Novel Method for the Production of Color-Compatible Ferrous Sulfate-Fortified Simulated Rice through Extrusion

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Ferrous sulfate heptahydrate (FSH), a highly bioavailable water-soluble compound, was used for the production of fortified simulated rice through extrusion of acidified rice flour. The acidic and/or reducing environment kept iron in the ferrous valence, diminishing or even eliminating discolorations associated with the use of water-soluble iron compounds such as FSH. Sensory tests and instrumental measurements showed that the color, size, and shape of simulated rice were compatible with the control Jasmine rice when mixed with it at 1:100 and 1:200 dilution ratios. *In vitro* tests demonstrated the high iron bioavailability of the product. Also produced were acidified iron-fortified rice samples containing ascorbic acid; they were color-compatible after extrusion, but extended storage resulted in discolorations. The FSH-fortified rice is an inexpensive and stable product that has the potential to combat anemia.

Keywords: *Iron fortification; ferrous sulfate; rice; extrusion; color*

INTRODUCTION

Iron deficiency anemia is the most prevalent nutritional deficiency, affecting at least 0.5−0.6 billion people worldwide (Baynes and Bothwell, 1990). The fortification of staples with iron compounds is a popular long-term attempt to alleviate anemia on a large scale (Hurrell and Cook, 1990). Readily water-soluble iron compounds exhibit a satisfactory bioavailability; however, their reactivity results in organoleptic deterioration of the vehicle food (discoloration, acceleration of lipid oxidation, metallic taste, etc.) (Hurrell, 1984). This constitutes a serious problem for the fortification of foods that have light color (such as rice and milk), moderate to high fat content (such as cheese), and/or subtle taste (such as rice).

Rice can serve as a suitable vehicle for iron fortification as it constitutes a very significant source of calories worldwide and especially in developing countries. However, only certain iron compounds characterized by chemical inertia and sparing solubility—such as iron orthophosphate and pyrophosphate—are currently used for the production of fortified rice, which is compatible with natural rice (Hunnell et al., 1985). Iron-fortified simulated rice prepared through extrusion was successfully produced in our laboratory using a variety of iron compounds (Zilberboim, 1994). Iron phosphates were the most compatible fortificants from an organoleptic standpoint (Zilberboim, 1994). However, the iron bioavailability of the iron phosphate-fortified rice was low, mainly due to the insolubility of the phosphates (Hurrell and Cook, 1990).

Ferrous sulfate heptahydrate (FSH) and ferrous sulfate monohydrate (FSM) are used in the fortification of several foods (bread, flours, pasta, infant formulas, etc.) as inexpensive sources of highly bioavailable iron (Cook and Reusser, 1983). Nevertheless, the high reactivity of such compounds combined with high water content, neutral pH values, and the presence of oxygen are responsible for quality deterioration of iron-fortified products, limiting the use of FSH and FSM strictly to foods with a short shelf life (Zoller et al., 1980). The major cause of the deterioration is oxidation of ferrous iron to the ferric state followed by formation of insoluble yellow-brown ferric hydroxide polymers (Nojeim and Clydesdale, 1981). However, it has been previously shown that acidic conditions and ascorbic acid can retard iron oxidation (Nojeim and Clydesdale, 1981). Our overall objective was to use ferrous sulfate in an acidic and/or reducing environment to produce stable iron-fortified simulated rice through extrusion. Furthermore, this final product was designed to be physically and organoleptically compatible with the control Jasmine rice before and after cooking (when mixed with it in appropriate ratios) and characterized by high content of both total and bioavailable iron that can fulfill the major portion of iron requirements for healthy individuals.

METHODOLOGY

Extruder Setup. The extruder setup comprised of a $^3/_4$ in. laboratory extruder (L/D = 1:20) (Model 2003, C. W. Brabender Instruments Inc., S. Hackensack, NJ), a screw-feed hopper assembly (Brabender), a 20:1 L/D ratio screw with 1:1 and 3:1 compression ratios (Brabender), a cooling jacket, a special die with three openings (dimensions 5.89 × 1.91 mm), and a cutter (all three made at Rutgers) was used for production of simulated rice (Zilberboim et al., 1994). The temperature at the two heat zones (positioned at the back and front ends of the cooling jacket) was monitored using two thermocouples (Omega Engineering, Stanford, CT), whereas the pressure at the end of the barrel was monitored by a pressure gauge (from Brabender) (Zilberboim et al., 1994).

