Bioavailability and Stability of Microencapsulated Ferrous Sulfate in Fluid Milk: Studies in Mice

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Summary Iron deficiency is the most important nutritional problem all over the world. Fluid milk is an attractive vehicle for iron fortification, since it is a food with a high nutritional value, accessible to the whole population and easy to be given to children. Fortification of this food with iron has the disadvantage of the interaction of the iron with the constitutive elements of milk, diminishing its bioavailability and changing its sensorial properties, making it unacceptable. Nowadays, this problem can be overcome by the implementation of a new technological procedure, which consists in the microencapsulation of the ferrous sulfate with lecithin, thus avoiding the interaction of iron with the food. The absorption obtained in mice for milk-ferrous sulfate was 7.9±3.2%, while for microencapsulated ferrous sulfate-milk the result was 11.6±4.5%. Comparing these data with those obtained with the ferrous ascorbate in water 13.1±4.9% and ferrous sulfate in water 13.2±4.3%, both of them considered as reference standards, no statistically significant difference between them and the microencapsulated ferrous sulfate in milk can be observed. However, this difference becomes significant (p<0.01) when these products are compared to the non-encapsulated ferrous sulfate in milk. On the other hand, we demonstrated that this product is stable to heat-processing (100°C, 30 min) and storage at a room temperature up to 6 months that lacteous products are usually submitted to.

Key Words milk, fortification, ferrous sulfate, mice, absorption, iron

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From a nutritional point of view, iron is one of the most studied elements, because its deficiency affects 24% of the world's population (1); this percentage is significantly higher in Latin America, affecting more than 80% of some the infants in some populations (2). According to Dallman (3), this deficiency is the most important on a world scale.

Diet is the best way to cover all the nutritional requirements for this element, but iron bioavailability, that is, its absorbed fraction, depends on the food composition, the chemical form in which iron is found and on the mechanism that controls its absorption through the intestinal mucous membrane, which depends on the physiological and metabolic necessities of the organism for this essential element.

The iron found in food can be highly bioavailable, as it happens with the hemic iron, which is part of red meat; this iron is part of the hem structure and its absorption is not affected by the composition of the diet (4). However, the cost of these products is too high for many people to afford them. The iron present in other products such as those of vegetal origin is non-hemic iron, having the disadvantage of interacting with some substances present in the food that inhibit its absorption, such as tannins, phytates, polyphenols, etc. Such substances drastically reduce the bioavailability of the iron. This kind of food is mainly consumed by the low social classes, making it difficult for them to meet their nutritional demands for this element.

When the diet does not satisfy the iron requirements, nutritional deficiency of this element may occur, and if this situation is not corrected a more serious condition called iron-deficiency anemia may occur. The potential health consequences for the population include the following: during pregnancy, a rise in premature births, and in severe circumstances mother and fetal death; in children born without an adequate amount of stored iron, an irreversible decrease of intellectual and psychomotor development; in adults, there is a decrease of the psychomotor and intellectual performance and this affects the work capacity and productivity, bringing very severe social and economic consequences (3-5).

The way to prevent all these problems is through the iron fortification of food (4). This is considered the best way to avoid the nutritional deficiency of this element; because it can be applied to certain groups or to the entire populations.

Compounds that are used in food fortification provide non-hemic iron. Therefore, it is important to carefully select the compound used to fortify the food and to take into account the kind of food that will be the nutritional matrix, because it can interfere with the iron absorption, diminishing its bioavailability (6).

The most common compounds used for this purpose can be classified according to its solubility degree into three groups. Group 1: freely water-soluble iron (ferrous sulfate, ferrous gluconate, ferrous lactate, ferric ammonium citrate), Group 2: poorly water-soluble iron and soluble in diluted acids (ferrous fumarate, ferrous succinate), Group 3: water-insoluble iron and poorly soluble in diluted acids (ferric orthophosphate, ferric pyrophosphate, elemental iron). The choice of the compound that will be used to fortify a certain food depends on its solubility in...
gastric juice and on the presence of activators or inhibitors. Another important point is the changes that can be provoked in the food sensorial characteristics; furthermore, the cost of the fortification is of paramount importance in the decision making, principally if the aim is the lower social classes (1).

The compounds described in group 1 can be completely dissolved, providing very highly bioavailable iron. However, they have the disadvantage of freely interacting with the food components, which may alter its sensorial properties. This is possible because this metal catalyzes oxidative processes and in consequence, provokes fat rancidity. This oxidation catalytic process may occur with other nutrients such as vitamins and amino acids, decreasing in this way, the nutritional value of the food.

