Preventing Iron Deficiency through Food Fortification

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Preventing Iron Deficiency Through Food Fortification

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One way to prevent iron deficiency anemia in developing countries is through the fortification of food products with iron. In addition to avoiding undesirable color and flavor changes, the main challenge is to protect the fortification iron from potential inhibitors of iron absorption present in commonly fortified foods.

Introduction

There is clear evidence of a high prevalence of iron deficiency anemia in developing countries and, to a lesser extent, in the more industrialized countries of the world. Most critically affected are infants, school-age children, and women of reproductive age. Approximately 50% of these populations suffer from anemia in the less-developed countries of South Asia and Africa, compared with about 25% in Latin America and approximately 10% in the industrialized countries of Europe.[1] In addition to the deleterious physiologic consequences of iron deficiency in individuals, the resulting public health consequences in developing countries can significantly impact economies in the form of health costs, wasted educational resources, and lost productivity.

Before considering an intervention strategy to prevent iron deficiency, its etiology must be understood. This is more complex in developing countries than in industrialized countries where the consumption of insufficient absorbable iron is usually the only cause or may be the major factor causing iron deficiency.[2] In developing countries, other possible causes are intestinal worm infections, malaria, and vitamin A deficiency.[3,4] The major causative factor in developing countries is not low iron intake, but, rather, low iron absorption. Iron intake is often relatively high, almost 20 mg/day,[3] and would easily meet the recommended dietary allowances for the United States (10–15 mg/day).[5] Unfortunately, much of the ingested iron is poorly bioavailable iron from plant sources or is contamination iron from soil and includes little bioavailable iron from animal tissues. Major cereals, legumes, and staple foods contain high levels of phytic acid, which is a potent inhibitor of iron absorption,[6,7] and some, such as sorghum, also contain phenolic compounds, which greatly impede iron absorption[8] by binding iron in the gut in unabsorbable complexes. The intake of foods that enhance iron absorption such as fruits and vegetables containing vitamin C[9] or muscle tissue[10] is often limited.

The fortification of foods is often regarded as the most cost-effective long-term approach to reducing the prevalence of iron deficiency.[11,12] This can be in the form of "mass medication" by fortifying foods such as cereals, milk, salt, and condiments that are widely consumed by both at-risk populations and others who have little or no need for extra iron. Alternatively, a targeted fortification program in which a food product preferentially consumed by one of the at-risk groups is fortified can be considered.

Although targeted fortification is relatively easy to design for infant foods such as formulas and commercial infant cereals, or for schoolchildren through school feeding programs including such foods as fortified drinks or cookies, it is more difficult to target a fortified food specifically for adult fertile women. For this group, the fortification of a widely consumed product would seem the best way to provide extra food iron, but other groups such as adult men and postmenopausal women, who do not require extra iron, will also consume the fortified food. In industrialized countries, there is concern that this excess iron may be detrimental and lead to increased incidence of atherosclerosis[13] and cancer[14] owing to increased oxidative stress.

In developing countries, however, where a lower intake of bioavailable iron occurs, these considerations might not apply. The prevalence of anemia in adult men has been reported to range from 2% in Europe and 4% in North America, to 13% in Latin America, 20% in Africa, and 32% in South Asia.[1]

Although widespread iron deficiency has been recognized for more than 50 years, intervention strategies including food fortification have been met with limited success. The only clear success story has been in industrialized countries, such as the United States and Sweden, where the steady drop in the prevalence of iron deficiency in infants and preschool children over the last 30 years[15] is

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considered to be the result of the consumption of iron-fortified infant formulas.[16] The current low incidence of iron deficiency anemia in fertile U.S. women, reported as 2.9% in the NHANES II survey,[17] could also be due in part to the high consumption of iron-fortified foods in the United States.[12] Fortification of products such as white bread, rolls, crackers, corn flour, corn grits, pasta, and breakfast cereals is widespread, and fortified iron from these products represented about 20% of the total iron intake as determined in NHANES II.[18,19]

Although pilot fortification trials in developing countries have given promising results, there are as yet no major success stories except perhaps for Chile.[20,21] This could be due to lack of political commitment, insufficient funding, too little technical support from local or multinational industries, poor distribution networks, or lack of nutrition education programs for the consumer, all of which are considered necessary for a successful fortification program.[22] Another reason could be that iron deficiency is due to factors other than insufficient absorbable iron. Hookworm infections, malaria, and vitamin A deficiency should be addressed simultaneously in any food fortification strategy. However, if low bioavailability of food iron is the major determinant of iron deficiency anemia in developing countries,[23] increasing the supply of absorbable food iron should decrease the prevalence of iron deficiency anemia.

This necessitates the careful selection of both the food product to be fortified and the iron fortification compound to be added. Clearly, the iron compound must be first optimized with respect to relative bioavailability.[24] However, if the food vehicle contains potent inhibitors of absorption, the added iron, like the native iron, will be poorly absorbed and will have little or no impact on the iron status of the consumer. The success of a food fortification program thus depends heavily on the absorbability of the added iron and its protection from major dietary absorption inhibitors.

This review focuses on the technical aspects governing the choice of food vehicle and iron compound with the aim of ensuring an adequate absorption of fortification iron. The optimization of the iron compound in relation to bioavailability and organoleptic problems is discussed first, followed by a description of methods that can be used to protect fortification iron from absorption inhibitors. These include the addition of ascorbic acid, the use of hemoglobin or dried blood, and the use of NaFeEDTA. Finally, the major foodstuffs that are used as iron fortification vehicles are discussed in relation to potential organoleptic problems, the presence of absorption inhibitors, and possible fortification compounds.

