Effects of Ingredients on Iron Solubility and Chemical State in Experimental Breads

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ABSTRACT

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Experimental white breads fortified with ferrous sulfate ($FeSO_4 \cdot 7H_2O$), ferric sodium pyrophosphate, electrolytic iron, and other breadmaking ingredients at two (usage) levels, were baked and analyzed for iron solubility and chemical state in aqueous slurry and in simulated stomach and duodenum conditions. The results showed that iron distribution was affected by the medium, amount, and type of ingredients in the bread. Besides the iron sources, the most active ingredients affecting the soluble iron were: shortening, NaCl, and nonfat dry milk in aqueous slurry; shortening and sugar in the stomach conditions; and yeast and NaCl in the simulated duodenum. Under duodenum conditions, soluble iron was decreased by high NaCl levels and increased by high yeast levels, indicating that a high level of NaCl in bread may decrease and high yeast levels may increase iron bioavailability in vivo. Further in vitro studies for estimating iron bioavailability confirmed the effects of high NaCl and yeast levels.

Iron deficiency anemia is a widespread nutritional problem throughout the world. Various nutritional surveys indicate that as much as 20% of the United States population, particularly infants, young children, and women of childbearing age, may have unacceptable blood iron levels (Combs 1974). Iron deficiency persists despite a plentiful and varied diet. The U.S. Food and Drug Administration (FDA) has required the enrichment of wheat flour, farina, bread, buns, and rolls with iron since the early 1940s (Fed. Regist. 1943) because of widespread iron deficiency. Recently the FDA has taken a number of actions (Fed. Regist. 1973, 1977, and 1982) that showed the need for additional research in the area of iron enrichment of foods. In 1973 the FDA approved an increase in iron levels of 13.0–16.5 mg to 40 mg per pound of enriched flour and of 8.0–12.5 mg to 25 mg per pound of enriched bread, buns, and rolls. In 1977 this approval was rescinded. Effective July 1, 1983, a single-level requirement of 12.5 mg per pound for enriched bread, rolls, and buns, and 20 mg per pound for enriched flour was established (Fed. Regist. 1982). Two factors persuaded the FDA to withdraw its proposal and continue the existing level of iron enrichment: the possibility of overloading certain individuals with iron and the lack of sufficient evidence that increased enrichment in bread alleviates iron deficiency.

There are two major sources of dietary iron in foods: heme and non-heme iron, each having different absorption mechanisms. The largest fraction is non-heme 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). Many factors influence iron absorption, including the individual's needs and the composition of the diet (Lee and Clydesdale 1978). The food value of dietary iron is affected more by the chemical state of iron in the food than by its total iron content (Leicheter and Josyln 1966, Fritz et al 1975). Processing of foods also changes iron bioavailability (Theuer et al 1971, 1973) and the chemical forms of iron (Hodson 1970). In 1980, Lee and Clydesdale reported that the baking process generated large amounts of insoluble iron, independent of the iron sources added to the baked food, suggesting that baking can affect iron bioavailability. In addition, large differences between iron sources before baking vanished in the final product.

Absorption of iron from bread has been studied by several workers (Leicheter and Josyln 1966, Callender and Warner 1968, Elwood et al 1968, Ranhotra et al 1971, Cook et al 1973, and

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Björn-Rasmussen 1974). These studies report a low iron absorption of 4–7% depending upon the source of iron used in fortification. Several in vitro methods have been reported for studying iron absorption (Narasinga Rao and Prabhavathi 1978, Miller et al 1981, Schricker and Miller 1982). Various ingredients in a food are also known to affect iron absorption (Morck and Cook 1981). Processing of bread itself has been shown to cause significant changes in iron forms (Lee and Clydesdale 1978, 1979). The particular iron form and the iron-food complexes have been correlated with the overall bioavailability from that food system (Forth and Rummel 1973, Monsen and Cook 1979, Nelson and Potter 1980). The present study reports the effects of standard bread ingredients and three iron sources used in experimental breads on the ionic forms of iron in aqueous slurries and under simulated stomach and duodenum digestion. It also correlates various iron forms with bioavailable iron as determined by an in vitro method (Miller et al 1981). This fundamental information is necessary to expand our current knowledge of the status of iron and its bioavailability in fortified bread.

