

SELECTION OF IRON SOURCES FOR CEREAL ENRICHMENT¹

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ABSTRACT

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Most cereal enrichment is accomplished with ferrous sulfate, reduced iron, ferric orthophosphate, or sodium iron pyrophosphate. Selection must be based on bioavailability, technological feasibility, and cost. Ferrous sulfate is the standard against which other iron sources should be compared for bioavailability. Great variation was found among samples of reduced iron and ferric orthophosphate. A sample of electrolytic iron was separated into fractions 7–10 μm and 27–40 μm . Relative biological value (RBV) of the finer was 68–75; that of the coarser was

27–29. Five samples of ferric orthophosphate had RBVs of 6–46. RBV was positively correlated with solubility in 0.1N HCl and negatively with particle size. Eighteen samples of unbleached white flour were enriched with different sources and levels of iron and stored in sealed containers. An untrained panel detected rancidity by smell in samples enriched with ferrous sulfate after 4 days at 50°C and after 11 to 28 days in samples with reduced iron. All samples stored at a constant temperature of $23^\circ \pm 3^\circ\text{C}$ were acceptable after 24 months' storage.

The history of cereal enrichment and recommendations for future policy were recently reviewed by a Committee of the Food and Nutrition Board of the National Academy of Sciences (1). The enrichment program was helpful in reducing the prevalence of vitamin-deficiency diseases, but had little effect on the prevalence of iron-deficiency anemia (2). One problem was the use of iron sources that were poorly utilized. Because information was lacking, the FDA regulations specified that iron could be added only in "forms which are harmless and assimilable" without defining assimilable (3). Later regulations used the term "safe and suitable" without defining suitable in terms of bioavailability (4).

Although a number of other iron sources are generally recognized as safe (5) or permitted by regulation (6), most cereal enrichment is accomplished with four iron sources: ferrous sulfate, reduced iron, ferric orthophosphate, and sodium iron pyrophosphate (7). An industry committee concluded that no single source was ideally suited for enrichment of all flour and flour products (8). Compromises are obviously necessary. These must include consideration of bioavailability, technological feasibility, and cost.

The bioavailability of iron sources used for enrichment and the methods of testing were reviewed by Waddell (7). Parallel tests with man and with the laboratory rat placed the common iron sources in the same order of biological availability although the numerical values did differ (9,10).

The purpose of this study was to investigate the variability between different lots of named iron sources, with consideration directed to bioavailability of the iron and its effect on the flour to which it is added. Meaningful specifications are needed.

MATERIALS AND METHODS

Bioavailability was measured by the rat hemoglobin repletion test (11,12). The

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animals are made anemic on a low-iron diet. They are then given the test materials for a 2-week repletion period. The final hemoglobin values are used as the criterion of response to the supplemental iron. Ferrous sulfate ($\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$) is used as the reference standard and relative biological value is calculated by the formula:

$$\text{RBV} = 100 \times \frac{\text{mg Fe/kg from FeSO}_4}{\text{mg Fe/kg from sample}}$$

to give equal curative effect.

An important consideration is that the iron supplement is fed as part of the diet. This minimizes the effect of other diet components on the absorption of the iron, and it makes little difference if the iron supplement is added directly to the test diet or if it is added as a component of a food (13,14).

A sample of electrolytic iron was separated into seven fractions by nitrogen elutriation in a particle segregation apparatus, the Infracizer (15). Two of the fractions, designated as 7–10 μm and 27–40 μm , were used in this study². The sample particle sizes were verified by microscopic examination.

The wide variability in relative biological value (RBV) of foods fortified with ferric orthophosphate (14) prompted a search for differences between samples of ferric orthophosphate. Five samples were obtained. These were examined microscopically to estimate particle size, tested for solubility in 0.1*N* HCl (13), and fed to rats in a bioavailability test (12).

A 45.4-kg bag of unbleached and unenriched all-purpose family flour was purchased from a local mill. This flour contained 8 mg iron per kg. Eighteen samples were removed and mixed with six iron sources to furnish 100, 200, or 300 mg iron per kg flour. The iron sources used included reagent grade ferrous sulfate ($\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$), hard fat-coated ferrous sulfate, samples of 325-mesh iron powders prepared by electrolytic or hydrogen reduction methods, 100-mesh iron powder reduced by hydrogen, and a carbonyl iron powder with 95% of the particles less than 10 μm . The iron powders were commercial products. Particle size information was taken from label descriptions and verified by microscopic examination.

Portions of each flour sample were placed in glass jars with screw caps and stored in a laboratory oven at 50° C. Other portions of the samples were stored at room temperature in plastic bags. The room temperature was 23° ± 3° C.

