Effects of Ingredients on Iron Distribution in Spray-Dried Experimental Soy Beverage[1]

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ABSTRACT

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Experimental soy beverages fortified with three iron sources and other additives at two levels were spray-dried and analyzed for metallic, complexed, and soluble iron in aqueous and acidic slurries. Analysis of variance showed that iron distribution differed according to the pH of the medium. In aqueous (pH 7) slurries, the metallic iron is affected by the presence of lipids, glucose, fiber, and ascorbic and citric acids, whereas the soluble iron is affected by the presence of $FeSO_4$, electrolytic iron, ascorbic and citric acids, and combinations of ingredients. In acidic slurries, the soluble iron is affected only by $FeSO_4$, electrolytic iron, and ascorbic acid. Ferrous ions of soluble iron in acidic slurries are increased by the presence of glucose, sucrose, $FeSO_4$, ascorbic acid, and the combinations of glucose and ascorbic acid. Some of these ingredients have been reported to increase bioavailability of iron in foods.

Iron deficiency is one of the most common nutritional disorders and the primary cause of anemia throughout the world. Of the factors that lead to iron deficiency, low availability of nonheme iron is considered to be one of the most important (Morck and Cook 1981). This is particularly so in populations subsisting on diets almost exclusively of plant foods. Many factors affect iron absorption, including individual need, composition of the diet, valency, solubility, ease of ionization, and the degree of chelation or complex formation of the iron and the food components (Conrad and Schade 1968, Forth and Rummel 1973, Jacob and Greenman 1969, Monsen and Cook 1979, Morck and Cook 1981, Saltman 1965). In addition, iron in foods undergoes changes during processing and storage. Hodson (1970) showed that ferric orthophosphate, an insoluble iron salt, became largely soluble and was reduced to the ferrous state when present during processing and storage of a canned liquid dietary food. Lee and Clydesdale (1980) have also reported significant conversion between the forms of iron during the spray-drying of an orange-flavored beverage.

Advances in food technology have produced a variety of new food ingredients to improve the nutritional and functional properties of the processed food products. Soy products, such as defatted flour, protein concentrate, and protein isolate, are being used increasingly to improve the protein quality of foods. Some of these products are spray-dried and offered to the baking industry as a replacement for dried milk. Recently, however, Cook et al (1981) reported substantial inhibition of iron absorption associated with soy products. Therefore, there is a need to know the effects of soy proteins in fabricated foods, such as spray-dried soy beverage, on iron distribution. This information can be useful in assessing the role of soy-proteins on iron bioavailability in processed foods.

The problem of determining the iron quality, hence iron bioavailability, in processed foods is well recognized. Animal and human absorption studies are expensive and tedious. Several in vitro methods of estimating iron bioavailability under simulated gastrointestinal conditions have been reported (Jacob and Greenman 1969, Lock and Bender 1980, Miller et al 1981, Narsinga Rao and Prabhavathi 1978). Another approach has been to quantify the chemical changes in iron forms as a result of processing operations (Lee and Clydesdale 1978, 1980). The particular iron form and iron food complex has been correlated with the overall bioavailability of the food system (Forth and

Rummel 1973, Monsen and Cook 1979, Nelson and Potter 1980). This study reports the effects of commonly used ingredients and three iron sources in an experimental spray-dried soy beverage on oxidative state and the forms of iron in aqueous slurry and under simulated stomach conditions. Evidence for significant changes in the chemical forms of iron (ie, solubility, valence, and complex formation) due to commonly used food components and different iron sources will expand our knowledge of iron status and hence its bioavailability in processed foods.

MATERIALS AND METHODS

A fractional factorial experimental design, having 1/16 replication of nine ingredients at two levels (low and high) was used to prepare 32 experimental spray-dried soy beverage samples. This statistical plan considers all possible treatment combinations of all nine ingredients, but only a fraction (1/16) are actually prepared by randomization (NBS 1957). Analysis of variance treatment of the data can measure the effect of each ingredient and the interactions between a majority of the combinations of two ingredients. However, it does not measure the interactions between combinations of three or more ingredients.

The 0.0% of the various ingredients was assigned as low levels. The high levels are: lipid (vegetable oil), 10.0%; glucose, 5.0%; sucrose, 20.0%; Avicel-RC, 20.0%; $FeSO_4$, 400 $\mu g/g$ on Fe basis; electrolytic iron, 400 $\mu g/g$ on Fe basis; Fe_2O_3, 400 $\mu g/g$ on Fe basis; ascorbic acid, 200 ppm; and citric acid, 200 ppm.