Optimization of the Iron Compound

Some characteristics of commonly used iron compounds are shown in Table 1.[12,24,25] They can be conveniently divided into four groups: (1) those that are freely water-soluble; (2) those that are poorly water soluble but soluble in dilute acids such as gastric juice; (3) those that are water

Table 1. Characteristics of Iron Sources Commonly Used to Fortify Food (adapted from Hurrell 1985, 1992; Bothwell & McPhail 1992)

	Approximate Fe content (%)	Average relative bioavailability		Approximate relative cost[a]
		Rat	Man	
Freely water soluble				
Ferrous sulfate $7H_2O$	20	100	100	1.0
Dried ferrous sulfate	33	100	100	0.7
Ferrous gluconate	12	97	89	5.1
Ferrous lactate	19	—	106	4.1
Ferric ammonium citrate	18	107	—	2.1
Poorly water soluble/soluble in dilute acid				
Ferrous fumarate	33	95	100	1.3
Ferrous succinate	35	119	92	4.1
Ferric saccharate	10	92	74	5.2
Water-insoluble/poorly soluble in dilute acid				
Ferric orthophosphate	28	6–46	25–32	4.1
Ferric ammonium orthophosphate (EKA Nobel, Sweden)	19	—	30–60	—
Ferric pyrophosphate	25	45–58	21–74	2.3
Elemental Fe powders: electrolytic	98	44–48	5–100	0.5
carbonyl	98	39–66	5–20	1.0
reduced	97	24–54	13–148	0.2
Protected compounds				
NaFe EDTA	14	—	28–416	6.0
Hemoglobin	0.34	—	100–700	—

Adapted from references 10, 12, and 25.

[a]Relative to ferrous sulfate $7H_2O$ = 1.0, for the same level of total iron.

insoluble but poorly soluble in dilute acid; and (4) protected iron compounds. The table gives guideline values for relative bioavailability in rat and man and a relative cost factor. A more detailed description of these and other compounds can be found in reference 25.

The cost of the more recent or experimental compounds such as NaFeEDTA, ferric ammonium orthophosphate,[26] and hemoglobin depends to some extent on the amounts ordered. In general, the freely water-soluble compounds are highly bioavailable in rodents and humans, as are compounds that are water insoluble but soluble in dilute acids. Compounds that are poorly soluble in dilute acid, however, have only a low to moderate bioavailability. This is because of variable dissolution in gastric juice owing to both the characteristics of the compound itself[27] and the meal composition.[28] Although it would be logical to always use iron compounds of highest bioavailability, they unfortunately often cause unacceptable color and flavor changes in many foods. Optimization, therefore, means selecting the iron compound with the highest potential absorption without causing subsequent organoleptic problems in the food vehicle.

Bioavailability

The absorption of fortification iron depends primarily on its solubility in gastric juice. Water-soluble compounds such as ferrous sulfate dissolve instantaneously in gastric juice, whereas more insoluble compounds, such as elemental iron, rarely dissolve completely. Once dissolved, fortification iron enters the common pool, where its absorption (like that of all pool iron) depends on the content of enhancing or inhibitory ligands in the meal and on the iron status of the subject. For example, phytate and polyphenols or a satisfactory iron status in an individual will diminish absorption, whereas vitamin C or low iron status will enhance absorption.

Because iron status and various food components may markedly affect iron absorption, the absorption of a single iron compound can vary from less than 1% to almost 100%. Therefore, when comparing different iron compounds, one must measure the bioavailability relative to a standard compound. The standard is usually ferrous sulfate, which has been designated as having a relative bioavailability (RBV) of 100. It has recently been demonstrated that the hemoglobin repletion test in rodents and the measurement of dialyzable iron in vitro are good predictors of iron bioavailability in humans.[29] The RBV of many commercial iron compounds is well known (Table 1[12,24,25]). New compounds can be screened by animal or in vitro assays, although human studies are ultimately necessary.

Compounds labeled with radioactive or stable isotopes can be prepared and used as confirmation for the more soluble compounds. For those compounds that are poorly soluble in dilute acids, however, such as phosphate and elemental iron powders, one is never absolutely sure that the labeled experimental compound made on a small scale has exactly the same physiochemical characteristics as the commercial compound.[29] The best confirmation of the utility of these compounds is intervention studies monitoring iron status.[30]

Organoleptic Problems

In addition to causing unacceptable changes in color and flavor when added to foods, iron compounds may also provoke precipitation, such as when added to fish sauce[31] or when iron-fortified sugar is added to tea.[32] Many iron compounds are colored and cannot be used to fortify light-colored foods. In addition, the more soluble iron compounds often react with substances in foods, causing discoloration. Infant cereals have been found to turn gray or green on addition of ferrous sulfate and dark blue if bananas are present.[27] Phenolic compounds have often been implicated, and Douglas et al.[33] reported that ferrous sulfate, ferrous lactate, ferrous gluconate, and ferric ammonium citrate, as well as the less soluble ferrous fumarate and ferric citrate, produce off-colors when added to a chocolate milk drink. Similarly, salt fortified with ferrous sulfate or other soluble iron compounds becomes yellow or brown.[34]

Off-flavor can also result from the metallic taste of the soluble iron itself, particularly in beverages. However, the catalytic effect of iron on fat oxidation in cereals during storage is the major problem. As in the case of product discoloration, the water-soluble compounds, such as ferrous sulfate, promote fat oxidation and reduce product shelf life. A convenient method to measure the potential of iron fortification compounds to promote fat oxidation in cereals is to measure pentane formation in the headspace of sealed cans containing the iron-fortified product.[35]

Pentane is the major hydrocarbon formed by the oxidative degradation of linoleic acid, and its formation correlates with the production of off-flavors. Figure 1 shows the rate of pentane formation during storage at 37 °C of a precooked whole wheat flour containing various iron salts (at a concentration of 15 mg iron per 100 g flour).[27] Ferrous sulfate and ferrous gluconate rapidly generated pentane and were judged unacceptable by a taste panel after 4 to 6 weeks of storage. Ferric pyrophosphate and reduced elemental iron generated far less pentane and were still organoleptically acceptable after 7 weeks of storage. A similar oxidative rancidity can occur in milk products when iron is added.[36,37]