An untrained panel of 20 volunteers was asked to examine the samples after various periods of storage and to judge each sample by smell according to the following scale: 1 = good, pleasant odor; 2 = acceptable; 3 = slightly rancid; 4 = very rancid; 5 = bad, unpleasant odor.

The containers were opened at the top with minimal disturbance to the contents, and the panel members were asked to smell the samples without handling the opened containers. The panel was also asked to examine the flour samples for any differences in color. The samples were examined under daylight

²I. Motzok, University of Guelph, obtained a sample of 325-mesh electrolytic iron from a food manufacturer, and arranged to have this sample separated by nitrogen elutriation into fractions of known particle size distribution. The Infracizer, described by H. E. T. Haultain in reference 15, was used. Particle size was further confirmed by microscopic examination using an ocular with a grid. The iron samples 7–10 μm and 27–40 μm were furnished by Motzok for a collaborative study.

fluorescent lighting. The samples were coded and the panel members did not know the identity or level of iron enrichment when judging the samples for odor and color. After judging for odor was completed, the panel members were asked to specifically compare the color of the unenriched flour sample and the color of the flour sample enriched with the highest level of carbonyl iron. For this comparison, small portions of each flour sample were placed side by side on a sheet of white paper.

Bread was baked from each of the flours enriched with the highest levels of the six iron sources (16). The panel members were asked to evaluate the bread samples for appearance, odor, and taste.

RESULTS AND DISCUSSION

The results of two tests on the reduced iron samples of known particle size are summarized in Table I. These data show that particle size has an important influence on bioavailability of this iron source when other factors such as method of preparation are constant. The difference in bioavailability between the samples of 7–10 μm and 27–40 μm was confirmed by a collaborative study in which these samples were included (11). These data show that 325-mesh iron powder is not sufficiently fine to assure optimum bioavailability since a 325-mesh screen can pass a 44 μm particle. It is recognized that surface area or oxide coating may also influence bioavailability (7).

Table II summarizes observations on five commercial samples of ferric orthophosphate. The relative biological value of these samples was highly

TABLE I
Effect of Particle Size on Relative
Biological Value of Electrolytic Iron

Particle Size μm	Relative Biological Value ^a (vs $\text{FeSO}_4 = 100$)	
	1st Test	2nd Test
7–10	68	75 \pm 7
27–40	27	29 \pm 2

^aAverage (1st test) or average \pm standard deviation (2nd test).

TABLE II
Observations on Five Commercial Samples of Ferric Orthophosphate

Estimated Particle Size μm	Solubility in 0.1N HCl %	Relative Biological Value (vs $\text{FeSO}_4 = 100$)
15	11.6	6
12	11.6	7
1	41.9	33
<1	45.5	33
<1	63.4	46

correlated with acid solubility ($r = 0.99$) and negatively correlated with particle size ($r = 0.95$). It is obvious that we need meaningful specifications if ferric orthophosphate is to be considered an acceptable source of supplemental iron.

If bioavailability were the only consideration, the selection of an iron source would be very easy. However, it is also important that the enriched food be acceptable to the consumer. This means that the food additive must have minimal adverse effects on processing and on color, odor, and taste of the food.

The predominantly identified form of iron added by bakers is ferrous sulfate, while the predominantly identified form of iron added when flour is enriched at the mill is reduced iron³. One-fifth of a series of flour samples examined in the FDA compliance program were enriched with ferrous sulfate.

Millers generally fear that ferrous sulfate will cause rancidity during prolonged storage of enriched flour. Several industry spokesmen said they wanted a storage life of 18 months. White, short extraction flour may contain up to 1% residual fat, and this fat may become rancid during the storage period, especially if ferrous sulfate or other prooxidants are present. Encapsulated products and a "stabilized" form of ferrous sulfate (17) are potentially available as aids in eliminating this objection to ferrous sulfate. The results of a study on the effect of different sources and levels of supplemental iron on stability of flour are summarized in Tables III and IV.

³Information about iron sources used for enrichment and compliance data were obtained in the course of FDA surveillance programs.

TABLE III
Average Length of Storage Period at 50°C that
Enriched Flour Samples Remained Acceptable

Iron source	Iron Enrichment of Flour		Maximum Acceptable Storage Period ^a days
	mg Fe/kg		
None	0		28+
Reagent grade FeSO ₄ ·7 H ₂ O	100		8
	200		4
	300		7
	300		2
Encapsulated FeSO ₄	200		4
	300		<2
	300		25
325-mesh electrolytic iron	100		25
	200		25
	300		18
325-mesh H ₂ -reduced iron	100		21
	200		16
	300		11
100-mesh H ₂ -reduced iron	100		25
	200		28+
	300		18
Carbonyl iron	100		20
	200		28
	300		25

^aFlour samples were scored for odor according to the scale: 1 = good, pleasant odor; 2 = acceptable; 3 = slightly rancid; 4 = very rancid; 5 = bad, unpleasant odor. An average score of 2.0 or less was considered to indicate an acceptable sample.