A commercially obtained soy-protein isolate was used to make the total 100%, d.b., as indicated by the plan. These ingredients were selected to make a simulated milk-type food. Avicel-RC was included as a convenient fiber source that can also be handled during spray-drying. Besides the two generally used iron sources ($FeSO_4$ and electrolytic iron), Fe_2O_3 was selected as a common contaminent from processing equipment. A 2-L, 10% (w/v) solution was prepared using deionized and distilled water, and the mixture was homogenized and kept in solution by an air-driven stirrer. The mixture was blanketed under nitrogen before spray drying. A Nerco Niro atomizer, laboratory-model spray-drier was used to dry the beverage mixture. The inlet and exhaust air temperature varied between 270–295° C and 88–95° C, respectively. The dried material was stored at −5° C under a blanket of nitrogen and analyzed within 10 weeks.

Iron distribution in the spray-dried material was determined in the aqueous (pH 7) slurry by using a modified approach of Lee and Clydesdale (1979) and by incubating in a pepsin-dilute HCl mixture (simulated stomach) as shown in Fig. 1. For iron distribution in aqueous slurries, 2 g of material was suspended in 50 ml of nitrogen-purged double-distilled water. Elemental iron was removed from the slurry magnetically, using two 5-cm teflon-coated magnetic stirring bars. Each sample was stirred for 20 min, magnets were removed by a teflon-coated forcep, rinsed in 25 ml of 3N HCl in an Erlenmeyer flask, and iron contents dissolved over a

mildly heated hot plate for about 15 min. The metallic iron thus extracted was analyzed for total iron content by atomic absorption spectroscopy (AA). The Fe^{+2} and Fe^{+3} content were determined by the bathophenanthroline reaction (Lee and Clydesdale 1979). Total iron was determined by wet-ashing the dried sample with concentrated HNO_3, picking up the residue in dilute HCl and analyzing the filtered solution by AA, using similar strength HCl. For measuring soluble and complexed (insoluble) iron, an aliquot of elemental iron-extracted slurry was centrifuged at $3,600 \times g$ for 15 min. The clear solution obtained was analyzed for Fe^{+2} and Fe^{+3} contents by bathophenanthroline color reaction. The precipitate was wet-ashed with concentrated HNO_3, the residue redissolved in dilute HCl, filtered, and analyzed by AA.

For measuring iron distribution under simulated stomach conditions, 2 g of spray-dried material was suspended in 50-ml mixtures of $0.1N$ HCl (pH 1.35) and 0.19 g of pepsin (Sigma Chemical Co., St. Louis, MO) and incubated at $37°$C in a shaking water bath for 120 min. After incubation, aliquots of the mixture were processed as described for the elemental iron-extracted aqueous slurry to obtain insoluble (complexed) and soluble iron for Fe^{+2} and Fe^{+3} distribution.

Sufficient time was initially devoted to develop satisfactory procedure to get $90 \pm 10\%$ repeatability on duplicate analysis of each iron species before proceeding with the experimental samples. Analysis of variance was performed on the average of duplicate values.

Fig. 1. Classification of the chemical forms of iron used in spray-dried experimental soy beverage.

RESULTS AND DISCUSSION

This statistical approach permits evaluating the combined effects of low and high levels of various ingredients in the presence of soy-protein isolate. It does not estimate the effect of soy proteins. The plan merely simulates fabricated foods made from soy-protein isolate. The important effects of various ingredients on iron forms, calculated as probability (P), are shown in Tables I-IV. For example a P value of 0.0500 is the probability of getting error at 5% or the chance of getting a correct answer at 95%. The ingredients affecting the amount of elemental iron are shown in Table I. The addition of lipid and electrolytic iron increased the amount of elemental iron in the spray-dried beverage, whereas the addition of glucose, Avicel-RC, and ascorbic acid had an opposite effect. This suggested that the presence of lipid inhibited the interaction between electrolytic (metallic) iron and the other components of the soy beverage. On the other hand, glucose Avicel-RC, and ascorbic acid promoted the interaction, thus decreasing the amount. The exact nature of the interaction is unknown. There was no interaction between the combination of ingredients and electrolytic iron. Even though the fresh elemental iron, when dissolved into dilute HCl by the technique mentioned above, resulted in $95 \pm 10\%$ ferrous ion. Magnetically extracted elemental iron from the spray-dried samples showed no significant difference between ferrous and ferric ions (not shown in Table I). This indicated that none of the ingredients used to make soy beverage protected electrolytic iron from oxidation during spray-drying and subsequent storage. The effects of ingredients on the amounts of insoluble iron, soluble iron, and the ion distributions in the soluble iron, are shown in Table II. Insoluble iron was increased by the addition of both $FeSO_4$ and Fe_2O_3 and was slightly affected (not significant at $P = 0.05$ level) by Avicel-RC. The other ingredients had no appreciable effect. This indicated that even though $FeSO_4$ is very soluble in water at approximately pH 7 (Forth and Rummel 1973), it nevertheless reacts with the soy-protein isolate, and most (90–95%) of it is precipitated. Fe_2O_3 has very low solubility at

