# Iron Status in Experimental Drum-Dried Rice Foods[1]

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## ABSTRACT

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Experimental rice foods fortified with iron salts, electrolytic iron (EI), and additives were drum-dried and analyzed for metallic, complexed, and soluble iron in aqueous slurries and under simulated stomach and duodenum conditions. The results showed that iron distribution varied, depending on the pH of the medium. In aqueous slurries, the metallic iron was affected only by the addition of EI, soluble iron, and its ferrous ($Fe^{+2}$) ion content by ascorbic acid. Under simulated stomach conditions, the high levels of EI, ascorbic acid, and combinations of $FeSO_4 \cdot 7H_2O$ and sucrose increased soluble iron. The $Fe^{+2}$ ions of soluble iron under simulated stomach conditions increased when EI, thin-kernel rice flour (TKRF), ascorbic acid, and combinations of ferric sodium pyrophosphate (FSPP), sucrose, lipid, and TKRF were added. None of the three iron sources alone increased soluble iron under simulated duodenum conditions. However, soluble iron was increased by combinations of FSPP and TKRF, FSPP and ascorbic acid, and sucrose and ascorbic acid. An in vitro method did not show any significant effect on bioavailable iron, due to any ingredients, including the three iron sources used. Data is presented to explain the possible unexpected shortcomings of this method.

Iron deficiency is a public health problem in many areas of the world. It is particularly severe in countries where cereals provide the bulk of the diet (WHO 1968). These dietary patterns have important implications for nutrition, because iron is generally poorly absorbed from cereals. The two major sources of iron in foods are heme and nonheme iron, each having different absorption mechanisms (Hallberg 1974). The largest fraction is nonheme iron, and its absorption is determined largely by the extent to which it remains soluble within the lumen of the upper intestinal tract (Forth and Rummel 1973, Cook 1983). The use of meat products with cereal foods has two beneficial effects on iron nutrition: First, the heme iron is highly bioavailable; second, the meat also potentiates the absorption of nonheme iron, which otherwise would have low iron absorption (Layrisse et al 1968). A similar stimulation of iron absorption from meals has been produced by foods that include an enhancer of iron absorption, such as ascorbic acid (Morris 1983, Sayers et al 1974).

Rice, an important food grain, makes up the bulk of the staple diet in many parts of Asia and other parts of the world where nutritional anemia is prevalent. Among various grains, iron bioavailability is the highest for wheat, intermediate for rice, and lowest for corn meals (INACG 1982). Harvested rice is milled to remove the hulls and bran, to produce polished or white rice. These processing steps remove much of the iron, along with other components, such as protein, lipids, and vitamins, which can also adversely affect iron absorption. The published values of iron bioavailability from rice vary from 1.2 to 11.6% (INACG 1982). However, these rice meals invariably contain some vegetables, spices, and differently milled rice samples (Batu et al 1976, Hallberg et al 1978, Sayers et al 1974).

Several approaches have emerged to increase the iron bioavailability of rice. One is to add iron and other nutrients to the milled rice. For example, the Food and Drug Administration has a standard of identity for enriched rice of not less than 13 mg and not more than 26 mg of iron per pound (U.S. FDA 1983). However, most rice is not enriched because of various technical problems. The other approach, practiced in many of the developing countries, is to limit the extent of milling and polishing. Another approach is to process polished rice, particularly broken rice, with added nutrients into ready-to-eat foods, such as drum-dried rice food. Such a food is available in the U.S. market.

It has become increasingly apparent that processing of foods changes iron availability (Theuer et al 1971, 1973) and the chemical forms of iron (Hodson 1970). Recently, Lee and Clydesdale (1980) reported that the baking process generated large amounts of insoluble iron, independent of the iron sources added to the baked product. In addition, large differences between sources before baking had vanished in the final product. Many factors affect iron absorption, including the individual's need, the composition of the diet, valency, solubility, ease of ionization, and the degree of chelation or complex formation of the iron and the food components (Forth and Rummel 1973, Jacob and Greenman 1969, Monsen and Cook 1979, Morck and Cook 1981, Saltman 1965). Several studies have correlated the soluble iron under simulated gastrointestinal conditions in processed foods with its iron bioavailability (Jacob and Greenman 1969, Lock and Bender 1980, Miller et al 1981, Narasinga Rao and Prabhavathi 1978, and Ranhotra et al 1971). This study reports the effects of two levels of various ingredients and three commonly used iron sources on iron distribution in aqueous slurries and under simulated stomach and duodenum conditions in rice foods made from polished rice and thin-kernel rice flours. It also correlates various iron forms with bioavailable iron determined by an in vitro method (Miller et al 1981). Evidence of significant changes in iron forms due to various ingredients and processing methods expands our knowledge of the iron status and its bioavailability in foods.