Freely Water-Soluble Compounds

Freely water-soluble compounds are the most bioavailable iron compounds, but also the most likely to promote unacceptable color and flavor changes. They are essential in liquid products, and there is often little difference between the compounds with respect to bioavailability, flavor, and organoleptic problems. Ferrous sulfate is the least expen-

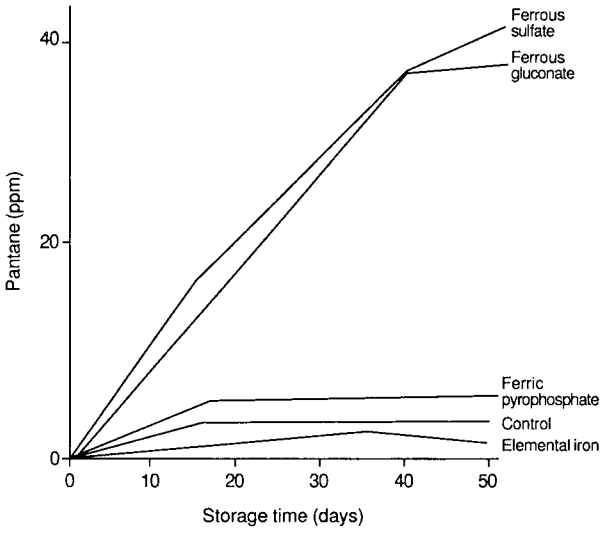

Figure 1. Pentane formation in stored wheat flour fortified with different iron compounds (adapted from reference 27).

sive compound and is widely used to fortify infant formulas and pasta and cereal flour that are stored for only short periods. Other possibilities are ferrous gluconate, ferrous lactate, and ferric ammonium citrate. Although there is no evidence that soluble ferric salts are absorbed to a lesser extent than soluble ferrous salts when iron is in an ionized form,[38] it is possible that ferric iron binds more strongly with inhibitors of absorption such as phytic acid and polyphenols.

Compounds Soluble in Dilute Acid

Recently, several compounds that are poorly soluble in water but readily soluble in dilute acids have been identified. These compounds are ferrous fumarate, ferrous succinate, and ferric saccharate. Their advantage is that they cause far fewer organoleptic problems than freely water-soluble compounds and still readily enter the common iron pool during digestion. They have been suggested for use in infant cereals[35] and chocolate drink powders.[39]

Studies have been conducted in which adult human subjects were fed a chocolate drink or an infant cereal fortified with ^{55}Fe-radiolabeled test compounds or ^{59}Fe-radiolabeled ferrous sulfate.[35,39] The chocolate drink contained 5 mg iron and 25 mg vitamin C per serving, and the infant cereal contained 7.5 mg iron and 35 mg vitamin C per serving. Absolute absorption from the ferrous sulfate control meals varied from 3% to 6%. The absorption from ferrous fumarate and ferrous succinate was at least as good if not better than from ferrous sulfate. Absorption from ferrous fumarate was twice as high as from ferrous sulfate in the chocolate milk drink, and the iron compound may have undergone some reactions during the manufacture of the chocolate drink powder, which included a vacuum drying stage. In the infant cereal, ferrous fumarate was dry-mixed into the product after processing and had an absorption equivalent to the ferrous sulfate. Ferric saccharate had a variable but moderate absorption (RBV 39–74), and ferric pyrophosphate had a variable but low absorption (RBV 20–39). It would seem that these iron compounds are less soluble in gastric juice in the presence of chocolate milk drink than in the presence of infant cereal, because the lowest absorption values were from the chocolate milk drink. Ferric pyrophosphate and ferric saccharate caused no organoleptic problems in either product. In the chocolate drink, ferrous succinate was satisfactory, but ferrous fumarate caused a color loss if the product was made with boiling water. Similarly, ferrous fumarate and ferrous succinate were organoleptically satisfactory when added to simple infant cereals, but color problems occurred in more acid fruit varieties.

Compounds Poorly Soluble in Dilute Acids

Compounds that are poorly soluble in dilute acids include ferric pyrophosphate, ferric orthophosphate, ferric ammonium orthophosphate, and the elemental iron powders made by carbonyl, electrolytic, or reduction techniques.[24,40] They are the most often-used compounds in food fortification and their main advantage is that they cause no organoleptic problems. Their disadvantage is that they have a variable absorption because they do not readily dissolve in gastric juice. Animal studies indicate that current commercial compounds are about half as well absorbed as ferrous sulfate.[35] Human studies, however, have given variable and conflicting results (Table 1[12,24,25]). This is either because the compounds tested had different physiochemical characteristics from the commercial compounds[29,41,42] or because of the influence of different meals on the dissolution of the iron compound in gastric juice. Hallberg et al.,[28] for instance, found that the RBV in humans of the same carbonyl iron powder varied from 5 to 20 and the RBV of ferric ammonium orthophosphate varied from 30 to60[28] simply because of the composition of the meal with which they were fed. When carbonyl iron is consumed without a meal in pharmacologic (100 mg) doses, it is reported to have a relative bioavailability in humans of about 70% that of ferrous sulfate.[43]

It seems probable that the low levels of elemental iron (40 mg/kg) added to wheat flour would have little impact on iron nutrition, but the much higher levels added to commercial infant cereals (200–550 mg/kg) together with vitamin C could contribute substantially to the prevention of iron deficiency anemia.