MATERIALS AND METHODS

A fractional factorial experimental plan for nine ingredients at two usage levels (low and high) was employed in the formulation of experimental breads. This plan encompassed all possible treatment combinations of the nine ingredients, but only a fraction (32 samples, 1/16th of the possible combinations) were actually prepared by complete randomization (NBS 1957). Analysis of variance of the data measures the effect of each ingredient which is biased (or confounded) with three factors or higher order interactions. Similarly, out of a total of 36 two-factor (two ingredients) interactions, only 21 were tested, and these were also biased by three or higher order interactions. The ingredients used to make the experimental bread are presented in Table I.

TABLE I
Experimental Bread Ingredients

Ingredients	Level (g)	
	Low	High
Shortening	0	6.0
Salt (NaCl A. R.)	0	8.0
Nonfat dry milk	4.0	12.0
Sugar (sucrose)	12.0	24.0
Yeast (dry)	2.0	4.0
Carboxy methyl cellulose	0	5.0
Ferrous sulfate ($FeSO_4 \cdot 7H_2O$ A. R.)	0	0.0448
Electrolytic iron	0	0.009
Ferric sodium pyrophosphate	0	0.075
Wheat flour (Pillsbury all-purpose enriched flour containing 5 mg/100 g EI)	212.0	170.9

Except for carboxy methyl cellulose (CMC), the various ingredients were selected because of their standard use in bread preparation. CMC was included as a convenient and pure source of fiber. All iron samples were food grade and met the Food Chemical Codex Specifications (NAS-NRC 1972). Ferrous sulfate ($FeSO_4 \cdot 7H_2O$) was an analytical reagent. Electrolytic iron (EI) (A-131) was obtained from Glidden-Durkee; ferric sodium pyrophosphate (FSPP) was from Mallinckrodt; and Redstar active dry yeast from Universal Foods.

The ingredients (230 g) were mixed with 120 g of deionized water and proofed for 160 min according to straight-dough procedures. The bread was baked at 215° C for 25 min, removed from the pan, allowed to cool overnight, freeze-dried, stored at −5° C under a blanket of nitrogen, and analyzed within 10 weeks. Preliminary studies had indicated that iron distribution in freeze-dried samples was comparable to that in fresh bread.

Iron distribution in bread samples was determined in aqueous slurry (pH 7) using a modified method of Lee and Clydesdale (1979) by incubating in pepsin-dilute HCl mixture (simulated stomach conditions) and by further digesting a portion of the simulated stomach-digested sample with pancreatin-bile mixture (Miller et al 1981) to simulate human duodenum conditions. The schematic of the analytical procedure is shown in Figure 1. In vitro iron bioavailability was also measured by the method of Miller et al (1981). The techniques of measuring iron distribution in aqueous slurries and in simulated stomach conditions have been described earlier (Kadan and Ziegler 1984). The EI in Figure 1 is the iron removed by magnetic procedure. When this iron was dissolved in dilute HCl, as described by Kadan and Ziegler (1984), both Fe^{+2} and Fe^{+3} ions were present, indicating the chemical changes undergone by the EI during processing. Unprocessed EI gave only Fe^{+2} ions. For measuring iron distribution in simulated duodenum conditions, a pepsin-dilute HCl-incubated sample was neutralized and further digested with pancreatin-bile extract, following the procedure of Miller et al (1981). At the end of the incubation period, the dialysis tubes were removed, rinsed with deionized water, weighed, and the Fe^{+2} and Fe^{+3} ion content determined by batho-