Preparation of Rice Slurry and Extrusion Conditions. Ferrous sulfate heptahydrate and sodium ascorbate were both obtained from Sigma Chemical Co. (St. Louis, MO). Rice flour was obtained from Gulf Rice Milling (Houston, TX). FSH (5−14 g) was dissolved in 75 mL of water, and the pH of the solution was adjusted to an acidic value (1 or 0.5) using 10 N HCl. Additionally, the occasional incorporation of sodium ascorbate was done during this initial step. The acidic solution was added dropwise to 300 g of rice flour (RF) and mixed in a heavy-duty KitchenAid mixer (iron content: 3.3−9.4 mg of

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Fe/g of RF). The resulting slurries contained 25% water and were extruded in less than 30 min to minimize oxidation. The amount of iron incorporated is adequate to provide up to 100% of the U.S. RDA for iron (15 mg) when mixed at appropriate ratios (1:100 to 1:200) with Jasmine rice.

The operating conditions for extrusion were as follows: screw speed, 60 rpm; temperature at first zone (T_1), 50 °C, and at second zone (T_2), 70 °C; cutter speed, 70−80 rpm (Zilberboim et al., 1994). The extrudates were left to air-dry for 2 days (final moisture: ∼8%), placed in Mason jars, and stored at room temperature, avoiding exposure to light.

Color Measurements. The $L^*a^*b^*$ tristimulus color coordinate system was used for the color evaluation of the rice extrudates. The parameter L^* represents the degree of lightness (or darkness) of the sample, whereas a^* and b^* serve as indices of green-red and yellow-blue color. $L^*a^*b^*$ values of the extrudates were measured using a Minolta CR-210 Chromameter (Minolta, Ramsey, NJ) equipped with a granular material attachment. The calibration of the instrument was performed against a standard Minolta white plate ($Y = 94.4$, $x = 0.3134$, $y = 0.3206$).

Determination of Iron from the Extrudates. The Laszlo (1988) method was used with minor modifications. Extrudates were ground briefly in a Glenn-Mills (Maywood, NJ) grinder with stainless steel parts. The resulting flour (0.3 g) was extracted for 2 h with 50 mL of 2 M HCl in a shaking water bath; such extraction conditions ensure 100% recovery of iron from the sample without altering the ratio of Fe(II)/Fe(III) in the sample (Laszlo, 1988). After centrifugation of the slurry to remove insoluble matter, the supernatant was filtered through a 0.45 μm Whatman filter; prior to injection in the HPLC column, the extract was diluted appropriately for the Fe(II) and Fe(III) concentrations to fall within the concentration range of the standard solutions. The determination of ferrous and ferric valences in the extrudates was carried out using a BioLC HPLC delivery system, with a gradient pump, an eluant degassing module, a postcolumn derivization system and a variable wavelength detector (all from Dionex, Sunnyvale, CA). The column used was a IonPac CS5 column equipped with a CS5 analytical precolumn (both from Dionex). The mobile phase was an aqueous solution of 6 mM pyridine-2,6-dicarboxylic acid and 8.6 mM LiOH (flow rate: 1 mL/min); the postcolumn reagent was an aqueous solution of 0.2 mM 4-(2-pyridylazo)resorcinol (PAR) in 1 M CH_3COOH and 3 M NH_4OH (flow rate: 0.5 mL/min). Calibration curves for Fe(II) and Fe(III) were generated using Fe(II) and Fe(III) standard solutions prepared fresh using Fe(II) and Fe(III) stock solutions (1000 ppm, from Aldrich). The retention times for Fe(II) and Fe(III) were 4.9 and 9.4 min, respectively. The determinations were conducted within 2 weeks from the production of extruded rice.