Data from an accelerated stability test, with the samples stored in an oven at 50°C, are presented in Table III. In this accelerated stability test, the acceptable shelf-life of flour enriched with unprotected ferrous sulfate did not exceed 8 days and some samples were rancid after 4 days. The hard fat coating of the encapsulated ferrous sulfate was broken by the heat and a bad odor developed very quickly. This adverse effect was confirmed in another study which also included ferrous sulfate coated with other materials or protected by the stabilization process (17). All samples enriched with nonfat coated ferrous sulfate or stabilized ferrous sulfate showed improved stability, with shelf-life of 18 to 21 days at 50°C. The flour samples enriched with reduced iron showed better shelf-life, with some samples considered acceptable after 28 days at 50°C. Samples were considered acceptable only as long as the average odor score did not exceed 2 on the scale described above.

Very different results were obtained with the enriched flour samples stored in plastic bags at room temperature (23° ± 3°C). No serious rancidity was detected during 2 years' storage and all samples were considered acceptable by the panel at the end of this test period. Average scores by the panel at the end of the 2-year storage period are shown in Table IV. These results demonstrate that ferrous sulfate can be satisfactorily used to enrich flour provided the enriched flour is then stored at a constant temperature of not more than 26°C.

Because of the density of reduced iron, questions were raised about possible settling during handling and storage. A field study by Fortmann *et al.* (18) showed that no such problem was encountered. The FDA surveillance program³ supported this view since all enriched flour samples were within the acceptable

TABLE IV
Stability of Enriched Flour Stored at Room Temperature

Iron Enrichment of Flour		Average Odor Scores ^a
Iron source	mg Fe/kg	after 24 months at 23 ± 3°C
None	0	2.1
Reagent grade FeSO ₄ ·7 H ₂ O	100	1.6
	200	2.2
	300	2.2
Encapsulated FeSO ₄	100	2.1
	200	2.2
	300	2.2
325-mesh electrolytic iron	100	1.9
	200	2.1
	300	2.0
325-mesh H ₂ -reduced iron	100	2.0
	200	1.8
	300	2.1
100-mesh H ₂ -reduced iron	100	2.0
	200	2.1
	300	2.0
Carbonyl iron	100	1.8
	200	1.7
	300	2.0

^aSee legend for Table III.

range, although a few samples of enriched bread contained less than the minimum required iron level.

Another cause for concern is the possible effect of the iron on color of the product. It may not be feasible to fortify some foods with any available iron source (19). Some millers oppose use of finely powdered reduced iron in the belief that such material will produce a gray color in the enriched flour and in enriched bread made from that flour. Discoloration of flour following addition of finely powdered carbonyl iron could be detected by the Pekar "slick" test (20), but the consumer panel could not detect any off-color even when directed to examine carefully the flour which contained 300 mg carbonyl iron per kg.

The panel members were asked to examine bread baked from each of the flours enriched with the highest levels of the six iron sources. Although no scoring system was used, the panel members could not detect obvious differences in appearance, odor, or taste of these breads. Each sample was considered acceptable. Data on bioavailability of iron in these breads have been reported elsewhere (13), and generally agreed closely with the relative biological values obtained on the iron sources used for enrichment.

Some bakeries, using the continuous process, have reported problems with bread quality and loaf volume when they used ferrous sulfate at a level to produce bread with 55 mg iron per kg. This is believed due to reaction between bromate and ferrous sulfate (21,22). Increasing oxidizing capacity, and properly scheduling the addition of bromate and ferrous sulfate, were reported to overcome the adverse effects of the higher level of ferrous sulfate.

RECOMMENDATIONS

Only iron sources with good bioavailability should be used in the cereal enrichment program. For enrichment of bread at the bakery, ferrous sulfate is the preferred iron source because of its bioavailability. Other water-soluble iron salts are equally satisfactory, but usually are more expensive (8).

For enrichment of flour, ferrous sulfate is recommended where storage conditions will permit its use without adverse effects on acceptability of the enriched flour. The authors recommend that bioavailability of other iron sources should be at least 50 (vs $\text{FeSO}_4 = 100$) by the AOAC test (12). Many lots of reduced iron with most of the material in particles of less than 10 μm will have satisfactory bioavailability. Some lots of ferric orthophosphate approach this degree of availability. Particle size distribution and solubility in 0.1N HCl may help in the selection of iron sources with satisfactory bioavailability.

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