## MATERIALS AND METHODS

A fractional factorial experimental plan with one-sixteenth replication of eight ingredients at low and high levels was selected to make experimental drum-dried rice foods. This plan considers all possible treatments of the eight ingredients, but only a fraction (1/16) are actually prepared by randomization (NBS 1957). Analysis of variance treatments of the data measures the effects of each ingredient and interactions between a majority of combinations of two ingredients, but it does not measure the interactions among three or more ingredients. Various ingredients used to make the experimental rice foods are shown in Table I. All iron sources were food grade and met Food Chemical Codex Specifications (NAS-NRC 1972). $FeSO_4 \cdot 7H_2O$ and ascorbic acid were analytical reagents. Electrolytic iron (EI) (A-131) was obtained from the Glidden-Durkee division of SCM Corp.; ferric sodium pyrophosphate (FSPP) was provided by Mallinckrodt; Crisco brand shortening was purchased locally; rice flour (RL-100), containing 7.5% protein, 0.75% lipids, 0.65% fiber, and approximately 89% starch, was purchased from Riviana Foods, Inc.; the thin kernel rice flour (TKRF), which was made from brown rice and contained 12.4% protein, 3.9% lipids, 2.1% fiber, and approximately 79% starch, was prepared in our laboratories

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(Matthews et al 1981); and lecithin (Centrolene A), a hydroxylated product, was provided by Central Soy Co. The water-soluble ingredients, including lecithin, were dissolved in deionized water, warmed if needed, and mixed with rice flour and other insoluble components, such as EI and FSPP, in a Hobart mixer. The concentration of the final slurry was 15% w/w. The mixture was then drum-dried on an Overton, chrome-plated double-drum drier at 75 psig steam pressure (160° C roll surface temperature). The processed food material was milled and stored at −5° C under a blanket of nitrogen and analyzed within 16 wk.

The iron status in drum-dried rice food was determined on two replicate (each analyzed in duplicate) samples, in an aqueous slurry (pH 7), using a modification of the method of Lee and Clydesdale (1979), by incubating the samples in a pepsin-dilute HCl mixture (simulated stomach conditions) and by further digesting part of the simulated stomach-digested sample with pancreatin-bile mixture (Miller et al 1981) to simulate digestion in human duodenum conditions. The chemical forms of iron used in drum-dried rice food are shown in Figure 1. In vitro iron bioavailability was also measured by the method of Miller et al (1981). For iron distribution in aqueous slurry, a 5-g sample was suspended in 100 ml of nitrogen-purged double-distilled water and technique by Kadan and Ziegler (1984) was used. For measuring iron distribution under simulated stomach and duodenum conditions, a 3-g sample was suspended in 20 ml of nitrogen-purged double distilled water and then 16 ml of 0.1 *N* HCl solution containing 0.16 g of pepsin (Sigma Chemical Co., St. Louis, MO) was added. The mixture was incubated at 37° C in a shaking water bath and iron distribution was determined, following the technique described above. A pepsin-dilute HCl-incubated sample was neutralized and further digested with pancreatin-bile extract to simulate duodenum digestion, following the procedure of Miller et al (1981), and iron distribution was determined on the contents of the dialysis tube by the method of Kadan and Ziegler (1984). An aliquot of the contents of the