Sensory Evaluation. A panel of 30 experienced but untrained panelists was used for the evaluation of the eating quality of uncooked and cooked mixtures of extrudates with Jasmine rice (dilution ratios 1:100 and 1:200). The majority of the panelists represented rice-eating populations, which constitute the target population for the fortified rice. A linear scale (0−150) was used for the evaluation. The following attributes were examined: size/shape uniformity, color uniformity, and smell for uncooked samples and color uniformity, flavor, stickiness, aftertaste, and overall acceptability for cooked samples. The sensory evaluation was conducted within 2 weeks from the production of extruded rice.

***In Vitro* Estimation of Iron Bioavailability.** The method of Miller et al. (1981) was used to evaluated the iron dialyzability (reliable index of iron bioavailability) of the extrudates. The extrudates were mixed in a ratio of 1:200 with Jasmine rice, cooked in an automatic rice cooker for 20 min, homogenized, and digested enzymatically. The bathophenanthroline disulfonic acid method was used for the determination of dialyzable iron, while the total iron content was determined after microwave acid digestion of the rice homogenate and atomic absorption spectrophotometry (Kapanidis, 1993).

Table 1. $L^*a^*b^*$ Values for Jasmine Rice and Several Extrudates Obtained by Minolta Chromameter within 2 Weeks from the Production of the Extrudates

	L^*	a^*	b^*
Jasmine rice[a]	70.83	−0.94	12.44
RF + W[a]	69.90	−0.83	12.35
RF + W + FSH	47.00	4.90	12.60
RF + W + FSH, pH 1[b]	64.20	0.40	12.82
RF + W + FSH, pH 0.5	71.64	−0.92	12.87
RF + W + FSH + AA	44.56	6.11	12.64
RF + W + FSH + AA, pH 1	54.61	3.18	8.43
RF + W + FSH + AA, pH 0.5	71.55	−0.92	12.87

[a] RF, Jasmine rice flour (300 g); W, water (75 mL added to each sample); FSH, ferrous sulfate heptahydrate (5 g); AA, ascorbic acid sodium salt (5 g). [b] The pH value cited is the pH to which the solution of ferrous sulfate and AA was adjusted before the addition to the RF.

RESULTS AND DISCUSSION

Preliminary Experiments. To test our hypothesis that manipulation of pH can affect the color of rice extrudates, we first observed the color of a ferrous sulfate solution (2.5 g in 37.5 g of water) at different pH values. At the pH of the resulting solution (3.2), we witnessed the rapid formation ($t < 5$ min) of a greenish-yellowish solution that precipitated over time. At a pH close to neutrality (6.7), a dark blue precipitate was formed along with a pale yellow suspension. However, in the case of the acidic environment (pH ∼1.8), we obtained an almost transparent solution which was stable (no precipitation) for more than a month at room temperature. This simple test demonstrated that low pH values can retard Fe(II) oxidation and subsequently retard brown color formation, as they favor the presence of ferrous ion (tested by HPLC, data not shown). Addition of ascorbate to the ferrous sulfate solution under very acidic conditions (pH <0.5) also resulted in a colorless solution. Other iron compounds tested exhibited low solubility (iron pyrophoshate) or formed colored solutions and/or precipitates (NaFe-EDTA, $Fe_2(SO_4)_3 \cdot 3H_2O$).

Effects of Acidic and Reducing Environment on the Color of the Extrudates. The $L^*a^*b^*$ color parameters of the extrudates produced under different conditions are shown in Table 1. Addition of FSH without acidification resulted in brown color formation documented by the substantially decreased lightness, L, and increased red color of the extrudate (increase of a^* parameter) compared to the control (RF + W). The discoloration reactions were partially or even fully inhibited by the addition of 1 and 3.3 mL of 10 N HCl to the FSH solution to achieve pH values of 1 and 0.5, respectively. The presence of ascorbate caused more severe discolorations at pH 3.3 (no pH adjustment after FSH dissolution) and pH 1, possibly due to the formation of purple iron ascorbate complexes (Gorman and Clydesdale, 1983). However, the use of low pH conditions has the potential to retard the formation of iron ascorbate complexes, resulting in extrudates with color similar to that of the actual rice.