Encapsulated Iron Compounds

Both ferrous sulfate and ferrous fumarate are available commercially in encapsulated form. Commonly, the coatings are partially hydrogenated oils, such as soybean and cottonseed, or ethyl cellulose. The coating has little influence

on the RBV as measured in rodent assays[25] and can prevent fat oxidation changes during storage of cereals or in infant formulas fortified with the easily oxidizable long-chain polyunsaturated fatty acids. Most coatings are heat labile, however, and at temperatures above 50–70 °C often do not prevent unwanted color reactions. Zinc stearate is the only coating proposed that has a high melting point (122 °C), and its bioavailability in rodent assays was reported to be 70% that of ferrous sulfate.[44]

Protecting and Enhancing the Absorption of Fortification Iron

Many food vehicles for iron fortification contain substances that inhibit iron absorption. Cereals contain phytic acid and occasionally polyphenols, milk contains calcium and casein, and chocolate drinks contain polyphenols. In addition, many diets in developing countries to which fortified salt, sugar, or other condiments are added are often high in phytate and polyphenols from cereal and legume foods. To ensure a level of absorption that is high enough to improve or maintain iron status, it is necessary to prevent the fortification iron from reacting with the absorption inhibitors. This can be accomplished by adding absorption enhancers. The most common enhancer is vitamin C. Alternatives would be bovine hemoglobin and NaFeEDTA where iron is in a protected form.

Vitamin C

Vitamin C can increase the absorption of both native iron and fortification iron severalfold when added to foods. Its effect appears to be related to both its reducing power and its chelating action. It can reduce ferric to ferrous iron and/or maintain ferrous iron in the ferrous state and so prevent or decrease the formation of insoluble complexes with absorption inhibitors or with hydroxide ion in the gut. In addition, it can form soluble complexes with iron at low pH that remain soluble and absorbable at the more alkaline duodenal pH. Thus, Layrisse et al.[45] reported a sixfold increase in iron absorption (1.4% to 7.9%) by adult peasants in Venezuela who consumed 100 g maize containing 2.8 mg iron and 70 mg added vitamin C. Similarly, Cook and Monsen[46] reported that iron absorption in young men fed a liquid formula meal containing 4.1 mg iron increased from 0.8% to 7.1% as vitamin C was increased from 25 to 1000 mg. More recently, Siegenberg et al.[47] reported that the effect of vitamin C on phytate and polyphenols was dose dependant and that as little as 30 mg vitamin C could completely overcome the effect of phytic acid (58 mg phytate phosphorus) in maize bran added to white bread, whereas >50 mg vitamin C overcame the negative effect of meals containing >100 mg polyphenols added as tannic acid.

Vitamin C increases the absorption of all fortification iron compounds to a similar extent.[29] Derman et al.[48] reported that iron absorption by adult women with low iron stores from infant cereal fortified with ferrous sulfate or ferrous ammonium citrate was only about 1% in the absence of vitamin C, but increased fourfold to 10-fold when vitamin C was added. Similarly, Forbes et al.[29] reported that iron absorption by adult men and women consuming a farina and milk meal containing 3 mg iron as ferrous sulfate, ferric orthophosphate, or electrolytic iron was only 1% to 4% in the absence of vitamin C but increased three- to fourfold in its presence.

In a milk-based infant formula fortified with 15 mg iron as ferrous sulfate per liter, iron absorption by infants was only 3% in the absence of vitamin C but increased to 5% with 100 mg vitamin C per liter and to 8% with 200 mg per liter.[49] The poor iron absorption from the product with no added vitamin C was cited as the reason for the relative ineffectiveness of a field trial conducted with this product,[20] but in subsequent field trials with the product containing 100 mg vitamin C per liter, the prevalence of iron deficiency anemia in children 15 months old was only 5.5% compared with 30% in infants receiving a non-iron-fortified formula.[50]

Hemoglobin

Hemoglobin is a form of food iron that is naturally protected from major inhibitors of iron absorption, such as phytic acid and polyphenols. The iron is contained within the porphyrin-ring of the heme molecule, which is split from the globin moiety during digestion, and is taken up intact into the mucosal cells.[51,52] The iron is released within the mucosal cell by the action of heme oxygenase[53] and is prevented from reacting with the inhibitory and enhancing ligands within the intestinal lumen. Hemoglobin iron, however, is better absorbed than heme iron without the globin and is further enhanced in the presence of muscle tissue.[54,55] The nature of the mechanism is not fully established, but it seems to be related to protein digestion products preventing the polymerization of heme molecules, thus reducing their absorption.[51]

When used as a food additive, hemoglobin is added in the form of dried red blood cells. Its main advantage is that iron absorption is relatively high and predictable. Absorption varies little with the composition of a meal, and although it varies to some extent with the iron status of the subjects,[56] this variation is far less than with nonheme iron. Monsen et al.[57] estimated that heme iron would be 15–35% absorbed depending on the iron stores; it is thus possible that if hemoglobin-fortified products are not targeted specifically to at-risk groups, tissue iron stores will gradually accumulate in iron-replete subjects. The main disadvantage of hemoglobin iron, however, is the very low iron content (0.34%) and its intense red-brown color. In infant cereal, 5 g dried bovine red blood cells per 100 g rice flour was necessary to provide 14 mg Fe/100 g,[58] making the product dark brown. Iron absorption was 14% in 8-month-old infants, and although the globin protein is lacking in isoleucine, it is high in lysine and is reported to

provide a useful amount of additional protein to a mixed diet.[58] Other disadvantages are the technical difficulties of collecting, drying, and storing animal blood and of obtaining animal blood in countries where meat is not widely consumed, as well as religions beliefs that forbid the consumption of blood.