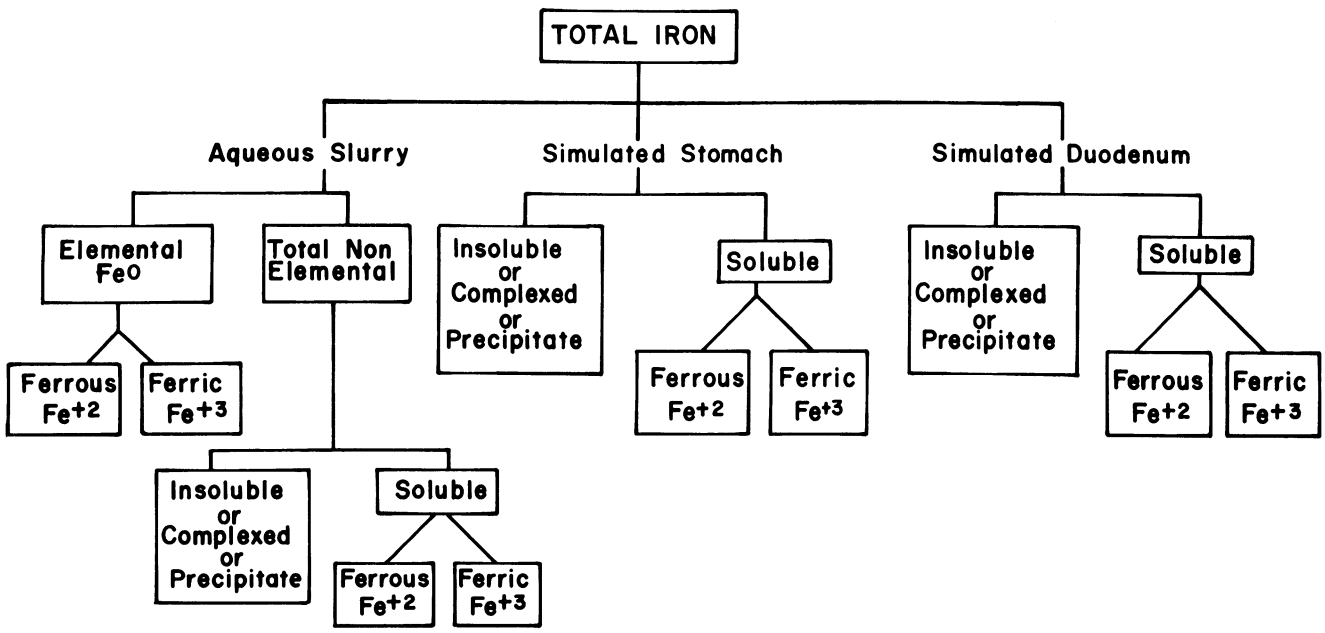

Fig. 1. Classification of the chemical forms of iron used in the experimental breads.

phenanthroline reaction, as described earlier (Kadan and Ziegler 1985). Aliquots of the contents of the dialysis tubes were treated with protein precipitating solution and by chromogen reagent to obtain in vitro available iron, i.e., colorimetric iron (Miller et al 1981).

Preliminary experiments had been used to develop satisfactory procedures for obtaining 90 ± 10% reproducibility on duplicate analyses of each iron species. Analysis of variance (NBS 1957) was performed on the averages of duplicate values of two replicates.