Ferrous and Ferric Iron in Extrudates. We used HPLC to monitor the concentration of the ferrous and ferric iron forms in the extrudates (Table 2). In the case of the control sample (RF + FSH, no acidification), a significant portion of total iron (40%) was converted to the ferric form after extrusion. Such oxidation of Fe(II) to Fe(III) did not occur only during extrusion; mixtures of RF with FSH prepared without acidification exhibited serious discolorations even shortly after the

Table 2. Concentrations of Fe(II), Fe(III), and Soluble Iron in the Extrudates Determined by HPLC

extrudate[a]	Fe(II) concn (ppm)	Fe(III) concn (ppm)	total soluble iron Fe(II) + Fe(III) (ppm)	Fe(III)/total soluble iron ×100 (%)	increase in soluble iron[b] (%)
Jasmine rice flour	0.05	0.00	0.05	0.00	N/A
RF + FS (control)[c]	1.66	1.08	2.74	39.24	0.00
RF + FSH, pH 1.0[d]	2.38	0.52	2.89	17.73	5.53
RF + FSH, pH 0.5	2.85	0.27	3.12	8.74	13.94
RF + FSH + AA, pH 1.0	3.27	0.09	3.36	2.59	22.54
RF + FSH + AA, pH 0.5	3.04	0.05	3.07	1.51	11.99

[a] RF, rice flour (300 g); FSH, ferrous sulfate heptahydrate (5 g); AA, ascorbic acid sodium salt (5 g); 75 g of water was added to each sample. [b] The increase of soluble iron in the extrudates is calculated after comparison to the control sample (RF + FSH). The values represent average of duplicates. [c] No pH adjustment (pH after FSH dissolution: 3.30). [d] The pH value cited is the pH to which the solution of ferrous sulfate and ascorbic acid was adjusted before the addition to the rice flour.

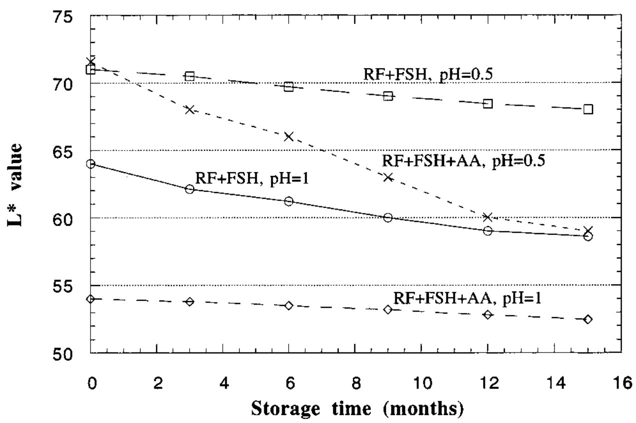

Figure 1. Effect of storage on the *L** value of the extrudates.

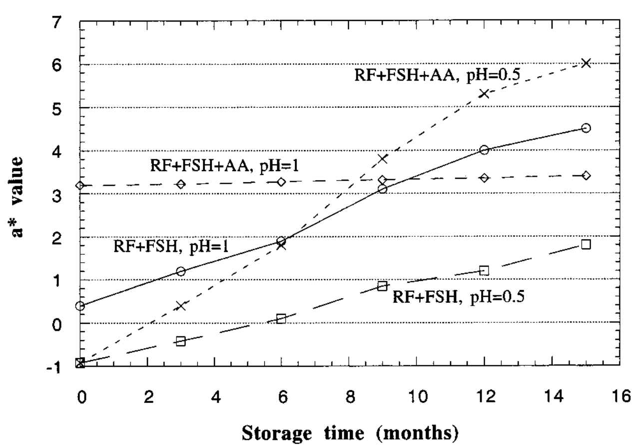

Figure 2. Effect of storage on the *a** value of the extrudates.

addition of water. Acidification decreased significantly the oxidation of ferrous to the ferric form and also increased the amount of soluble iron in the extrudates. Utilization of ascorbic acid sodium salt (AA) in the mixture resulted in the reduction of Fe(III) and increased the total soluble iron (possibly via the chelating properties of AA). The higher soluble iron concentration at pH 1, compared to pH 0.5, indicates that iron chelation is stronger at higher pH values at which AA is ionized. The elimination of the ferric form and the increased iron solubility in the extrudates may prove to be beneficial nutritionally as these factors can positively affect iron bioavailability.

