A New Procedure to Fortify Fluid Milk and Dairy Products with High-Bioavailable Ferrous Sulfate

June 1997: 240–246

José R. Boccio, Marcela B. Zubillaga, Ricardo A. Caro, Ph.D., Carlos A. Gotelli, Mariano J. Gotelli, and Ricardo Weill

The behavior of SFE-171 used for food fortification was studied. The biological and nutritional properties of this new iron source are discussed in this work.

Introduction

From a nutritional point of view, iron is one of the most studied elements because its deficiency affects about one-third of the world's population.[1] This percentage rises significantly in Latin America, affecting more than 80% of some infant populations.[2] According to Dallman this deficiency is the most important on a global scale.[3]

Diet is the best way to meet all of the nutritional requirements for this element. Iron bioavailability, that is, its absorbed fraction, depends on 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 physiologic and metabolic necessities of the organism for this essential element.[3]

The iron found in food can be highly bioavailable, as is the case with heme iron, which is found in red meat; this iron is part of the heme 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. The iron present in other products, such as those of vegetable origin, is nonheme iron, but it has the disadvantage of interacting with some substances in food that inhibit its absorption, such as tannins, phytates, and polyphenols; that is, it is iron with very low bioavailability. Much of this kind of food is consumed by people in the lower socieconomic classes, who thus cannot meet their physiologic needs for iron.

When the diet does not satisfy the body's iron requirements, nutritional deficiency of this element may occur. If this situation is not reversed, a more serious state called ferropenic anemia may result. The consequences for public health are a rise in premature births and, in severe circumstances, maternal and fetal death; in children born without an adequate amount of iron, a decrease in intellectual and psychomotor development that is irreversible; and in adults, a decrease in psychomotor and intellectual performance that affects work capacity and productivity, with potentially severe social and economic consequences.[3-5]

The best way to prevent these problems is through the iron fortification of food for the whole population or only for certain groups. Compounds used in food fortification provide nonheme iron, so it is important to select fortification compounds and foods, or vehicles, that will not diminish iron bioavailability.[4–7]

The most common iron fortification compounds can be classified into three groups according to solubility: group 1, freely water-soluble iron (ferrous sulfate, ferrous gluconate, ferrous lactate); group 2, poorly water-soluble iron or soluble in diluted acids (ferrous fumarate, ferrous succinate); and group 3, water-insoluble iron or poorly soluble in diluted acids (ferric orthophosphate, ferric pyrophosphate, elemental iron). The choice of iron compounds depends on its solubility in gastric juice and on the presence of activators or inhibitors in the fortification food. What also must be considered are the changes that can occur in the fortified food's sensory characteristics and the cost of fortification, the latter of which is of significance if the target populations are those of limited financial means.[1,6]

Group 1 compounds can be completely dissolved and thus provide very high bioavailable iron. However, they have the disadvantage of freely interacting with the fortified food, which may alter its sensory properties. This can happen because iron catalyzes oxidative processes and thus provokes fat rancidity. This catalytic oxidation process may occur with other nutrients such as vitamins and amino acids, thus decreasing the nutritional value of the food.

Mr. Boccio, Mrs. Zubillaga, and Dr. Caro are at the Radioisotope Laboratory, Physics Department, School of Pharmacy and Biochemistry, University of Buenos Aires, 1113 Buenos Aires, Argentina. Mr. C.A. Gotelli and Mr. M.J. Gotelli are at the Center for Toxicological Research, Buenos Aires. Mr. Weill is in the Center for Research and Development, La Serenisima, Buenos Aires.

Part of the research reported herein was carried out under a Specific Agreement between Facultad de Farmacia y Bioquímica de la Universidad de Buenos Aires and Mastellone Hnos under Resolution (C.D.) 340/95.

Group 2 compounds have good solubility and thus good bioavailability. However, they have the disadvantage of being used only in solid dehydrated food because they do not dissolve in neutral liquids, in which they precipitate. In this last situation, the free fraction of iron interacts with the constitutive elements of the food to decrease its nutritional value and alter its sensory characteristics.

Group 3 compounds have a very low solubility. Thus, although they do not change the sensory properties or nutritional value of the food, they have the disadvantage of having very low bioavailability.

Therefore, the ideal product for food fortification is one that supplies high-bioavailable iron, does not diminish the nutritional value of the food vehicle through nutrient oxidation, does not alter its sensory properties, can be used to fortify solid and liquid foods, is resistant to food technology processes, and is low in cost so that it can be accessible to the whole population. Until recently a product having these properties was unknown.