The compounds of group 2 have good solubility, and therefore good bioavailability; however, they have the disadvantage of being used only in solid dehydrated food, because as they do not dissolve in neutral liquids, they precipitate. In such situations, the free fraction of iron interacts with the constitutive elements of the food, decreasing its nutritional value, and also changing its sensorial characteristics.

The compounds of the third group have a very low solubility, and therefore, although they do not change either the sensorial properties of the food or its nutritional value, they have the disadvantage of very low bioavailability.

Therefore, the ideal product for food fortification should be one that supplies a highly bioavailable iron, that does not diminish the nutritional value of the food by its nutrient oxidation, that does not alter its sensorial properties, that could be used to fortify solid and liquid food, that is stable during food processing, and that has a low cost, in order to be accessible for the whole population.

A new procedure allows the production of a compound that has the desirable properties mentioned above. This compound, SFE-1711, is ferrous sulfate micro-encapsulated with lecithin. In this way, a product with the same bioavailability as ferrous sulfate was obtained with the advantage that as it is wrapped in a phospholipid membrane so it does not allow the iron to contact the food, avoiding the interaction and the undesirable changes that happen when unencapsulated ferrous sulfate is added.

This new product added to fluid milk and milk products means that iron can be more accessible to a broader population group, with a high nutritional value and at a lower cost.

In this work, we evaluated the iron bioavailability of this product and its stability during conventional manufacturing processes that milk is submitted to during the different industrial processes of production and commercialization.

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1 Japanese patent #178712/94; SFE stands for stabilized ferrous sulfate in Spanish; 171 is the number of the experiment.

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MATERIALS AND METHODS

Animals. We used 390 female mice from the Swiss strain, with weights ranging from 30 to 40 g. They were divided into 13 groups of 30 mice; each group was maintained in a stainless steel cage of 315 mm by 445 mm by 240 mm high with a stainless steel grated floor and a collecting tray of the same material, which prevents the excrements from coming into contact with the animals. They were maintained with free access to water and nourished with a normalized diet having the following composition: protein, 20.0% w/w minimum; fat, 2.0% w/w minimum; fibers, 11.0% w/w maximum; ashes, 10.0% w/w maximum; calcium, 0.6% w/w; phosphorus, 0.7% w/w; iron, 50 ppm (7); moisture, 13.0% w/w. The animals were maintained with cycles of 12 h with light and 12 h in darkness throughout the experiments.

Administration of the preparations. Before administration of the products, the animals were deprived of any solid food for 10 h, which was not provided until 1 h after the products intake. The preparations were administered by means of a syringe coupled to a plastic gastric catheter which allowed the intake volume to be standardized (0.1 ml). The amount of iron administered to each mouse was 4 µg and the activity of $^{59}$Fe (NEN, DuPont, catalogue no. NEZ 037), was 3 µCi per mouse for all the products.

Radioactive measurements. The absolute activities of each preparation were determined by means of an ionization chamber (RADX Model 255 Remote). The activity retained by the mice as a function of time was measured by a gamma spectrometer with a 5 cm × 5 cm NaI(Tl) well crystal with optimal electronic conditions. The measured activity was always compared to a $^{60}$Co standard in order to detect any eventual efficiency fluctuation. The efficiency of the measurements (2.4%) remained constant during all the experiments.

The iron absorption was determined by measuring the $^{59}$Fe radioactivity in the mice, using a whole-body geometry, introducing each animal in a covered lucite box, the size of which was adapted to the animal size and to the detector geometry. In this way, it was possible to minimize detection errors during the measurements, which could be attributed to eventual movements of the animal. The radioactivity determinations of an $^{59}$Fe standard in the same geometric and electronic conditions demonstrated the reliability of this technique. Taking into account the measurement efficiency and the $^{59}$Fe activity, the mass of absorbed iron was calculated. By this method, the percentage of absorbed iron with regard to the administered iron could be determined.

Statistical analysis of the experimental results. The data are presented as the $\text{M} \pm \text{SD}$. To test for differences among all the products under study, we evaluated the results by a one-way analysis of variance (ANOVA). To test the differences among the means, the Student-Newman-Keuls method was used. Only probability levels less than 0.01 were considered statistically significant (8).
RESULTS AND DISCUSSION

Absorption studies

In the first place, studies were carried out to evaluate the iron bioavailability of SFE-171 and its interaction with fluid milk, as compared to other iron compounds. Four groups of mice were used and the obtained results are shown in Table 1.

Table 1. Iron absorption from different compounds.

<table>
<thead>
<tr>
<th>Ferrous ascorbate in water¹</th>
<th>Ferrous sulfate in water²</th>
<th>Ferrous sulfate in milk</th>
<th>SFE-171 in milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.1±4.9%</td>
<td>13.2±4.3%</td>
<td>7.9±3.2%</td>
<td>11.6±4.5%</td>
</tr>
</tbody>
</table>

¹ Molar ratio Fe/Ascorbic acid = 1.
² Under nitrogen atmosphere.