TABLE I
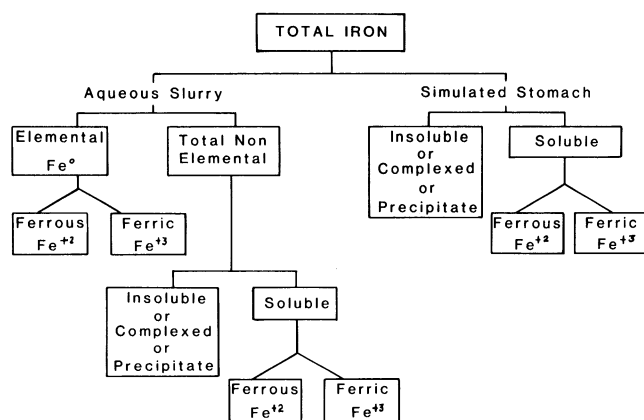Ingredients Affecting the Amount of Elemental Iron
in Reconstituted Soy Beverage (pH 7.0)

Ingredients Added	Effect[a]	Significance[b]
Lipid	Increases	.0550
Glucose	Decreases	.0212
Avicel-RC	Decreases	.0328
Electrolytic iron	Increases	.0001
Ascorbic acid	Decreases	.0550

[a] High amounts added.
[b] Significant at $P = 0.05$.

TABLE II
Ingredients Affecting the Forms of Iron in Reconstituted
Soy Beverage (pH 7.0)

Iron Form	Ingredients	Effect[a]	Significance[b]
Insoluble (complexed)	Avicel-RC	Decreases	0.0699
	$FeSO_4$	Increases	0.0040
	Fe_2O_3	Increases	0.0003
Soluble (ionic)	Lipid	Decreases	0.0719
	$FeSO_4$	Increases	0.0001
	Electrolytic iron	Increases	0.0098
	Ascorbic acid	Decreases	0.0098
	Citric acid	Increases	0.0593
	Lipid + ascorbic acid	Decreases	0.0355
	Sucrose + citric acid	Increases	0.0489
	$FeSO_4$ + ascorbic acid	Increases	0.0521
Fe^{+2} in soluble	$FeSO_4$	Increases	0.0076
Fe^{+3} in soluble	Lipid	Decreases	0.0585
	$FeSO_4$	Increases	0.0001
	Electrolytic iron	Increases	0.0614
	Ascorbic acid	Decreases	0.0105

[a] High amounts added.
[b] Significant at $P < 0.05$.

TABLE III
Ingredients Affecting the Forms of Iron in Reconstituted Soy
Beverage Under Simulated Stomach Condition (pH 1.5)

Iron Form	Ingredients	Effect[a]	Significance[b]
Insoluble (complexed)	$FeSO_4$	Increases	0.0005
	Electrolytic iron	Increases	0.0005
	Fe_2O_3	Increases	0.0002
Soluble (ionic)	Sucrose	Increases	0.0605
	$FeSO_4$	Increases	0.0270
	Electrolytic iron	Increases	0.0156
	Ascorbic acid	Increases	0.0017
Fe^{+2} in soluble	Glucose	Increases	0.0365
	Sucrose	Increases	0.0351
	Electrolytic iron	Increases	0.0316
	Ascorbic acid	Increases	0.0010
	Glucose + ascorbic acid	Increases	0.0389
Fe^{+3} in soluble	None	···	···

[a] High amounts added, except Fe^{+3} in soluble iron.
[b] Significant at $P < 0.05$.

neutral pH and, as expected, accumulates in the precipitate. Even at pH 1.35 (Table III) where all three iron sources are very soluble, soy-protein complexes nearly 90% of added iron, as is also evidenced by the significant (at $P = 0.05$ level) increase in complexed iron by all three sources. Avicel-RC probably decreases the iron contents of the precipitate by dilution rather than by any complex formation, as it does not affect either the soluble iron or the ion distribution at both neutral and acidic pHs. Nelson and Potter (1979) have reported that soy proteins can complex with iron and thus take up large amounts of both ferrous and ferric ions over a wide range of pH. Insoluble soy proteins were reported to tie appreciable amounts of ferrous and ferric ions, both at neutral and acidic pH. The data in Table III show that soy-protein-iron complex is stable even in the presence of pepsin and at low pH. However, little is known about the ultimate nutritional significance of such protein-iron complex.