In recent years, a compound called SFE-171 (SFE-171 is its generic and experimental name; its commercial name is Biofer™), a ferrous sulfate microencapsulated with lecithin, has been produced and studied in animals and humans. This new product has the same bioavailability as ferrous sulfate but has the advantage of being coated with a phospholipid membrane. This keeps the iron from coming in contact with the food vehicle, preventing the undesirable interactions that happen when conventional ferrous sulfate is used.

Absorption and Stability Studies

Absorption

Studies were carried out to evaluate the iron bioavailability of SFE-171 and its interaction with fluid milk compared with other iron sources. Four groups of mice were used and the results are shown in Table 1.

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. To evaluate the effect of the milk components on the absorption of ferrous sulfate, we administered milk with ferrous sulfate to another group of mice and obtained a result of 7.9 ± 3.2%. Finally, we evaluated the bioavailability of SFE-171 in milk. The last group of animals was administered this preparation, and the obtained result was 11.6 ± 4.5%.

Statistical studies show that there is no significant difference between SFE-171 in milk and ferrous ascorbate and ferrous sulfate in water. However, the difference becomes significant with $p < 0.01$ between the group that was supplied with ferrous sulfate in milk and the other three groups mentioned above. These results show that the constitutive elements of the milk such as casein, whey protein, and calcium interact negatively in the absorption of the ferrous ion when it is added to milk, diminishing in consequence its bioavailability.[8–10] This negative interaction effect of the nutritional matrix on the iron absorption can be avoided when SFE-171 is used.[11]

After evaluating the absorption of iron from SFE-171 in fluid milk, its stability was determined under the different conditions of the technological processes of elaboration and manufacturing. These procedures, which are usually those to which milk is submitted, 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. Milk to which SFE-171 was added was heated to 100 ºC for 30 minutes (sterilization) and its iron bioavailability was determined. The product was administered to several groups of 30 mice each and the iron absorption percentage was determined (see Table 2). These results can be compared with those obtained without any thermal treatment and with sterilized milk to which ferrous sulfate has been added.

Experimental results show that when SFE-171 is added to milk, iron absorption values do not differ significantly between sterilized and nonsterilized products. In this way, we were able to demonstrate that the absorption, and thus the bioavailability, of microencapsulated ferrous sulfate is not modified when SFE-171-fortified milk is submitted to different thermal treatments during processing.[11]

Stability as a Function of Time (Shelf Life)

Milk that has been sterilized and adequately packaged can be sold over a several-months-long period, as is the case with "long-shelf-life" milk. However, over time, because of its chemical instability, the iron in milk can be altered and

Table 1. Iron Absorption from Different Iron Sources

Iron Source	Ferrous Ascorbate in Water[a]	Ferrous Sulfate in Water[b]	Ferrous Sulfate in Milk	SFE-171 in Milk
Absorption (% ± SD)	13.1 ± 4.9	13.2 ± 4.3	7.9 ± 3.2[*]	11.6 ± 4.5
Animals (*n*)	30	30	30	30

[a]Molar ratio Fe/ascorbic acid = 1.
[b]Under nitrogen atmosphere.
[*]Value statistically different from the other groups.

Table 2. Effect of Heat Treatment on the Iron Absorption from SFE-171 in Milk and Other Iron Sources

	Ferrous Ascorbate in Water[a]	Ferrous Sulfate in Water[b]	Ferrous Sulfate in Milk	Sterile[c] (Ferrous Sulfate in Milk)	SFE-171 in Milk	Sterile[c] (SFE-171 in Milk)
Absorption (% ± SD)	(13.1 ± 4.9)	(13.2 ± 4.3)	(7.7 ± 3.1)*	(7.9 ± 3.2)*	(12.1 ± 4.4)	(11.6 ± 4.5)

[a]Molar ratio Fe/ascorbic acid = 1.
[b]Under nitrogen atmosphere.
[c]Sterile: 100 °C for 30 minutes.
*Value statistically different from the other groups.

Table 3. Iron Absorption from SFE-171 in Milk as a Function of Storage Time

Time (months)	0	1	2	3	4	5	6
Iron absorption (%)	(12.4 ± 3.9)	(12.0 ± 4.5)	(12.3 ± 3.1)	(12.0 ± 4.2)	(12.6 ± 4.5)	(11.9 ± 3.4)	(11.6 ± 4.3)

its bioavailabitity decreased. For this reason, SFE-171-fortified milk was first sterilized and afterward stored for 6 months at room temperature. During this period, its stability was ascertained monthly. SFE-171-fortified milk was given to several groups of 30 mice each and the percentage of iron absorbed was determined, as can be seen in Table 3.

