and as beneficial to health (15).

**Technology evaluation: iron bioavailability.** For an iron fortification program to be effective in improving the iron status of the target groups, the iron from the fortified product has to be absorbed. During the “Unlock” stage of the “Lock-Unlock” model, the iron fortification technology is designed to make the unreactive iron become available for absorption after ingestion. The bioavailability of iron from the lecithin-encapsulated ferrous fumarate was evaluated using the hemoglobin depletion–repletion assay in young rats (17). Comparison was made against the standard ferrous sulfate and ferrous fumarate. The hemoglobin gains and relative bioavailability values after a 2-week repletion period are presented in Table 3. The encapsulated ferrous fumarate has the same bioavailability as that of the nonencapsulated ferrous fumarate. Consistent with the published data, the relative bioavailability value of ferrous fumarate was comparable to that of ferrous sulfate (3,4).

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**TABLE 1**

**Oxidative status of ferrous iron from ferrous bis-glycinate**

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Absorbance, 560 nm</th>
<th>%Fe²⁺ recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferrous bis-glycinate</td>
<td>0.097 ± 0.003</td>
<td>20.09 ± 0.68</td>
</tr>
<tr>
<td>Redox-stabilized ferrous bis-glycinate</td>
<td>0.463 ± 0.006</td>
<td>101.09 ± 0.28</td>
</tr>
<tr>
<td>Redox-stabilized ferrous bis-glycinate in fortified powder fruit beverage</td>
<td>0.477 ± 0.009</td>
<td>104.72 ± 1.94</td>
</tr>
</tbody>
</table>

* Samples contain 4.8 mg iron dissolved in 200 mL water. After 1 h, 1 mL sample was diluted to 25 mL with ferrozine solution. Absorbance was measured at 560 nm.

**FIGURE 2** Effect of iron on off-color development. Ferrous bis-glycinate was added to water, a chocolate-containing beverage, and a baby cereal (prepared as a slurry) with warm deionized water. Photos were taken after 1 h.

**TABLE 2**

**Effect of encapsulated ferrous fumarate on chocolate milk’s color**

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Hunter “a” value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No iron (control)</td>
<td>6.8</td>
</tr>
<tr>
<td>Ferrous fumarate</td>
<td>3.2</td>
</tr>
<tr>
<td>Encapsulated ferrous fumarate</td>
<td>7.5</td>
</tr>
</tbody>
</table>

* Values are color measurements using the Hunter L, a, b scale, where white is L = 100, black in L = 0, red is positive a, green is negative a, yellow is positive b, and blue is negative b.

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**TABLE 3**

**Bioavailability of iron from encapsulated ferrous fumarate**

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Average hemoglobin gain, g/dL</th>
<th>Relative bioavailability value (RBV)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferrous sulfate (standard)</td>
<td>3.42 a</td>
<td>100</td>
</tr>
<tr>
<td>Ferrous fumarate without encapsulation</td>
<td>3.95 b</td>
<td>107</td>
</tr>
<tr>
<td>Encapsulated ferrous fumarate</td>
<td>4.40 b</td>
<td>98</td>
</tr>
</tbody>
</table>

* Values with different superscript letters are significantly different at P < 0.05.
† RBV = Hemoglobin regeneration efficiency (HRE) relative to HRE for ferrous sulfate × 100.
The bioavailability of iron from the chelation-redox modulation—stabilized ferrous bis-glycinate, which was delivered in the form of fortified powdered fruit beverage, was determined in nonpregnant women using a double isotope labeling technique (18). The treatments included an iron-fortified powdered beverage alone and with rice. The absorption values were normalized to the 40% absorption of the reference iron compound, ferrous ascorbate. The bioavailability of iron from the fortified powdered fruit drink when consumed alone was 23.4%. However, when the beverage is taken with rice, the bioavailability of iron decreased from 23.4% to 10.7% (7). Even though the bioavailability of redox-stabilized ferrous bis-glycinate in a fruit beverage was quite good when consumed alone, simultaneous consumption with rice reduced absorption by about 50%. Foods of plant origin do contain components (e.g., phytates, tannins, dietary fibers, and calcium) that interfere with iron absorption (3,4,19) Repeated bioavailability studies have shown that both intrinsic and extrinsic iron are poorly absorbed from foods of plant origin (19). The percentage of iron absorbed from rice in human subjects was extremely low (about 1%) compared with that from the other plant foods (19). Thus, even in the presence of a full serving of rice, the 10.7% absorption from the chelation-redox—stabilized iron (in powdered fruit beverage) is comparable to that from fish (19) and milk fortified with ferrous sulfate and vitamin C (20).

**Product efficacy evaluation.** The effectiveness of the redox-stabilized ferrous bis-glycinate added to a multimineral-fortified powdered fruit beverage has been evaluated with randomized, double-blind, placebo-controlled clinical trials in school children (21), adolescent girls (22), and pregnant women (23). The school children, adolescent girls, and pregnant women received 5.4 mg, 7 mg, and 10.8 mg of iron daily, respectively. The duration of the intervention phases were 6 mo for the school children, 6 and 12 mo for the adolescent girls, and 8 weeks for the pregnant women. The changes in hemoglobin concentration among the subjects who were anemic at the baseline are shown (Fig. 3). In all 3 studies, there was a significant increase in hemoglobin among the groups that received the powdered beverage fortified with the redox-stabilized iron as compared with the groups that received the placebo. A similar significant increase in ferritin was observed in the group that received the iron-fortified product but not in the placebo group (16,21,22). The results from these efficacy trials do show that the iron stabilized through amino acid chelation and redox modulation is effective in improving iron status.

**Conclusion and remarks.** Although successful iron fortification programs have been implemented in many countries, fortifying foods with meaningful levels of bioavailable iron without the development of undesirable taste and appearance remains a challenge. Iron sources stabilized with either the chelation-redox modulation or vesicle-based encapsulation approaches have the potential to contribute to the eradication of iron-deficiency anemia. The iron fortification technology based on chelation-redox modulation has been demonstrated to 1) solve the problem of the iron-mediated off-color and off-flavor development, and 2) deliver clinically proven bioavailable iron. In addition, stability data in both the powdered and beverage form showed that it has no effect on the stability of the multiple vitamins and iodine that were codelivered with the iron (7). The technology has been shown to be robust during manufacturing, storage, distribution, and consumption of a fortified powdered fruit beverage (7). Note that there is a limitation in the use of the redox-stabilized iron. Because it is pH dependent, it works only in products with pH <5.

The lecithin-based encapsulation of ferrous fumarate has been shown to be effective in preventing the development of off-color, off-flavor, and metallic aftertaste without compromising bioavailability. Also, because it is compatible with products with pH above 5, it has potential for broad food fortification applications. However, the technology’s robustness is yet to be demonstrated during scaling up, storage, distribution, and consumption. Currently, there are a number of encapsulated iron sources (e.g., ferrous sulfate, ferrous fumarate, and micronized ferric pyrophosphate) available in the market (23). Hydrogenated oils are used to encapsulate the iron sources. However, the technology has limitations in products that are likely to be exposed to higher temperatures during processing, storage, or preparation. Products fortified with the encapsulated iron have been shown to cause off-color development when the preparation is carried above the melting point of the hydrogenated oils (45–65°C) (7,23). Also, salts fortified with hydrogenated oil—encapsulated iron sources (ferrous sulfate, ferrous fumarate) were observed to develop undesirable appearance when stored in a relatively humid environment (23).

**LITERATURE CITED**