In Latin American countries where the supply of animal blood is plentiful, two field trials demonstrated the potential usefulness of dried red blood cells as a food fortificant. In the first,[59] extruded rice containing 5% bovine hemoglobin concentrate was fed to infants 4 to 12 months old and their iron status was compared with that of infants fed regular solid foods (vegetables and meat). In the control group at 12 months, the prevalence of iron deficiency anemia was 17% compared with only 6% in infants who consumed more than 30 g fortified cereal per day. In a second study,[21] three 10 g wheat flour cookies containing 6% bovine hemoglobin concentrate were fed as part of the Chilean school lunch program over a period of 3 years. In a survey of 1000 participating children, significantly higher serum ferritin and hemoglobin levels were found in children who consumed the fortified cookies than in those who did not. However, the prevalence of anemia in 10- to 16-year-old schoolchildren was surprisingly low, and in girls the prevalence fell from 1.3% to 0.5%, compared with a fall from 0.8% to 0.4% in boys. The authors concluded that the program would have had a larger impact on iron status in regions where the prevalence of iron deficiency in schoolchildren is higher.

Sodium Iron EDTA

The use of NaFeEDTA as a food additive has recently been reviewed by the International Nutritional Anemia Consultative Group (INACG)[60] and was strongly recommended as the most suitable iron fortificant for use in developing countries. The provisional acceptance of the compound by the Joint FAO/WHO Expert Committee on Food Additives[61] for use in supervised fortification programs in iron-deficient populations has cleared the way for large-scale fortification trials. Other EDTA-containing compounds, i.e., Na_2EDTA and $CaNa_2EDTA$, are widely used in manufactured foods in industrialized countries as protection against metal-induced organoleptic changes. The EDTA molecule forms FeEDTA in the intestinal tract,[60] so that combinations of Na_2EDTA and ferrous sulfate or other iron compounds can also be considered for fortification purposes.

Chemistry. EDTA (ethylene diamine tetraacetic acid) is a hexadentate chelate binding through its four negatively charged carboxylic acid groups and two amine groups. It can combine with virtually every metal in the periodic table. Its effectiveness as a chelate depends on the stability constant between EDTA and the metal. This is influenced by pH and molar ratio, and any metal capable of forming a stronger complex with EDTA will at least partially displace another. Of the nutritionally important metals, Fe^{3+} has the highest stability constant log k of 25.1, followed by copper (Cu) at 18.4, zinc (Zn) at 16.1, Fe^{2+} at 14.6, calcium (Ca) at 10.7, magnesium (Mg) at 8.7, and sodium (Na) at 1.7. The less desirable metals such as mercury (Hg, 20.4), lead (Pb, 17.6), and aluminum (Al, 15.5) and perhaps manganese (Mn, 13.5) also have fairly high stability constants. The situation is somewhat complicated by having an optimum pH for complex formation between 1 and 10. The optimum pH for complex formation between Fe^{3+} and EDTA is pH 1, Cu is 3, Zn is 4, Fe^{2+} is 5, Ca is 7.5, and Mg is 10.[62]

Based on the pH optima, the predicted effect in the intestine of NaFeEDTA and $CaNa_2EDTA$ in food would be as follows. In the stomach, Fe^{3+} from NaFeEDTA would remain firmly bound to EDTA, whereas Ca and Na from $CaNa_2EDTA$ would dissociate and EDTA would bind Fe from the common pool. So even with the addition of $CaNa_2EDTA$, iron EDTA would form in the stomach. In the duodenum, the iron would be released and absorbed[63] and the EDTA would presumably bind in succession to Cu (pH 3), Zn (pH 4), and Fe^{2+} (pH 5), but most of the metals are released for absorption as <5% of the metal–EDTA complexes are absorbed (<1% FeEDTA)[64] and excreted directly in the urine. More than 95% of the EDTA molecule is excreted in the stool. Theoretically, in the ileum and colon, it could bind to Ca, which has a pH optimum of 7.5 for complex formation. Mg, with a low stability constant and a high pH optimum of 10.5, probably would not react.

Absorption of Iron from NaFeEDTA. The major advantage of NaFeEDTA over other iron fortification compounds is that it prevents iron from binding with the phytic acid present in many cereal and legume grains. Thus, in cereal foods or meals containing a considerable quantity of phytic acid, the absorption of iron from NaFeEDTA is two- to threefold that from ferrous sulfate. With less inhibitory foods, such as potato, there is little difference between the iron absorption from the two iron compounds. With neutral foods, such as sugar cane syrup, consumed on their own, iron absorption when fortified with NaFeEDTA was only 30% of that from ferrous sulfate (for detailed review see reference 60).

In a way similar to vitamin C, Na_2EDTA could be considered an absorption enhancer. It has the added advantage of being stable during processing and storage. It must, however, be added at an equivalent or slightly lower molar ratio to iron in the meal. El-Guindi et al.[65] added equimolar quantities of ferrous sulfate and Na_2EDTA to Egyptian *baladi* bread and increased iron absorption from 2.1% to 5.3%. Earlier work suggested that increasing the ratio of Na_2EDTA to iron is associated with a progressive reduction in iron absorption.[66] MacPhail and Bothwell[60] recently reported that adding Na_2EDTA to a ferrous sulfate–fortified rice meal significantly increased absorption at EDTA-to-iron ratios of 1:4 to 1:1, with a maximum absorption at

1:2. EDTA-to-iron ratios of 2:1 to 4:1 did not significantly increase or decrease iron absorption.

Possible Reactions of EDTA with Other Dietary Minerals. Considering the possible impact of EDTA from NaFeEDTA (10 mg iron per day) on the nutritional status of other minerals assumed to be in the diet at levels equivalent to their RDAs, it can be calculated that on a molar ratio basis there are 50 times more magnesium and 80 times more calcium than EDTA, so there would be no likely impact of EDTA on magnesium or calcium metabolism. With copper and zinc, however, there could be a possible effect, since on a molar basis there are eight times more EDTA than copper and equivalent amounts of EDTA and zinc.

