0163-4984/05/10800–0000 $30.00

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

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Received April 30, 2005; Accepted May 5, 2005

ABSTRACT

In this research, we measured the iron bioavailability of ferrous gluconate stabilized with glycine (SFG) when it is used to fortify petit suisse cheese using the prophylactic–preventive method in rats. Three groups of male, weaned rats received a basal diet (control diet; 5.2 ppm Fe), a reference standard diet (SO_4Fe; 9.2 ppm Fe), and a basal diet using iron-fortified petit suisse cheese as the iron source (cheese diet; 8.8 ppm Fe) for 22 d. The iron bioavailability was calculated as the ratio between the mass of iron incorporated into hemoglobin and the total iron intake per animal during the treatment. These values (BioFe) were 68% and 72% for SFG and ferrous sulfate, respectively. The value of the Relative Biological Value (RBV) was 95% for SFG in petit suisse cheese. These results show that according to this method, the iron bioavailability from industrial fortified petit suisse cheese can be considered as a high bioavailability rate.

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

INTRODUCTION

Iron deficiency is one of the most widespread nutritional problem. This situation justifies the generalized efforts undertaken to reduce this nutritional-health problem. Food fortification with iron has been recog-

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nized as a cost-effective and sustainable strategy for overcoming this malnutrition *(1).* Developing countries have a higher prevalence of iron deficiency, especially in children, who are considered one of the most important risk groups. However, fortification of these types of food is more than adding an iron compound because it produces alterations of the sensorial characteristics of the food *(2).* The prophylactic–preventive method has the advantage, over other methods, of allowing the evaluation of 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 using the prophylactic–preventive method the iron bioavailability in infant dessert that was fortified with ferrous gluconate stabilized with glycine (SFG).

MATERIALS AND METHODS

The protocol of the prophylactic method *(3)* was adapted for this study *(4).* Thirty male, inbred, Sprague–Dawley rats weaned at the age of 25 d were individually weighed (Wi, initial weight) and their initial hemoglobin concentrations (HbCi) were determined by the cyanomethahemoglobin method *(5).* The animals were housed in stainless-steel cages in a temperature- and light-controlled environment. Three 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 *(6)* but modified because the final iron content was 5.22 ppm. The other two diets were also prepared as AIN-93G recommendations, but modified because the 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 9.2 ppm. The test diet was prepared using fortified petit suisse cheese (Danonino; Danone, Argentina) with SFG under an industrial procedure. SFG (Bioferroso; Lipotech, Argentina) as the iron source was added at a final iron content of 8.8 ppm (cheese diet). All diets were available ad libitum to the different 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 an atomic absorption spectroscope from Buck Scientific *(7,8).*

The animals were treated for 22 d, and after that, 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 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 *(4):* 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) *(9).*

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	5.2	0.6±0.3[§]	50.4±7.1	9.3±1.7	1.1±0.3
Standard	10	9.2	1.3±0.4	51.9±4.3	9.9±1.4	1.2±0.2
Cheese	10	8.8	1.1±0.3	54.3±4.8	10.5±0.9	1.3±0.2

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 ($p<0.05$).

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

Group	Wf (g)	Hbf (g/dL)	HbFef (mg)
Control	103.0±20.2	5.7±1.1[§]	1.4±0.4[§]
Standard	113.4±21.3	8.4±1.8	2.1±0.5
Cheese	117.6±18.1	7.7±1.4	2.0±0.4

Note: Results are given as mean ± standard deviation

§ Significantly different from the other groups ($p<0.05$).

Statistical analysis of the results were carried out by a one-way analysis of variance (ANOVA) followed by the Tukey test, fixing $p<0.05$ as the limit for significance *(10)*.

RESULTS

As shown in Table 1, body weight, hemoglobin concentration, and hemoglobin iron concentrations among the animals of the three groups were not statistically different. However, because the dietary iron concentration was different, the total iron intake of the control group was lower ($p<0.05$) with regard to the other groups. The total iron intake per animal

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

Group	BioFe %	RBV %
Control	48.3±4.2§	67
Standard	71.3±6.8	100
Cheese	68.5±7.1	95

Note: Results are given as mean ± standard deviation.
§ Significantly different from the other groups ($p<0.05$).

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 the case of hemoglobin concentration and hemoglobin iron concentration, the values of the control group are lower than those of the other three groups ($p<0.05$).

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 the standard group, as this was the reference group. Table 3 shows the BioFe and the RBV of each group. These results show that according to this method, iron bioavailability in petit suisse cheese can be considered as a high bioavailability rate.

DISCUSSION

Iron deficiency widely affects the world's population. For many years now, iron food fortification has been recommended as one of the long-term strategy for eradicating iron deficiency *(2).* 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 gluconate, for instance, have high bioavailability but strong interaction. This interaction causes the development of undesirable taste (metallic aftertaste and rancidity) and off-color *(2).* Other iron compounds with lower bioavailability like ferric orthophosphate and elemental iron are usually the first choice for the industry because of their low interaction with the food, making

them useful from a technological but not from a nutritional point of view because they have a low bioavailability *(2).* In order to overcome this problem, in the last few years some food companies have implemented the use of some protected iron compounds in order to prevent the interaction of this element with the molecules of the fortified food.

The aim of this study was to evaluate the bioavailability of SFG when it is used to fortify petit suisse cheese at industrial scale. No interaction with the nutritional matrix was detected, and from a nutritional point of view, the RBV of 95% was found, a value that indicates a high iron bioavailability.

The same study was previously performed using microencapsulated ferrous sulfate (SFE-171), a protected iron compound developed to be used in the fortification for dairy products *(11,12),* and micronized ferric orthophosphate used for the same purpose *(13).* The RBVs found for microencapsulated ferrous sulfate and micronized ferric orthophosphate were 95% and 69%, respectively, in petit suisse cheese *(12,13).* Whereas this microencapsulated compound is useful from a nutritional and technological point of view, its high cost is a limiting factor for its widespread use in food fortification. On the other hand, micronized ferric orthophosphate is also useful from a technological point of view but it is not the most appropriate because of its medium bioavailability *(13).*

For this reason, SFG would be considered a solution to be used in the fortification of petit suisse cheese without changing the sensorial properties at a low cost and a high bioavailability.

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