No statistically significant differences were observed between any of the groups. These results show that during 6 months of storage at room temperature, the absorption and bioavailability of iron from sterilized milk fortified with SFE-171 are unchanged.[11] They also show that SFE-171 added to fluid milk provides nonheme iron with high bioavailability, that the fortified milk is resistant to the technological processes that dairy products are usually submitted to, and that neither the bioavailability of the iron nor the sensory properties of the fortified milk are altered. Thus, a low-cost food with high nutritional value can be offered to broad segments of the population.[11]

Influence of Additives

Milk is usually consumed with other foods, such as tea, coffee, maté (or Argentine green herbs tea), and cereals. For the purpose of evaluating the effect of these and other products on the absorption of the iron in SFE-171 in fluid milk, we gave different foods to 16 groups of 25 mice each and determined the iron absorption in each case. For comparative and reference-standard purposes, we conducted the same experiments in milk fortified with nonencapsulated ferrous sulfate (Table 4).

As can be seen, in each case the absorption of iron provided by SFE-171 was similar to or higher than that provided by ferrous sulfate.[12]

Absorption Mechanism

Iron homeostasis is controlled at the level of the intestinal mucous membrane cells. By a specific transport mechanism, these cells determine iron absorption according to

Table 4. Statistical Comparison Between the Iron Absorption of Each of the Products Fortified with $FeSO_4$ or with SFE-171

Studied Products	Retention Percentage	Difference Limit: $p < 0.01$
Milk + SO_4Fe	7.7 ± 2.7	
Milk + SFE-171	12.3 ± 2.9	S
Cacao 10% + milk + SO_4Fe	10.0 ± 3.1	
Cacao 10% + milk + SFE-171	20.1 ± 3.3	S
Cacao 1% + milk + SO_4Fe	11.4 ± 2.7	
Cacao 1% + milk + SFE-171	12.5 ± 3.1	NS
Yogurt + SO_4Fe	6.9 ± 2.9	
Yogurt + SFE-171	8.1 ± 3.0	NS
Coffee 5% + milk+ SO_4Fe	6.5 ± 2.0	
Coffee 5% + milk + SFE-171	7.9 ± 3.1	NS
Tea 5% + milk + SO_4Fe	4.4 ± 2.1	
Tea 5% + milk + SFE-171	4.6 ± 1.5	NS
Maté 5% + milk + SO_4Fe	9.5 ± 3.0	
Maté 5% + milk + SFE-171	11.0 ± 2.0	NS
Cereals 5% + milk + SO_4Fe	6.7 ± 3.0	
Cereals 5% + milk + SFE-171	8.3 ± 2.2	NS

S: significant; NS: not significant.

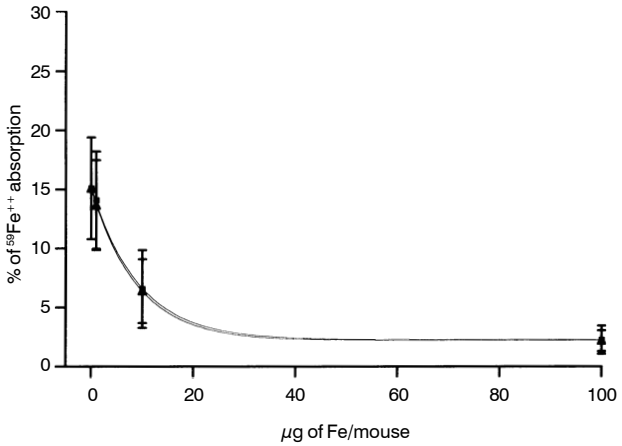

Figure 1. In vivo self-displacement studies. (▲): displacement of $^{59}Fe^{++}$ by increasing doses of Fe^{++} from ferrous sulfate as a reference iron compound. (■): displacement of $^{59}Fe^{++}$ by increasing doses of Fe^{++} from SFE-171 as the studied iron source.

the physiologic and metabolic needs of the organism. For this reason, it is important that the compound used in food fortification has a high bioavailability and follows the specific mechanism of intestinal regulation that protects the organism from iron overload.

We designed an experiment to study whether the iron from SFE-171 follows the same absorption mechanism as the ferrous ion provided by ferrous sulfate. Seven groups of 30 mice each received 1 mg Fe^{++} labeled with ^{59}Fe and different iron doses administered as ferrous sulfate or as SFE-171. Figure 1 summarizes the results.

