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Iron Bioavailability from Fortified Fluid Milk and Petit Suisse Cheese Determined by the Prophylactic–Preventive Method

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ABSTRACT

In this research, we measure the iron bioavailability of micronized ferric orthophosphate when it is used to fortify low-fat fluid milk enriched with calcium and petit suisse cheese using the prophylactic–preventive method in rats. Four groups of male weaned rats received a basal diet (control diet; 6.5 ppm Fe), a reference standard diet (SO_4Fe; 18.2 ppm Fe), a basal diet using iron-fortified fluid milk as the iron source (milk diet; Fe ppm 17.9), and a basal diet using iron-fortified petit suisse cheese as the iron source (cheese diet; 18.0 ppm Fe) for 22 d. The iron bioavailability of the different sources was calculated as the ratio between the mass of iron incorporated into hemoglobin during the experiment and the total iron intake per animal. The relative biological values with regard to the reference standard (RBV%) were 61% and 69% for the milk and cheese diet, respectively. These results show that according to this method, the iron bioavailability in both fortified foods can be considered as medium bioavailability rates.

Index Entries: Iron; bioavailability; milk; infant dessert; fortification.

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INTRODUCTION

It is well known that iron deficiency is a worldwide nutritional-health problem. Developing countries have a higher prevalence of iron deficiency, especially in children, who are considered one of the most important risk groups *(1).* Food fortification has largely been demonstrated to be a cost-effective strategy to overcome this nutritional problem. Nevertheless, because of the oxidative properties of his element, several technological problems usually arise when iron is used in food fortification *(1).* The prophylactic–preventive method has the advantage, over other methods, that it allow one to evaluate the relative bioavailability of an experimental iron source that has been synthesized and added into foods under industrial procedures. In this way, the objective of this research was to determine by means of the prophylactic–preventive method the iron bioavailability in two different foods: fluid milk and infant dessert that were fortified with micronized ferric orthophosphate.

MATERIALS AND METHODS

The protocol of the prophylactic method *(2)* was adapted for this study *(3).* Forty male, inbred, Sprague–Dawley rats weaned at age of 25 d were individually weighed (Wi, initial weight) and their initial hemoglobin concentrations (HbCi) were determined by the cyanomethahemoglobin method *(4).* The animals were housed in stainless-steel cages in a temperature- and light-controlled environment. Four experimental diets were prepared in our laboratory and given to the animals for 22 d. A basal diet of low-iron content (control diet) was elaborated as AIN-93G diet for rodents *(5)* but modified because the final iron content was 6.5 ppm. The other three diets were also prepared as AIN-93G recommendations but modified because iron sources were different. The standard diet was prepared adding ferrous sulfate to the mineral mix as the iron source, to a final iron content of 18.2 ppm. The tests diets were prepared using low-fat fluid milk enriched with calcium (1500 ppm) and vitamin C (55 ppm) and fortified with micronized ferric orthophosphate (particle size: 2–6 μm; STR-15, Biodar, Israel) as the iron source at a final iron content of 17.9 ppm (milk diet). Petit suisse cheese was enriched with zinc (20 ppm) and vitamin C (180 ppm) and fortified with the same iron source to a final iron content of 18.0 ppm (cheese diet). All diets were available ad libitum to the different groups of rats and no other type of nourishment was offered. Food consumption was registered daily and the animals had free access to deionized water (Ametek, Plymouth, MA, USA). The iron concentration of each diet was determined by the ferrozine technique modified for foods *(6).*

The animals were treated for 22 d, and after that period, they were weighed, treated with 1500 IU heparin/kg body weight, anesthetized with ethyl ether, and bled by means of retro-orbital sinus puncture, collecting

Table 1
Dietary Iron Content and Total Iron Intake, Initial Weight, Initial Hemoglobin Concentration, and Initial Hemoglobin Iron Concentration of the Animals

Group	N	DIC (ppm)	ToFeIn [ψ] (mg/rata)	Wi (g)	Hbi (g/dL)	HbFei (mg)
Control	10	6.5±0.50[§]	1.93±0.40[§]	40.50±2.08	10.77±1.87	0.99±0.10
Standard	10	18.2±2.00	5.14±1.30	40.83±1.32	10.13±1.17	0.94±0.15
Milk	10	17.9±1.90	5.75±1.30	40.94±2.80	9.61±1.37	0.90±0.10
Cheese	10	18.0±2.10	4.87±1.20	41.05±2.28	10.88±1.54	1.02±0.17

Note: Results are given as mean ± standard deviation.

[ψ] ToFeIn/animal was determined as the product of the DIC multiplied by the amount of food consumed by each animal during the experiment.

[§] Significantly different from other groups ($p<0.01$).

between 3 and 4 mL of blood per animal. The hemoglobin concentration in the collected blood was determined in the same way as for HbCi.