Effect of Storage on the Color of Extrudates. We followed the color of the extrudates for 15 months. The changes in the *L**, *a**, and *b** values can be seen in Figures 1, 2, and 3, respectively. The RF + FSH (pH 0.5) sample was very stable over time with color parameters that were very close to those of Jasmine rice, even after 15 months of storage. On the other hand, the presence of AA was associated with serious discolorations (probably due to the oxidation of AA coupled with the formation of a color complex with iron) that make samples with AA incompatible with Jasmine rice. Storage under conditions of low or no oxygen may retard color deterioration.

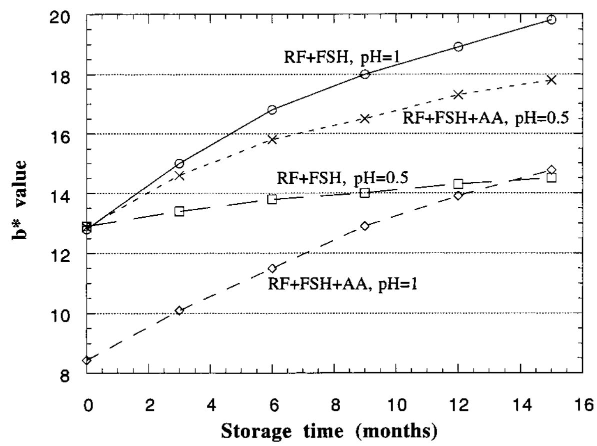

Figure 3. Effect of storage on the *b** value of the extrudates.

Table 3. Mean Scores[a] for Several Quality Attributes of the Rice Samples Used in Sensory Evaluation

	Jasmine rice	FSH (1:100)	FSH (1:200)
	Before Cooking		
size/shape uniformity[b]	106.3[a] ± 5.08	109.5[a] ± 4.92	109.9[a] ± 5.24
color uniformity	91.7[a] ± 6.43	94.5[a] ± 6.82	96.9[a] ± 6.52
smell	100.4[a] ± 5.13	102.7[a] ± 4.44	105.7[a] ± 4.85
	After Cooking		
color uniformity	110.7[a] ± 6.42	99.4[a] ± 6.06	102.0[a] ± 6.35
flavor	83.1[a] ± 7.25	78.0[a] ± 6.06	88.5[a] ± 6.57
stickiness	79.8[a] ± 6.44	69.8[a] ± 6.98	75.7[a] ± 6.60
aftertaste	66.0[a] ± 6.17	76.0[a] ± 7.05	84.1[b] ± 6.29
overall acceptability	94.5[a] ± 5.25	77.9[b] ± 6.06	73.7[b] ± 6.12

[a] The values represent averages and standard deviations for 30 panelists; different superscripts represent samples with a significantly different sensory attributes. [b] Score definition for each attribute: uniformity, 0, not uniform at all, 150, very uniform; smell/flavor/aftertaste, 0, no smell/flavor/aftertaste; 150, strong smell/flavor/aftertaste; stickiness, 0, not sticky at all; 150, very sticky; overall acceptability, 0, unacceptable, 150, acceptable.

Sensory Evaluation. The results can be seen in Table 3. The extrudates contained 7 and 14 g of FSH/300 g of RF and were mixed, respectively, in 1:100 and 1:200 extrudate/rice ratios with rice. The sensory results showed that, for the attributes tested, there is no significant difference among the control and the fortified samples before cooking. The color uniformity is retained after cooking, and all of the scores for most attributes are comparable to the scores for the control. However, the overall acceptability of the fortified samples is still significantly different after cooking, possibly due to a metallic aftertaste. Reducing the amount of iron and increasing the dilution ratio may eliminate the problem. Preliminary experiments indicated that rice fortified with ferrous sulfate monohydrate may improve the organoleptic quality (Hurrell, 1984).