A comparison of soluble iron in both aqueous slurry (Table II) and acidic solution (Table III) indicates that the addition of FeSO$_4$ and electrolytic iron resulted in a significant increase of soluble iron. The addition of Fe$_2$O$_3$ did not affect soluble iron in either neutral or acidic pH. It should be fairly soluble in water at pH 1.5 (Forth and Rummel 1973) but apparently it has complexed with beverage components so strongly, particularly with soy protein (Nelson and Potter 1979) during spray-drying, that it does not dissociate, even under simulated stomach conditions. It is interesting that the addition of electrolytic iron, which was added in the form of fine (dendritic) crystals and should be practically insoluble in water (neutral pH), also increased soluble iron in aqueous slurry. It showed that this iron source can react with various food components during processing and thus converts into water-soluble form. The addition of ascorbic acid decreased soluble iron in aqueous slurry but increased it under simulated stomach conditions. Also, some combinations, such as ascorbic and citric acid, increased, whereas others decreased soluble iron in aqueous slurry. These interactions suggest the importance of various ingredients in overall iron bioavailability in processed foods. Several earlier reports (Camire and Clydesdale 1982, Conrad and Schade 1968, Saltman 1965) have also recognized the role of iron-food complexes in iron metabolism.

The ferrous- and ferric-ion distributions of soluble iron in aqueous slurries and under simulated stomach conditions showed two distinct patterns. In neutral pH, only ferric ion is significantly affected (Table II), and in acidic solution (Table III), the ferrous ion attains significance. A perusal of oxidation/reduction reactions (Milazzo and Caroli 1978) involving metallic iron, FeSO$_4$ and Fe$_2$O$_3$, shows that the equilibrium between ferrous and ferric ion would increase in favor of ferrous ion with the decrease in pH rather than at higher pH. Apparently, at neutral pH, the ferrous ion is very prone to oxidation and, thus, the equilibrium favors accumulation of the ferric ion. The presence of other ingredients in food, particularly ascorbic acid, also affects the equilibrium. It decreased significantly the ferric ion in aqueous slurry but increased the ferrous ion in acidic solution. The effect of ascorbic acid on food iron distribution can be related to its reducing effect, preventing the formation of insoluble ferric hydroxide and to its formation of soluble complexes with ferric ion. The presence of ascorbic acid in food is known to increase iron bioavailability (Jacob and Greenman 1969, Lee and Clydesdale 1978, Monsen and Cook 1979, Morck and Cook 1981) and is reported to inhibit the binding of ferrous ion to other food components (Camire and Clydesdale 1982). Table IV shows the significant correlations between the various forms of iron. The data show an irregular pattern between aqueous and simulated stomach conditions. These differences also highlight the difficulty of correlating a certain chemical form (in vitro tests) with actual iron bioavailability. No significant correlation was observed between the soluble iron in aqueous slurry and under simulated stomach conditions. The iron form, hence the nonheme iron bioavailability in processed food, is dependent upon the composite effect of various ingredients, along with the particular processing conditions.

The results of this study indicate that the iron added to a

TABLE IV
Significant Correlation Coefficients Between Various Iron Forms

Condition	Form	Significance[a]
Aqueous slurry	Insoluble (complexed)	
	vs total iron	0.0001
	vs ionic (soluble)	0.0177
	vs Fe^{+3} in ionic	0.0131
	Ionic (soluble) iron	
	vs Fe^{+2} in ionic	0.0001
	vs Fe^{+3} in ionic	0.0001
Simulated stomach	Insoluble (complexed)	
	vs total iron added	0.0001
	vs electrolytic iron added	0.0175
	Ionic (soluble)	
	vs Fe^{+2} in ionic	0.0001
Simulated stomach		
	vs in aqueous slurry	
	Insoluble (complexed)	
	vs ionic (soluble) iron	0.0031
	vs Fe^{+3} in ionic iron	0.0021

[a]Significant at $P < 0.05$.

simulated soy beverage does not remain in the original form. Apart from the spray-drying process, ingredients such as lipid, glucose, sucrose, ascorbic acid, citric acid, and combinations of these ingredients, also affect the iron distribution. Even electrolytic iron, which is essentially a fine iron powder, reacted with several ingredients and increased the soluble iron, even in aqueous slurry. The effects of ingredients on soluble iron are different in aqueous and acidic conditions. For example, ascorbic acid decreased soluble iron in aqueous slurry but increased it under acidic conditions.

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