Six parameters were calculated as described elsewhere *(3):* dietary iron concentration (DIC), total iron intake (ToFeIn), initial hemoglobin iron content (HbFei), final hemoglobin iron content (HbFef), the BioFe%, and the relative biological value (RBV) *(7).*

Statistical analysis of the results were carried out by a one-way analysis of variance (ANOVA) followed by the Scheffé test, fixing $p<0.01$ as the limit for significance *(8).*

RESULTS

As shown in Table 1, body weight, hemoglobin concentration, and hemoglobin iron concentrations among the animals of the four groups were not statistically different. However, because dietary iron concentration is different, the total iron intake of the control group is significantly lower ($p<0.01$) with regard to the other groups. The total iron intake per animal was calculated as the product of the DIC multiplied by the amount of food consumed by each animal during the experiment.

Table 2 shows the values of body weight, hemoglobin concentration, and hemoglobin iron concentration at the end of the treatment. In all of the cases, the values of the control group are significantly lower than those of the other three groups ($p<0.01$).

The BioFe values were calculated as the percentage ratio between the hemoglobin iron content during the treatment and the total iron intake. The relative biological value was calculated as the percentage ratio between the BioFe value of each group and that of the standard group as

Table 2
Final Weight, Final Hemoglobin Concentration, and Final Hemoglobin Iron Concentration of the Animals

Group	Wf (g)	Hbf (g/dL)	HbFef (mg)
Control	88.35±11.72§	7.26±2.50§	1.46±0.10§
Standard	108.38±9.47	10.67±0.91	2.63±0.40
Milk	118.13±12.67	7.62±1.07	2.05±0.30
Cheese	113.56±10.63	8.23±0.77	2.13±0.30

Note: Results are given as mean ± standard deviation

§ Significantly different from the other groups (p<0.01).

Table 3
Iron Bioavailability and Relative Biological Values of the Iron Sources Under Study

Group	BioFe %	RBV %
Standard	32.9±3.2§	100
Milk	20.0±2.1	61
Chesse	22.8±2.2	69

Note: Results are given as mean ± standard deviation.

§ Significantly different from the other groups (p<0.01).

is the reference group. Table 3 shows the BioFe and the RBV of each group. These results shown that according to this method, iron bioavailability in both fortified foods can be considered as medium bioavailability rates.

DISCUSSION

Iron deficiency strongly affects the world's population. According to the World Bank and several United Nation organizations, food fortification has demonstrated to be one of the most effective and inexpensive

strategy to overcome this nutritional problem *(1).* Nevertheless, in the particular case of iron, because of its high reactivity with several components of the nutritional matrix, food fortification with this element is especially difficult. Several compounds like ferrous sulfate and ferrous fumarate, for instance, have high bioavailability but, at the same time, strong interaction with the nutritional matrix, provoking off-taste and off-color *(1).* Other iron compounds with lower bioavailability, like ferric pyrophosphate and elemental iron, are usually the first choice for the industry because of their low interaction with the food, making them useful from technological but not from a nutritional point of view *(1).* In order to overcome this problem, in the last few years, some food companies have been implemented some new strategies. One of them is using some protected iron compounds; the others involve the use of some micronized insoluble iron compounds that are relatively stable in the food, but, at the same time, are more bioavailable than nonmicronized form. This decrease in the particle size increases iron bioavailability by improving the dissolution in the gastric juice *(1).* The aim of this study was to evaluate the bioavailability of micronized ferric orthophosphate when it is used to fortify fluid milk and petit suisse cheese on an industrial scale. No interactions with the nutritional matrix were detected in both cases, and from a nutritional point of view, RBVs of 61% and 69% were found, respectively, values that agree with a medium iron bioabailability. Nevertheless, micronized iron compounds have a higher bioabvailability with regard to nonmicronized compounds, which is a significant advantage from a nutritional viewpoint. The same study was previously performed using microencapsulated ferrous sulfate (SFE-171), a protected iron compound developed to be used in the fortification of dairy products at the beginning of the 1990s *(9,10).* The RBVs found for microencapsulated ferrous sulfate were significantly higher, giving values of 98% and 95% for milk and petit suisse cheese, respectively *(9,10).* Even this microencapsulated compound is useful from a nutritional and technological point of view; its high cost is a limiting factor in its massive used in food fortification. The development of new micronized iron compounds with a smaller and homogeneous particle size look like as a promising strategy in the near future, if it is possible to find a good balance between a small particle size with a good stability in fortified foods and with a high bioavailability. Perhaps a combination of previous strategies learned in the past, as it could be the development of an adequate micronized-protected iron compound, would allow us to fortify different kinds of food with a stable and bioavailable iron compound.

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