**TABLE I**
**Experimental Rice Food**

| Ingredient | Level (g) Low | Level (g) High |
|---|---|---|
| $FeSO_4{\cdot}7H_2O$ | 0 | 0.200 |
| Electrolytic iron | 0 | 0.040 |
| Ferric sodium pyrophosphate | 0 | 0.333 |
| Sucrose | 0 | 90.800 |
| Shortening (Crisco brand) | 0 | 72.6 |
| Thin-kernel rice flour | 0 | 181.6 |
| Ascorbic acid | 0.046 | 0.272 |
| Lecithin | 6.0 | 6.0 |
| Rice flour (Riviana RL-100) | 885.0 | 557.0 |

**TABLE II**
**Ingredients Affecting the Amount of Elemental and Nonelemental Iron in a Reconstituted Drum-Dried Product[a]**

| Iron Form | Ingredients Added | Effect[b] on Iron | Probability[c] |
|---|---|---|---|
| | **Elemental (Metallic)** | | |
| | Electrolytic iron | Increased | 0.0016 |
| | $FeSO_4{\cdot}7H_2O$ and sucrose | Decreased | 0.0496 |
| | Sucrose and thin-kernel rice flour | Increased | 0.0165 |
| | Thin-kernel rice flour and ascorbic acid | Increased | 0.0120 |
| $Fe^{+2}$ in elemental | Ascorbic acid | Decreased | 0.0254 |
| $Fe^{+3}$ in elemental | Electrolytic iron | Increased | 0.0057 |
| | $FeSO_4{\cdot}7H_2O$ and sucrose | Decreased | 0.0289 |
| | Sucrose and thin-kernel rice flour | Increased | 0.0165 |
| | Thin-kernel rice flour and ascorbic acid | Increased | 0.0120 |
| | **Insoluble (Complexed)** | | |
| | $FeSO_4{\cdot}7H_2O$ | Increased | 0.0001 |
| | Electrolytic iron | Increased | 0.0001 |
| | Ferric sodium pyrophosphate | Increased | 0.0001 |
| | Crisco brand shortening | Increased | 0.0447 |
| | Thin-kernel rice flour | Increased | 0.0354 |
| | Electrolytic iron and sucrose | Increased | 0.0441 |
| | Ferric sodium pyrophosphate and sucrose | Increased | 0.0066 |
| | Ferric sodium pyrophosphate and thin-kernel rice flour | Increased | 0.0183 |
| | Sucrose and ascorbic acid | Increased | 0.0138 |
| | Thin-kernel rice flour and ascorbic acid | Increased | 0.0106 |
| Soluble (ionic) | None | | |
| $Fe^{+2}$ in soluble | Ascorbic Acid | Increased | 0.0035 |
| $Fe^{+3}$ in soluble | None | | |

[a] pH 7.
[b] High ingredient level added.
[c] Significant at $P < 0.05$.