RESULTS AND DISCUSSION

The statistical approach used here permits the evaluation of the effects of various ingredients on iron distribution during the baking process. It does not estimate the effect of wheat flour itself but rather compares the effect of each ingredient or combination of ingredients with the wheat flour alone. The plan merely simulates bread processing made from this source of flour. The statistically significant effects of various ingredients on iron forms are shown in Tables II–VI. The *P* value is the measure of the probability of an error. For example, a *P* value of 0.05 is the probability of getting an error at 5% or the chances of getting a correct answer at the 95% confidence level. The bread components significantly affecting the amount of EI and nonelemental iron (NEI) are shown in Table II. The EI is further characterized for its Fe^{+2} and Fe^{+3} ion content and NEI for insoluble and soluble iron. The high levels of sucrose and EI increased the amount of elemental iron in the experimental bread, whereas high levels of $FeSO_4$ had an opposite effect. This indicated that the presence of sucrose inhibited interactions between the EI (metallic) and the other components of the bread. On the other hand, the addition of $FeSO_4$ promoted the interaction, thus decreasing the amount of EI. There was also significant interaction between sucrose and FSPP and highly significant interaction between EI and $FeSO_4$ as well as $FeSO_4$ and FSPP. Further, distribution of Fe^{+2} and Fe^{+3} ions in EI iron indicated that high amounts of EI and nonfat dry milk (NFDM) increased the Fe^{+2} ion (i.e., protected the EI ion from oxidation). By contrast, the addition of $FeSO_4$ decreased Fe^{+2} ion or accelerated the oxidation of EI. Only NFDM increased Fe^{+3} ions in EI. Insoluble iron was increased by the addition of both $FeSO_4$ and FSPP. EI was earlier removed as metallic iron by the magnet. The other ingredients had no significant effect. This indicated that even though $FeSO_4$ is very soluble in water around pH 7 (Forth and Rummel 1973), it nevertheless reacted with something in the wheat flour during processing and most (90–95%) of it was precipitated. FSPP has very low solubility at neutral pH and, as expected, accumulated in the precipitate. Even under simulated stomach and duodenum conditions (not shown in the tables), nearly 70–90% of added iron was found in the precipitate. Nelson and Potter (1979) have reported that wheat gluten can complex iron and thus take up large amounts of both Fe^{+2} and Fe^{+3} ions over a wide range of pH. Insoluble wheat gluten was reported to tie up appreciable amounts of Fe^{+2} and Fe^{+3} ions, both at neutral and acidic pH. Their further work showed that wheat-gluten-bound iron should be readily freed for absorption within the gastrointestinal tract (Nelson and Potter 1980). The soluble iron and its Fe^{+2} and Fe^{+3} ion contents were affected by several more experimental bread components than the insoluble iron. High levels of shortening and NFDM decreased and a high level of NaCl increased the soluble iron contents. The addition of both $FeSO_4$ and FSPP did not affect the Fe^{+2} ion of soluble iron but increased Fe^{+3} ions, probably due to oxidation of Fe^{+2} to Fe^{+3} under the neutral pH of the bread. The high level of NaCl increased both Fe^{+2} and Fe^{+3} ions, whereas a high level of shortening increased Fe^{+2} but decreased Fe^{+3} ions.

Soluble iron under simulated stomach and duodenum conditions has been suggested as a means of estimating iron bioavailability from processed foods (Ranhotra et al 1971, Narasinga Rao and Prabhavathi 1978). Miller et al (1981) improved the method of Narasinga Rao and Probhavathi (1978) by making a gradual and reproducible adjustment of pH from gastric to duodenum conditions. Table III presents the ingredients which significantly ($P < 0.05$) affect the soluble iron under simulated stomach conditions. Besides the three iron sources, high levels of

TABLE II
Ingredients Affecting the Elemental Iron and Nonelemental Iron in Aqueous (pH 7.0) Bread Slurry

Iron Form	Ingredients	Effect	P[a]
Elemental iron	High sucrose[b]	Increased	0.0105
	High $FeSO_4$[c]	Decreased	0.0421
	High EI[d]	Increased	0.0001
Fe^{+2} ions found in elemental iron	High $FeSO_4$	Decreased	0.0356
	High EI	Increased	0.0001
	High NFDM[e]	Increased	0.0466
Fe^{+3} ions found in elemental iron	High NFDM	Increased	0.0439
Insoluble (complexed iron) in nonelemental iron	High $FeSO_4$	Increased	0.0381
	High FSPP[f]	Increased	0.0001
Ionic (soluble iron)	High shortening[g]	Decreased	0.0173
	High salt[h]	Increased	0.0013
	High NFDM	Decreased	0.0011
	High $FeSO_4$	Increased	0.0001
	High FSPP	Increased	0.0001
Fe^{+2} in soluble iron	High shortening	Increased	0.0306
	High salt	Increased	0.0386
Fe^{+3} in soluble iron	High shortening	Decreased	0.0070
	High salt	Increased	0.0062
	High NFDM	Decreased	0.0038
	High $FeSO_4$	Increased	0.0001
	High FSPP	Increased	0.0001

[a] $P < 0.05$ (F) is significant at the 5% level.
[b] High sucrose = 24.0 g.
[c] High $FeSO_4$ = 0.0448 g ferrous sulfate.
[d] High EI = 0.009 g electrolytic iron.
[e] High NFDM = 12.0 g nonfat dry milk.
[f] High FSPP = 0.075 g ferric sodium pyrophosphate.
[g] High shortening = 6.0 g.
[h] High salt = 8.0 g NaCl.