We have investigated this effect in both rodents and adult women. In rodents, increasing levels of EDTA in the diet increased zinc absorption and, to a lesser extent, also increased copper absorption but had no effect on calcium absorption.[67] In adult women fed iron-fortified bread rolls, zinc absorption was increased from 20% with ferrous sulfate to 34% with NaFeEDTA, although there was no effect on calcium absorption. Urinary zinc excretion was also increased from 0.3% to 0.6%, but this had little or no effect on overall zinc metabolism.[68] The EDTA molecule from added NaFeEDTA can therefore increase both iron and zinc absorption from meals containing phytic acid. It might also increase the absorption of copper, as well as the potentially toxic elements Pb, Hg, Al, and Mn. However, it would be expected to have no effect on calcium and magnesium absorption.

Intervention Studies. Three intervention studies have been made with NaFeEDTA by Garby and Areekul[69] in Thailand, Viteri et al.[70,71] in Guatemala, and Ballot et al.[72] in South Africa. All were controlled studies, but only the South African study was double blinded. The number of subjects varied from approximately 600 to 17,000 and the study time from 12 to 32 months. None of the food vehicles—fish sauce, sugar, curry powder—contained phytic acid. The amounts of iron provided per day were 4.3 mg in sugar, 7.7 mg in curry powder, and 10–15 mg in fish sauce. All showed a positive effect on iron status. In the fish sauce study, packed cell volume increased in men, women, and children. In the sugar study, even with a fairly low level of fortification and a relatively modest compliance, there was an increase in serum ferritin (iron stores) in all subjects receiving the fortified product but not in subjects receiving the unfortified product. In the curry powder study, there was an increase in red cell hemoglobin levels and serum ferritin in all subjects, and anemia in women fell dramatically from 22% to 5%.

Organoleptic Considerations. Iron combined in NaFeEDTA causes fewer organoleptic problems than other water-soluble iron compounds. It can, however, cause unwanted color changes. We have found it to be unsuitable for the fortification of chocolate drink powders and infant cereals containing banana and other fruits. Viteri et al.[71]

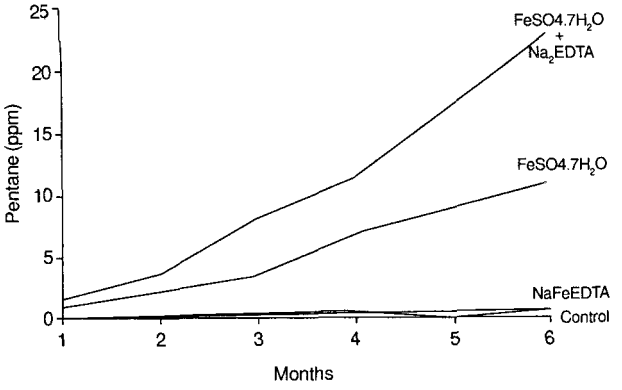

Figure 2. Pentane formation in stored wheat flour fortified with NaFeEDTA.

reported that NaFeEDTA-fortified sugar is slightly yellow in color and, when added to tea, turned the tea black. Similarly, when added to corn starch puddings and gruels, it turned them a pinkish-violet color.

NaFeEDTA does have an advantage, however, when added to stored cereals, because unlike ferrous sulfate, it does not provoke the fat oxidation reactions that lead to rancid, oxidized products. We stored (unpublished results) dry white wheat flour mixed with NaFeEDTA, ferrous sulfate, or ferrous sulfate plus equimolar Na_2EDTA (15 mg Fe/100 g) in closed aluminum cans as described by Hurrell et al.[35] Fat oxidation was quantified by measuring the accumulation of pentane in the headspace. The results (Figure 2) show that stored wheat flour underwent little or no fat oxidation during 6 months storage at 37 °C when unfortified or fortified with NaFeEDTA. In contrast, when the flour was fortified with $FeSO_4$ $7H_2O$ (hepta hydrate), or $FeSO_4$ $7H_2O$ plus Na_2EDTA, lipids in the wheat flour were progressively oxidized during the storage periods and progressively more pentane accumulated in the headspace.

Regulatory Issues and the Current Use of EDTA in Foods. The Joint FAO/WHO Expert Committee on Food Additives (JECFA)[73] permitted the use of $CaNa_2EDTA$ and Na_2EDTA up to 2.5 mg/kg body weight/day with a maximum acceptable daily intake (ADI) set at 150 mg/person/day. The ADI was extrapolated from the rodent study by Oser et al.[74] as the highest no-effect level (250 mg/kg), applying a safety factor of 100. Unfortunately, this study did not include higher levels of EDTA. These compounds are now permitted by local food and drug authorities for use in many countries in Asia, Africa, the Middle East, Europe, and America as a sequestering agent for metals to prevent flavor changes, rancidity, discoloration, turbidity, and texture loss. They are most often added to foods such as mayonnaise, canned vegetables (peas, beans, potatoes), canned fish and shell fish, carbonated beverages, beer, and margarine. In the United States, they are permitted in 34 different foods at levels varying from 33 to 800 mg/kg (Table 2), although the estimated daily intake is only 25

Table 2. Examples of Approved $CaNa_2EDTA$ Use in Foods in the United States

Food Products	Purpose	Amount Permitted (mg/kg)
Lima beans, canned	Retain color	310
Pinto beans, dried	Retain color	800
Cabbage, pickled cucumber	Retain color, flavor texture	200
Carbonated beverages	Retain flavor	33
Crabmeat, clams, shrimps	Retain color	250–340
Egg products	Preservative	200
Margarine	Retain color	75
Mayonnaise	Retain color	75
Mushrooms, canned	Retain color	200
Potatoes, canned	Retain color	110
Sandwich spread	Preservative	200

mg/person/day,[60] 10 times less than the ADI.

Although other regions, such as Malaysia and the Philippines, also allow EDTA in a wide range of foods, the European Union takes a more restrictive view and only allows addition to canned crab, canned shrimp, pickles, canned mushroom, glacé cherries, and sauces. EDTA compounds are currently not allowed in foods consumed by infants and young children.