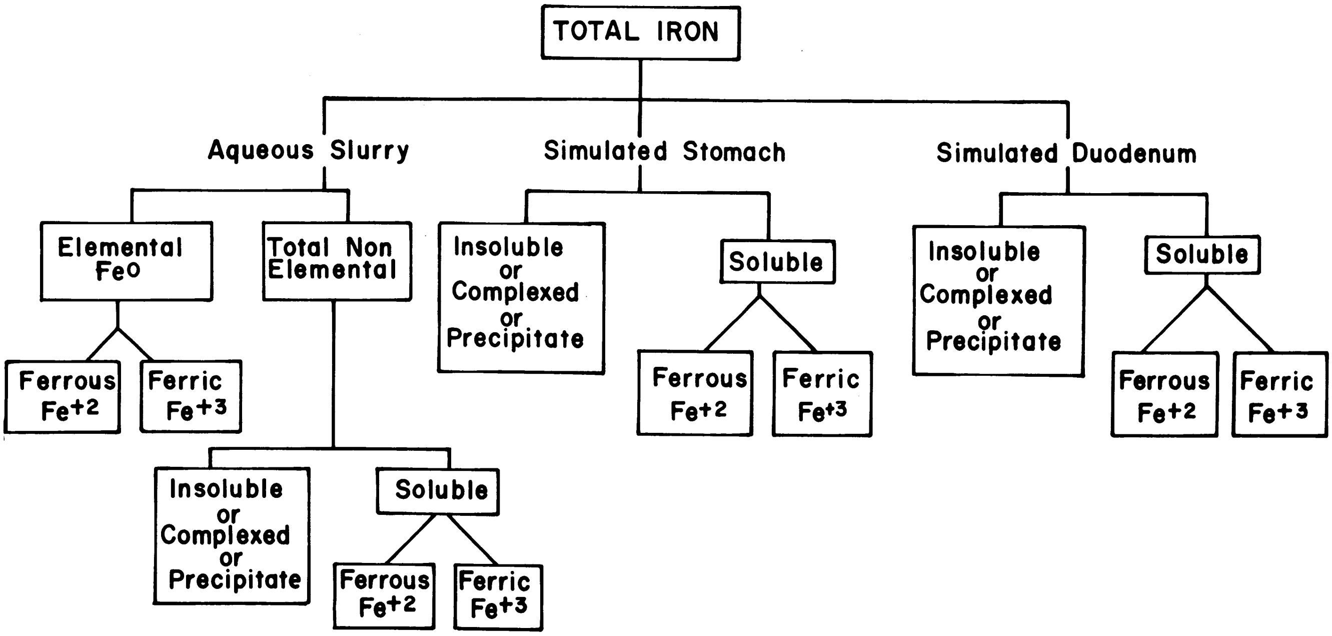

**Fig. 1.** Classification of the chemical forms of iron used in drum-dried rice food.

dialysis tube was also treated with protein-precipitating solution and chromogen reagent to obtain in vitro bioavailable colorimetric iron (Miller et al 1981). The dialysis tube contents were analyzed for total nitrogen, reducing sugars (Miller 1959), and carbohydrate (Dubois et al 1956). The data were analyzed by analysis of variance.

**TABLE III**
**Ingredients Increasing[a] the Amount of Insoluble and Soluble[b] Iron by Simulated Stomach[c] and Duodenum Assays of a Reconstituted Drum-Dried Product**

| Iron Form | Ingredients Added | Probability[d] |
|---|---|---|
| | **Stomach Assay** | |
| Insoluble (complexed) | $FeSO_4 \cdot 7H_2O$ | 0.0001 |
| | Electrolytic iron | 0.0001 |
| | Ferric sodium pyrophosphate | 0.0001 |
| | Ferric sodium pyrophosphate and thin-kernel rice flour | 0.0219 |
| | Sucrose and ascorbic acid | 0.0181 |
| Soluble (ionic) | Ferric sodium pyrophosphate | 0.0189 |
| | Electrolytic iron | 0.0005 |
| | Crisco brand shortening | 0.0286 |
| | Thin-kernel rice flour | 0.0352 |
| | Ascorbic acid | 0.0001 |
| | Ferric sodium pyrophosphate and sucrose | 0.0240 |
| | $FeSO_4$ and sucrose | 0.0089 |
| | Thin-kernel rice flour and sucrose | 0.0358 |
| $Fe^{+2}$ in soluble iron | Electrolytic iron | 0.0007 |
| | Crisco brand shortening | 0.0086 |
| | Thin-kernel rice flour | 0.0472 |
| | Ascorbic acid | 0.0001 |
| | Ferric sodium Pyrophosphate and sucrose | 0.0011 |
| | Crisco brand shortening and thin-kernel rice flour | 0.0049 |
| | Electrolytic iron and thin-kernel rice flour | 0.0290 |
| | Ferric sodium pyrophosphate and thin-kernel rice flour | 0.0401 |
| | Sucrose and thin-kernel rice flour | 0.0223 |
| $Fe^{+3}$ in elemental iron | Ferric sodium pyrophosphate | 0.0038 |
| | **Duodenum Assay** | |
| Insoluble (complexed) | $FeSO_4 \cdot 7H_2O$ | 0.0001 |
| | Electrolytic iron | 0.0004 |
| | Ferric sodium pyrophosphate | 0.0002 |
| | Sucrose and ascorbic acid | 0.0026 |
| | Ferric sodium pyrophosphate and ascorbic acid | 0.0007 |
| | Electrolytic iron and ascorbic acid | 0.0447 |
| | Ferric sodium pyrophosphate and sucrose | 0.0432 |
| Soluble (ionic) iron | Sucrose | 0.0102 |
| | Ferric sodium pyrophosphate and thin-kernel rice flour | 0.0573 |
| | Ferric sodium pyrophosphate and ascorbic acid | 0.0109 |
| $Fe^{+2}$ in soluble iron | Ferric sodium pyrophosphate and thin-kernel rice flour | 0.0350 |
| | Ferric sodium pyrophosphate and ascorbic acid | 0.0292 |
| | Sucrose and ascorbic acid | 0.0076 |
| $Fe^{+3}$ in elemental iron | Sucrose and thin-kernel rice flour[e] | 0.0358 |

[a] High ingredient level added.