TABLE III
Ingredients Affecting the Soluble Iron in Bread, Under Simulated Stomach Conditions

Iron Form	Ingredients	Effect	P[a]
Soluble iron	High shortening[b]	Increased	0.0259
	High sucrose[c]	Increased	0.0001
	High $FeSO_4$[d]	Increased	0.0001
	High EI[e]	Increased	0.0001
	High FSPP[f]	Increased	0.0001
Fe^{+2} in soluble iron	High shortening	Increased	0.0471
	High sucrose	Increased	0.0001
	High CMC[g]	Decreased	0.0452
	High $FeSO_4$	Increased	0.0001
	High EI	Increased	0.0001
	High FSPP	Increased	0.0001
Fe^{+3} in soluble iron	High salt[h]	Decreased	0.0001
	High sucrose	Decreased	0.0028
	High $FeSO_4$	Increased	0.0001
	High EI	Increased	0.0002
	High FSPP	Increased	0.0015

[a] $P < 0.05$ is significant at the 5% level.
[b] High shortening = 6.0 g.
[c] High sucrose = 24.0 g.
[d] High $FeSO_4$ = 0.0448 g ferrous sulfate.
[e] High EI = 0.009 g electrolytic iron.
[f] High FSPP = 0.075 g ferric sodium pyrophosphate.
[g] High CMC = 5.0 g carboxy methyl cellulose.
[h] High salt = 8.0 g.

shortening and sucrose increased the soluble iron. The soluble iron was also increased significantly (not shown in tables) by combinations of low and high levels of shortening with a high level of NFDM, high levels of shortening and yeast, low and high levels of sucrose with high level of FSPP, and low and high levels of $FeSO_4$ with high level of FSPP. A perusal of oxidation-reduction reactions involving iron salts (Milazzo and Caroli 1978) shows that the equilibrium between Fe^{+2} and Fe^{+3} ions would shift in favor of Fe^{+2} with a decrease in pH. Nearly 80–90% of the soluble iron was found (not shown in the tables) in the Fe^{+2} form under simulated conditions. The Fe^{+2} ions in the soluble iron fraction were increased by high levels of $FeSO_4$, EI, FSPP, shortening, and sucrose but were decreased by a high level of CMC. As expected, there were significant interactions between ingredients. The Fe^{+2} ions in soluble iron were affected (not shown in tables) by combinations of shortening and NaCl levels, shortening and NFDM levels, shortening and yeast levels, shortening and EI levels, shortening and FSPP levels, NaCl and NFDM levels, NaCl and yeast levels, NFDM and EI levels, sucrose and FSPP levels, and yeast and EI levels. The Fe^{+3} in soluble iron was increased only by the three iron sources and combinations with NFDM, sucrose, yeast, and CMC (not shown in the tables). Apparently, any increase in soluble iron equilibrates between Fe^{+2} and Fe^{+3} ions, as most of these components also increased Fe^{+2} ions, as discussed earlier. The high levels of both NaCl and sucrose resulted in a decrease of Fe^{+3} ions, indicating that these components prevented the oxidation of Fe^{+2} to Fe^{+3} under acidic conditions of the medium.

The data regarding effects of various ingredients on soluble iron under simulated duodenum conditions are presented in Tables IV and V. The only difference between the two sets of data is in the technique of measuring soluble iron. The data in Table IV was obtained by the modified method of Kadan and Ziegler (1984). Beside the three iron sources, two commonly used bread ingredients, i.e., high levels of NaCl and yeast, were found to significantly affect the soluble iron. Even though the *P* values for high yeast levels are slightly above the 0.05 level, the data were included because in subsequent controlled studies this effect was confirmed (Table VI). The high level of NaCl decreased, whereas the high level of yeast increased the soluble iron. As expected (Milazzo and Caroli 1978), nearly all the soluble iron was in the Fe^{+3} form in the alkaline medium of duodenum. The Fe^{+2} ions were not significantly affected by any of the bread components, and the ingredients affecting the soluble iron had a similar effect on Fe^{+3} ions. Surprisingly, no significant interactions between the ingredients were observed. The Miller et al (1981) method, on the other hand, did not detect the effect of high levels of NaCl and yeast by themselves (Table V). However, the two ingredients were effective in bringing about the same result in the presence of NFDM (not shown). It should be mentioned that the high levels of both NaCl and yeast were slightly higher than normal levels used in white bread. This was done to accentuate the effect of these ingredients. Additional experiments (Table VI) have confirmed the effects of NaCl and yeast levels under simulated duodenum conditions.