***In Vitro* Bioavailability Experiments.** The dialyzability of the FSH and the FSH + AA sample was close to 7% (average of duplicates), a value higher than the one obtained for the control $FeCl_3$ sample (5%) and certainly satisfactory for a non-heme iron form (Kapanidis, 1995). Although the results of *in vitro* experiments can serve only as an index of iron bioavailability and require support by subsequent *in vivo* studies, they confirm that the biological value of FSH incorporated in RF is better than or at least similar to the value assigned for the FSH in other foods and meals.

Conclusions. We developed a new method to produce an inexpensive and nutritious rice product that is relatively stable under a variety of conditions. The product is ready for *in vivo* studies that should certify its nutritional value. Optimization of the preparation methods and extrusion conditions can further improve the quality of the simulated rice. The concept of acidification as a process capable of reducing discolorations due to iron fortification is also applicable to other foods, such as breakfast cereals and baked foods, to design products with improved iron nutritional value. In conclusion, we believe that such fortified products can be extremely helpful in fighting anemia, especially in the developing world.

LITERATURE CITED

Baynes, R. D.; Bothwell, T. H. Iron deficiency. *Annu. Rev. Nutr.* **1990**, *10*, 133−148.

Cook, J. D.; Reusser, M. E. Iron fortification: an update. *Am. J. Clin. Nutr.* **1983**, *38* (10), 648−659.

Gorman, G. E.; Clydesdale, F. M. The behavior and stability of iron-ascorbate complexes in solution. *J. Food Sci.* **1983**, *48*, 1217−1220, 1225.

Hunnell, J. D.; Yasumatsu, K.; Moritaka, S. Iron enrichment of rice. In *Iron Fortification of Foods*; Clydesdale, F. M., Wiemer, K. L., Eds.; Academic Press: New York, 1985.

Hurrell, R. F. Bioavailability of different iron compounds used to fortify formulas and cereals: technological problems. In *Iron Nutrition in Infancy and Childhood;* Steckel, A., Ed.; Vevey/Raven Press: New York, 1984.

Hurrell, R. F.; Cook, J. D. Strategies for iron fortification of foods. *Food Sci. Technol.* **1985**, *1* (3), 56−61.

Kapanidis, A. N. *In vitro* iron bioavailability of three raw and processed Cruciferous vegetables. M.S. Thesis, Rutgers, The State University of New Jersey, New Brunswick, 1993.

Kapanidis, A. N.; Lee, T-C. Heating of Cruciferous vegetables increases the *in vitro* dialyzability of intrinsic and extrinsic iron. *J. Food Sci.* **1995**, *60* (1), 128−131, 141.

Laszlo, J. A. Content and stability of ferrous iron in soybean hulls. *Cereal Chem.* **1988**, *65* (1), 20−23.

Lee, K.; Clydesdale, F. M. Iron sources used in food fortification and their changes due to food processing. *CRC Crit. Rev. Food Sci. Nutr.* **1979**, *11* (2), 117−153.

Miller, D. D.; Schricker, B. R.; Rasmussen, R. R.; Van Campen, D. An *in vitro* method for estimation of iron availability from meals. *Am. J. Clin. Nutr.* **1981,** *34*, 2248−2256.

Nojeim, S. J.; Clydesdale, F. M. Effect of pH and ascorbic acid on iron valence in model systems and in foods. *J. Food Sci.* **1981**, *48*, 606−611, 616.

Zilberboim, R. Rice fortification through extrusion. Ph.D. Dissertation, Rutgers, The State University of New Jersey, New Brunswick, 1994.

Zoller, J. M.; Wolinsky, I.; Raden, C. A.; Hoskin, J. C.; Lewis, K. C.; Lineback, D. R.; McCarthy, R. D. Fortification of non-staple food items with iron. *Food Technol.* **1980**, *34* (1), 38−47.

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