[b] None of the ingredients had a significant effect, if measured by the method of Miller et al (1981).

[c] pH 1.5.

[d] Significant at $P < 0.05$.

[e] Low sucrose and thin-kernel rice flour increased, but high sucrose and thin-kernel rice flour decreased.

## RESULTS AND DISCUSSION

The experimental design permits the evaluation of low and high levels of ingredients on the iron status as a result of the drum-drying process. The technique compares the effects of each ingredient and its level of usage with rice flour; it merely simulates the drum-drying process used to make food products from flour. The significant effects of ingredients on iron forms are shown in Tables II and III. The ingredients significantly affecting the amount of metallic iron and its ferrous ($Fe^{+2}$) and ferric ($Fe^{+3}$) ions are shown in Table II. None of the ingredients alone, except EI, affected the amount of metallic iron recovered by magnets. An increase in the amount of metallic iron recovered indicated that rice food components inhibited the interactions between EI and food ingredients. Similarly, a decrease denoted significant interaction. Only a combination of $FeSO_4 \cdot 7H_2O$ and sucrose promoted interaction; combinations of sucrose and TKRF, and TKRF and ascorbic acid inhibited interaction during drum drying. Little information is available regarding the nature of this interaction and its ultimate nutritional significance. The EI used was a minus 325-mesh powder, which had nearly 80% of the particles less than 20 $\mu$ and irregular dendritic shape. It is a popular source of iron for food enrichment, because unlike water-soluble iron salts, it does not discolor the final product. Therefore, any significant decrease of metallic iron during processing denotes a potential discoloration problem. The $Fe^{+2}$ and $Fe^{+3}$ ion distribution of metallic iron indicated that only the addition of ascorbic acid decreased the $Fe^{+2}$ ion of the metallic, whereas the $Fe^{+3}$ ion was increased by EI and combinations of sucrose and TKRF, and TKRF and ascorbic acid. On the other hand, the combination, $FeSO_4 \cdot 7H_2O$ and sucrose, decreased $Fe^{+3}$ ion. Electrolytic iron, when dissolved in dilute HCl, gives nearly 100% $Fe^{+2}$ ion. Any significant increase in $Fe^{+3}$ ion during drum drying, therefore, indicates the oxidation of EI.

The ingredients significantly affecting the distribution of nonmetallic iron in a reconstituted aqueous slurry are also shown in Table II. None of the ingredients, including $FeSO_4 \cdot 7H_2O$, which is very soluble in water at neutral pH (Forth and Rummel 1973), significantly affected soluble iron or its ion distribution. Instead, most of the ingredients increased insoluble or complexed iron. Little is known about iron-rice food complexes. Iron is known to complex with cereal proteins (Nelson and Potter 1980), with sugars (Bachran and Bernhard 1980), and with cereal fibers (Camire and Clydesdale 1982, Mod et al 1981).

Iron distribution under simulated stomach conditions is shown in Table III. Besides the three sources, some ingredients, such as TKRF, sucrose, and ascorbic acid, in combinations with other ingredients significantly increased insoluble iron. Soluble iron, measured under simulated stomach conditions, has been suggested as an indirect method to estimate iron bioavailability (Jacob and Greenman 1969, Ranhotra et al 1971) in processed foods. The following components significantly increased soluble iron: FSPP, EI, TKRF, Crisco brand shortening, and ascorbic acid. Surprisingly, $FeSO_4 \cdot 7H_2O$, a commonly used water-soluble (at pH 7 and 1.5) iron salt, was not found to increase soluble iron. Most of