No suitable explanation is available in the literature to explain the mechanism of action of increased levels of NaCl and yeast on soluble iron under simulated duodenum conditions and hence on iron bioavailability. Limited observations have indicated that increased yeast levels resulted in progressive increases in loaf volume without significantly affecting the final pH of baked bread. Increased NaCl levels were found to increase the brown color of the product. It is probable that increased yeast activity resulted in accumulation of higher amounts of organic acids, which in turn resulted in increased soluble iron by reducing or sequestering action. Increased NaCl levels probably decreased yeast activity. However, the volatiles profiles (Dupuy et al 1977) by gas-liquid chromatography did not show any significant differences between the samples.

NaCl is an important ingredient of nearly all foods (Anonymous 1980). Its effects on color, flavor, texture, and other functional properties are well known. The inhibitory effect of NaCl on soluble iron under simulated duodenum conditions, and apparently on iron bioavailability, is unique and important. This observation, if confirmed by actual animal and human feeding studies, will give better insight for understanding the mechanism of action of iron bioavailability in foods and perhaps add an extra dimension to its role in human health. The effects of yeast levels in bread on soluble iron would also help to explain the causes of widespread anemia among populations consuming unleavened wheat products in many of the mideastern and far eastern countries.

The results of this study indicate the complex role of various ingredients on iron bioavailability in processed foods. Research approaches such as a fractional factorial experimental plan and appropriate in vitro and subsequent in vivo methods to measure iron bioavailability provide a practical means for identifying ingredients or processing techniques that can potentially affect iron bioavailability.

TABLE IV
Ingredients Affecting the Soluble Iron in Bread Under Simulated Duodenum Conditions

Iron Form	Ingredients	Effect	P^a
Soluble (total) iron	High salt[b]	Decreased	0.0001
	High yeast[c]	Increased	0.0552
	High $FeSO_4$[d]	Increased	0.0154
	High EI[e]	Increased	0.0046
	High FSPP[f]	Increased	0.0004
Fe^{+3} ions in soluble iron	High salt	Decreased	0.0002
	High yeast	Increased	0.0573
	High $FeSO_4$	Increased	0.0109
	High EI	Increased	0.0106
	High FSPP	Increased	0.0002

[a] $P<0.05$ is significant at the 5% level.
[b] High salt = 8.0 g.
[c] High yeast = 4.0 g.
[d] High $FeSO_4$ = 0.0448 g ferrous sulfate.
[e] High EI = 0.009 g electrolytic iron.
[f] High FSPP = 0.75 g ferric sodium pyrophosphate.

TABLE V
Ingredients Affecting the Soluble Iron in Bread Under Duodenum Conditions Using the Method of Miller et al (1981)

Iron Form	Ingredients	Effect	P^a
Soluble iron	High $FeSO_4$[b]	Increased	0.0024
	High EI[c]	Increased	0.0140
	High FSPP[d]	Increased	0.0009

[a] $P<0.05$ is significant at the 5% level.
[b] High $FeSO_4$ = 0.0448 g ferrous sulfate.
[c] High EI = 0.009 g electrolytic iron.
[d] High FSPP = 0.75 g ferric sodium pyrophosphate.

TABLE VI
Mean Values of Percent Iron Bioavailability of Experimental White Bread Made from Enriched Wheat Flour[a] Using the Method of Miller et al (1981)

	Common Salt (%)			
	0	1	2	4
Yeast, %				
1	10.4	15.2	12.6	10.1
2	18.0	15.4	12.6	9.7

[a] Standard error for all cell means is ±0.273. The effect of salt, yeast and salt, and yeast is significant at $P<0.01$.

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