Present Status of NaFeEDTA. Although NaFeEDTA would appear, at present, to be the most appropriate iron fortificant for use in developing countries, it is still about six times more expensive than ferrous sulfate. However, it is two- to threefold better absorbed than ferrous sulfate, and relatively expensive vitamin C does not need to be added as an absorption enhancer. Additional savings can be made in the packaging material, because less sophisticated packaging can be used for a NaFeEDTA-fortified food than for one fortified with ferrous sulfate (or other iron salts) and vitamin C. The better packaging material must be designed to protect vitamin C from degradation during storage.

However, before general use of NaFeEDTA can be recommended, more systematic studies are necessary to ascertain potential organoleptic problems in a variety of foods. Additionally, its influence on the absorption of the potentially toxic metals (Pb, Hg, Al, Mn) must be investigated and the physiologic importance of any demonstrated influence must be ascertained.

Food Vehicles for Iron Fortification

Cereal Products

Cereal flours are currently the most frequently used vehicles for iron fortification that reach the entire population. The amount of iron added is usually relatively low because it is added only to restore the iron level in milled flour to that of the whole grain. With true fortification, a higher amount than is usually present would be added. Wheat flour enrichment is mandatory in many countries, and the native level in 70% extraction flour (11–12 mg/kg) is enriched up to 44 mg/kg, which is the approximate content of whole-wheat grains. This is the situation in the United States. Other countries add even lower amounts of iron. In Denmark, the enrichment level is 30 mg/kg and in the United Kingdom it is 16.5 mg/kg, as the iron content in white flour is restored to that of 80% extraction flour.

In the United States, corn (maize) meal, corn grits, and pasta products also have federal standards for voluntary iron enrichment, and these commodities are mostly enriched by manufacturers similarly to other baked goods such as crackers, rolls, cookies, and doughnuts but to a lesser extent.[75] The contribution of fortified iron to iron intake is highest in the United States, where it accounts for 20–25% of total iron intake.[76,77] The contribution of fortified iron to iron intake in the United Kingdom is much lower, around 6%.[19]

Technology also exists for fortifying whole grains such as rice. This can be done by coating, infusing, or by using extruded grain analogues. The fortified grains are then mixed 1:100 or 1:200 with the normal grains. Hunnell et al.[78] described a sophisticated method of preparing fortified rice grains by first infusing B vitamins and then adding iron, calcium, and vitamin E in separate layers of coating material. The cost of these procedures together with the difficulty of completely masking the fortified grains is the main reason why no successful programs have been implemented in developing countries. Although iron fortification of rice is mandatory in the Philippines, it has never been enforced.[11]

Other commonly fortified foods are breakfast cereals and infant cereals. In industrialized countries, breakfast cereals can potentially provide a significant amount of iron, particularly to children and adolescents. In the United Kingdom, for instance, they can provide up to 15% of total iron intake in 11–12-year-olds.[79] The contribution of fortified iron from infant cereals is potentially much greater because they often provide the major source of iron at a critical time in a child's growth and brain development.

There are two major disadvantages to using cereal

products as vehicles for iron fortification. First, they contain high levels of phytic acid, a potential inhibitor of iron absorption[6]—up to 1% in whole grains and about 100 mg/100 g in high-extraction flours. Second, they are extremely sensitive to fat oxidation during storage when highly bioavailable iron compounds such as ferrous sulfate are added.[35] For organoleptic reasons, cereal flours such as wheat and maize are usually fortified with poorly absorbed elemental iron powders, and rice with ferric orthophosphate or ferric pyrophosphate.[78] Only bread, wheat flour stored for less than 3 months, and pasta products, because of their low moisture content, can be fortified with the more highly available ferrous sulfate.[80] However, even with these foods, iron absorption will be inhibited by the presence of phytic acid unless an absorption enhancer is present. This is rarely the case, although NaFeEDTA would appear to be ideally suited to the fortification of cereal flours and perhaps even pasta products. The usefulness of the fortification of these cereal foods can therefore be questioned, because rather low levels of poorly absorbed iron compounds are added without absorption enhancers to products containing phytic acid.

Breakfast cereals are similarly fortified with reduced elemental iron,[80] and in the absence of vitamin C, the usefulness of this fortification is also doubtful. Infant cereals, by contrast, are fortified with much higher levels of iron (200–500 mg/kg) in the presence of large amounts of vitamin C. More bioavailable iron compounds such as ferrous fumarate are also often used,[35] and even with the electrolytic form of elemental iron, the efficiency of infant cereals to provide a nutritionally useful source of iron has been demonstrated.[30]

Salt

Iodine-fortified salt has successfully eradicated iodine deficiency in many countries,[81] so salt would also seem a highly suitable vehicle for iron fortification. However, iron fortification of salt poses many technical problems, and for developing countries, an efficient production and distribution system must also exist.

Almost all of the development work for the fortification of salt with iron has been conducted in India.[82-85] Color changes during storage have been the main problem, because salt in India is relatively crude and contains up to 4% moisture. All soluble iron compounds and vitamin C caused unacceptable color changes. Fortification was possible only with insoluble iron compounds, and ferric orthophosphate was recommended at 1 mg iron per gram salt so as to provide about 15 mg extra iron per day. When $NaHSO_4$ was added as an absorption promoter,[34] absorption was reported to be 80% that of ferrous sulfate. A small-scale fortification trial in which the fortified salt was included in a school feeding program demonstrated an improvement in iron status.[84]

Salt that contains fewer impurities would undoubtedly be easier to fortify, but the extra cost to the consumer is always a major consideration in developing countries. In addition, there is always the possibility that the iron-fortified salt will cause unacceptable color reactions if added to vegetables in a meal. This was one of the explanations offered for the failure of a salt fortification program in the Seychelles and Mauritius in the early 1960s.[86] The other reasons were the relatively poor bioavailability of the ferric pyrophosphate used and the fact that it separated from the salt and sank to the bottom of the salt barrels.