**TABLE IV**
**Nitrogen, Phosphorus, and Carbohydrate Contents of Dialysate from Unprocessed Rice Flours and Foods**

| Sample | Nitrogen (mg/ml) | Phosphorus (ppm) | Reducing Sugar (mg/ml) | Carbohydrate Total (mg/ml) | Carbohydrate Reducing Sugar (mg/ml) |
|---|---|---|---|---|---|
| Rice flour (Riviana RL-100) | 0.58 | 113.6 | 3.27 | 11.1 | 7.8 |
| Thin-kernel rice flour (unprocessed) | 0.75 | 185.6 | 2.71 | 8.7 | 6.0 |
| Drum-dried rice food from Riviana RL-100 | 0.33 | 72.2 | 1.20 | 23.5 | 22.3 |
| Drum-dried rice food from Riviana RL-100 and unprocessed thin-kernel rice flour | 0.45 | 9.7 | 1.76 | 26.8 | 25.0 |

the same ingredients, except FSPP, also increased $Fe^{+2}$ ion of the soluble iron. FSPP was effective only in the presence of sucrose and TKRF. The $Fe^{+3}$ ions were increased only when FSPP was added. The data support the general principle that the $Fe^{+2}$ and $Fe^{+3}$ ion equilibrium would shift in favor of $Fe^{+2}$ ion with decrease in pH (Milazzo and Caroli 1978).

Table III also shows the iron distribution under simulated duodenum conditions. Besides the three iron sources, sucrose and ascorbic acid in combinations with other ingredients increased insoluble iron. Soluble iron, however, was not increased by any of the three iron sources used. Only FSPP in the presence of either TKRF or ascorbic acid increased soluble iron. Recent work by Miller et al (1981) has reported an excellent correlation between the soluble iron under simulated duodenum conditions of a food and its iron bioavailability. Therefore, it appears that something in the chemical composition of regular rice flour makes it a poor source of iron before and after enrichment. The fact that such ingredients as TKRF, ascorbic acid, and sucrose are effective in increasing soluble iron (i.e., $Fe^{+2}$ ion) suggests the importance of incorporating these and other ingredients in rice foods. However, little is known about the constituents of rice that inhibit iron absorption. TKRF had higher amounts of proteins, lipids, and fiber than the regular rice flour, but the mechanism of actions of these components on iron during processing and human digestion is not clear. The method of Miller et al (1981) did not detect any significant increase in soluble iron due to added iron salts or other ingredients (not shown in the tables). The only difference between the assay for soluble iron under simulated duodenum conditions and that by Miller et al (1981) is the way the final color is developed. In the case of Miller et al (1981), the dialysate is treated with a protein precipitant solution, apparently to remove any soluble proteins, and color is developed by using a bathophenanthroline sulfonate chromogen solution to determine its soluble iron contents. Soluble iron and its $Fe^{+2}$ and $Fe^{+3}$ ion distribution under simulated duodenum conditions were determined by using a slightly modified method of Lee and Clydesdale (1979). This method uses chloroform to extract bathophenanthroline-iron color, whereas the Miller et al (1981) method does not use a solvent to remove color. Apparently, the chloroform extraction step removes any interfering component, thus increasing the accuracy of the test.

Other food components, such as phytic acid and phosphates, can also complex with iron (Cheryn 1980) and thus, decrease its bioavailability. The data in Table IV indicated that this apparently is not the case with rice food. The dialysate from drum-dried rice food had more carbohydrates than from the starting rice flours. Other components, such as nitrogen (protein), phosphorus, and reducing sugars, tend to decrease during processing, which suggests that soluble iron is probably complexed with the carbohydrate moiety, and the Miller et al (1981) in vitro method somehow does not recover it; the Lee and Clydesdale (1979) method, however, apparently can recover it. The exact nature of the carbohydrate moiety and its possible complex formation with soluble iron remains to be investigated. Further study of this complex can possibly explain the reasons for poor iron bioavailability from rice. Also, no significant correlations were observed among the iron distribution in aqueous slurries and simulating stomach and duodenum conditions.

In summary, this study showed that none of the commonly used iron sources alone increased soluble iron under simulated duodenum conditions. Only FSPP in combination with sugar and ascorbic acid was effective in increasing soluble iron, thus highlighting the importance of these and perhaps other ingredients in rice foods to increase their iron bioavailability.

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