Two groups were supplied with ferrous ascorbate and ferrous sulfate in water. These groups were used as reference standards and their absorption was 13.1±4.9% and 13.2±4.3%, respectively. With the purpose of evaluating the effect of the milk components on the absorption of ferrous sulfate, we administered to another group of mice milk with ferrous sulfate and obtained a result of 7.9±3.2%. Finally, we wanted to evaluate the bioavailability of SFE-171 in milk. For this purpose, the last group of animals was administered with this preparation, and the obtained result was 11.6±4.5%. Statistical analysis showed that there is no significant difference between SFE-171 in milk and ferrous ascorbate and ferrous sulfate in water. However, this difference becomes significant (p<0.01) between the three groups mentioned above and the one which was supplied with ferrous sulfate in milk.

These results show that the constitutive elements of the milk interact negatively in the absorption of the ferrous ion when it is added to milk, thus diminishing its bioavailability (9–11). This negative interaction effect of the nutritional matrix on iron absorption can be avoided when this new fortification procedure is used, providing in this way, a highly bioavailable non-hemic iron.

After evaluating the absorption of iron from SFE-171 in fluid milk, its stability was determined under the different conditions encountered during the processes of elaboration and manufacturing. These procedures, which are usually those which milk is submitted to, are the thermic and long-term storage processes.

Thermic stability

The technological processes milk is submitted to are pasteurization and sterilization, which consist of heating the milk to different temperatures at different times. In order to evaluate the thermic effect on this product, milk added with SFE-171 was heated at 100°C for 30 min (sterilization) and its iron bioavailability was
determined. For this purpose this product was administered to several groups of mice and the iron absorption percentage was determined. These results can be compared to those obtained without any thermal treatment and also when the milk with ferrous sulfate is sterilized, as seen in Table 2.

Table 2. Effect of heat-treatment on the iron absorption from SFE-171 in milk and other iron compounds.

<table>
<thead>
<tr>
<th>Ferrous ascorbate in water&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Ferrous sulfate in water&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Ferrous sulfate in milk</th>
<th>Sterile&lt;sup&gt;3&lt;/sup&gt; (Ferrous sulfate in milk)</th>
<th>SFE-171 in milk</th>
<th>Sterile&lt;sup&gt;3&lt;/sup&gt; (SFE-171 in milk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.1±4.9%</td>
<td>13.2±4.3%</td>
<td>7.7±3.1%</td>
<td>7.9±3.2%</td>
<td>12.1±4.4%</td>
<td>11.6±4.5%</td>
</tr>
</tbody>
</table>

<sup>1</sup>Molar ratio Fe/Ascorbic acid = 1.
<sup>2</sup>Under nitrogen atmosphere.
<sup>3</sup>Sterile: 100°C for 30 min.

Experimental results show that when SFE-171 is added to milk, the iron absorption values do not differ significantly between the sterilized and the non-sterilized products. In this way, we could demonstrate that the microencapsulation does not modify the absorption, and consequently the bioavailability of ferrous sulfate when SFE-171-fortified milk is submitted to the different thermic processes during its manufacture.

**Stability as a function of time (Shelf-life)**

Once the milk has been sterilized and adequately packed, it can be sold over a period of several months as in the case of “long life” milk. During the time in which the milk is stored at room temperature, the iron, because of its chemical instability, can be altered and consequently its bioavailability decreases as a function of time. For this reason, SFE-171-fortified milk was first sterilized and afterwards it was stored for 6 month at room temperature. During this period, its stability was determined monthly. The preparation was given to several groups of mice and the percentage of iron absorption was determined, as can be seen in Table 3.

Table 3. Iron absorption from SFE-171 in milk as a function of storage time.

<table>
<thead>
<tr>
<th>Time (months)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron absorption (%)</td>
<td>12.4±3.9</td>
<td>12.0±4.5</td>
<td>12.3±3.1</td>
<td>12.0±4.2</td>
<td>12.6±4.5</td>
<td>11.9±3.4</td>
<td>11.6±4.3</td>
</tr>
</tbody>
</table>

In none of the cases was a statistically significant difference observed. These results show that, after storage for 6 months at room temperature, the iron from
SFE-171 fortified and sterilized milk does not modify its absorption or bioavailability during the study period.

These results show that SFE-171 added to fluid milk provides a non-hemic iron with high bioavailability (12, 13), that resists the technological processes of production and manufacturing that lacteous products are usually submitted to with no alterations, neither of the bioavailability of this element nor the sensorial properties of the fortified milk. In this way, a low-cost food with high nutritional value can be made available to the population.

REFERENCES