Sugar

Sugar is an alternative vehicle for iron fortification in regions of the world where it is produced, such as the Caribbean and Central America, but in other developing countries refined sugar consumption is more common in the middle and upper socioeconomic segments of the population.[11] Iron from fortified sugar would be expected to be well absorbed if consumed with citrus drinks but poorly absorbed from coffee and tea owing to phenolic compounds or, if added to cereal products, owing to phytate.

As with salt, the main technical problem is to select a bioavailable iron compound that does not cause unwanted color changes in less pure sugar products. In Guatemala, this was overcome by adding NaFeEDTA.[71] Commercial white cane sugar would appear easier to fortify, and Disler et al.[32] reported the successful addition of several different ferric and ferrous compounds (100–200 mg iron/kg) together with vitamin C. There were, however, unacceptable color reactions when added to coffee and tea[32] or to certain maize products.[71] A successful fortification trial was reported in Guatemala, where NaFeEDTA added to sugar at 13 mg iron/kg to provide an extra 4 mg iron/day per person increased iron stores in all population groups receiving the fortified product.[71]

Milk

Infant formulas are usually milk based with added vegetable oils, minerals, and vitamins. Iron is almost always added as ferrous sulfate from 5 to 12 mg per liter,[87] and its absorption can be improved considerably by the addition of 100–200 mg vitamin C per liter.[49] The relatively low iron bioavailability from milk products can be assumed to be due to the presence of two inhibitory factors, calcium[88] and the milk protein casein.[89] In a series of fortification trials in Chile in which iron-fortified formulas were fed to infants, the improvement of iron status was only modest in the absence of vitamin C but improved considerably when it was added to formula.[20] The widespread consumption of iron-fortified (and vitamin C-fortified) formulas by infants in the United States is regarded as the reason for the dramatic fall in the prevalence of anemia over the last 30 years.[16]

Whole milk could also be considered as a vehicle for iron fortification, but because of the presence of calcium and casein, an absorption enhancer should be added to

improve absorption. Unfortunately, it is difficult to add vitamin C to fluid milk and it has been reported to degrade rapidly to diketogluconic acid leading to changes in flavor.[90] Many soluble iron compounds rapidly produce off-flavors when added to milk, owing to the promotion of lipolytic rancidity, oxidative rancidity by the oxidation of free fatty acids, and the partial or complete loss of vitamins A, C, and ß-carotene.[91]

After evaluation of a series of compounds, the addition of ferric ammonium citrate has been proposed for liquid milk[36,92] and for skim milk, skim milk concentrate, and dry milk powder.[93] The addition of NaFeEDTA would appear to be an interesting alternative, but it has not been evaluated extensively for organoleptic properties in milk. The usefulness of milk as a vehicle for iron fortification has been demonstrated in a Mexican school feeding program.[94] The hemoglobin level of children fed 200 mL milk containing 20 mg iron as ferrous chloride improved by 1 g/dL in 3 months. This study demonstrated that with high levels of added iron, the addition of vitamin C was not essential. As with iron-fortified sugar, when iron-fortified milk is added to tea, coffee, or cocoa, the beverages undergo unacceptable color changes.

Iron-fortified milk-based chocolate drinks are also food products that can be usefully targeted to children and adolescents. A variety of products are commercially available, although the phenolic compounds present in cocoa powder readily undergo color changes with soluble iron[33] and also bind iron in the gut and inhibit its absorption. Compounds such as ferrous fumarate, ferrous succinate, ferric saccharate, and ferric pyrophosphate have shown acceptable organoleptic properties,[39] with fumarate showing the highest absorption. The addition of vitamin C would presumably be necessary to overcome the inhibitory factors in the cocoa and milk.

Condiments

Condiments that are traditionally used in developing countries, such as monosodium glutamate, fish sauce, curry powder, and bouillon cubes, could be useful fortification vehicles. Monosodium glutamate is widely used as a flavor enhancer in Asia and has been successfully fortified with ferric orthophosphate and ferrous sulfate encapsulated in zinc stearate.[44] The latter compound had 70% of the relative bioavailability of ferrous sulfate in rodents and the capsule had a melting point of 122 °C. Pilot fortification trials with iron-fortified fish sauce[69] or curry powder,[72] both fortified with NaFeEDTA, resulted in significant improvement in iron status in the population consuming the fortified products. The success of fortified condiments presumably depends both on the absence of adverse color reactions and on the addition of an absorption enhancer, such as EDTA.

Coffee

In some populations coffee is consumed by most adults as well as some children, and it is technically and economically feasible to fortify coffee with iron. Johnson and Evans[95] reported the use of ferrous fumarate in roasted and ground coffee, in which one cup (200 mL) provided 1 mg added iron. The addition of iron to soluble coffee is also relatively easy; Klug et al.[96] reported that the addition of a range of soluble ferrous and ferric compounds was possible. Flavor and color changes, however, are a potential problem, and coffee, like tea and cocoa, contains phenolic compounds that strongly inhibit iron absorption.[97]

Conclusion

One strategy to overcome the high prevalence of iron deficiency anemia in developing countries is to fortify various food products with iron. There are several options with respect to the iron compound used and the food product to be fortified. Various factors, including cost effectiveness of the fortification in raising absorbable iron intake in the targeted population, the palatability of the fortified food, and the etiology of iron deficiency must be considered before initiating a fortification program. As most iron-fortified foods contain potential absorption inhibitors, it is essential to protect the fortification iron so as to ensure adequate absorption. This can be achieved easily in the food industry by adding vitamin C, although EDTA and, possibly, hemoglobin would seem better options for developing countries.

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