As the administered iron mass (either from SFE-171 or from ferrous sulfate) rises, the absorption of $^{59}Fe^{++}$ decreases. Thus, the iron from both compounds competes and displaces the ferrous ion from its binding site, confirming that both compounds have the same specific and saturable absorption mechanism and that the organism will absorb only the amount of iron necessary for its physiologic requirements.[13]

Metabolic and Biochemical Studies

We used four groups of 30 mice each to study whether the iron in SFE-171 follows the same metabolic pathway as that in ferrous sulfate. To the first group we administered ferrous sulfate in milk, to the second SFE-171 in milk, to the third ferrous ascorbate in water, and to the fourth ferrous sulfate in water. The last two groups were used as reference standards.

Fifteen days after administration—the time during which iron is absorbed and distributed in different tissues and organs according to its metabolic and biochemical functions—the animals were sacrificed and the biodistribution of iron was determined (Table 5).

As can be observed, a higher percentage of absorbed iron is found in blood because the iron becomes part of the

Table 5. Percentage of Activity in Different Organs for Each Group

Group	Blood	Carcass[a]	Liver	Spleen	Lung	Heart	Kidney	Gut[b]	Brain	Uterus
Ferrous ascorbate[c]	66.56 ± 7.42	17.91 ± 5.52	8.11 ± 2.28	0.87 ± 0.39	0.69 ± 0.43	0.32 ± 0.15	1.92 ± 0.59	3.22 ± 1.12	0.23 ± 0.16	0.20 ± 0.17
Ferrous sulfate[d]	67.17 ± 6.33	17.40 ± 4.93	8.22 ± 1.71	0.91 ± 0.37	0.66 ± 0.51	0.30 ± 0.18	1.85 ± 0.55	3.00 ± 0.61	0.28 ± 0.24	0.22 ± 0.21
Ferrous sulfate[e]	65.92 ± 5.68	18.45 ± 5.33	8.45 ± 1.60	0.77 ± 0.31	0.76 ± 0.45	0.30 ± 0.17	1.90 ± 0.54	3.00 ± 0.54	0.27 ± 0.21	0.17 ± 0.16
SFE-171[f]	67.12 ± 6.46	16.96 ± 3.82	8.32 ± 1.61	1.04 ± 0.46	0.69 ± 0.47	0.31 ± 0.25	1.80 ± 0.56	2.91 ± 0.65	0.34 ± 0.28	0.28 ± 0.25

Note: All values are expressed as Mean ± SD. In all cases, the sum of the whole organ's activity was considered equal to 100% for each animal.

[a]Muscle + bone + skin.

[b]With content.

[c]In water and equimolecular ratio ascorbic acid, iron.

[d]In water and under nitrogen atmosphere.

[e]In milk and heated at 100 °C for 30 minutes.

[f]In milk and heated at 100 °C for 30 minutes.

hemoglobin present in the red blood cells, carrying out one of the principal functions related to oxygen transport. Although lower than in blood, the amount of iron found in the carcass (muscles and bone) was high because in muscle iron is part of myoglobin and in the liver iron is in the form of ferritin and hemosiderin. We observed no significant differences between iron absorption from SFE-171 and from the other iron compounds in all tissues and organs.[14] Consequently, we can conclude that the iron provided by SFE-171 follows the conventional metabolic, physiologic, and biochemical pathways of this element.

To determine whether these results could be repeated, a similar study was done using another animal species: Sprague-Dawley rats. We obtained results very similar to those for mice.[15]

Mother-to-Fetus Iron Transfer

Pregnancy is one of the physiologic conditions in which iron requirements rise, because during this period there is a significant increase in the synthesis of different molecules that contain iron in their structure, e.g., hemoglobin. As a consequence of the development and growth of the fetus, iron has to increase. Thus, adequate iron transfer through the placenta must take place to meet the requirements for this element.

To study the metabolism of the iron from SFE-171 during pregnancy, we administered to a group of 30 female mice of the Swiss strain milk fortified with SFE-171 throughout gestation. On the day of birth, we determined the percentage of iron transferred through the mother to the offspring. All animals—mother and offspring—were sacrified and the distribution of the iron from SFE-171 to all the tissues and organs was measured. Figure 2 summarizes our findings.

As can be seen, 45% of the absorbed iron from the milk fortified with SFE-171 was transferred through the mother to the pups. In both cases, mother and pups, the highest percentage of iron is found in blood, where it forms part of the hemoglobin, playing one of the principal metabolic roles relative to oxygen transport. A high percentage of iron is also found in liver and the carcass (muscle and bone). In the liver iron is deposited as ferritin and hemosiderin.[16] Thus, the iron in SFE-171-fortified fluid milk is efficiently transferred from mother to offspring, following the known metabolic pathway for this element.

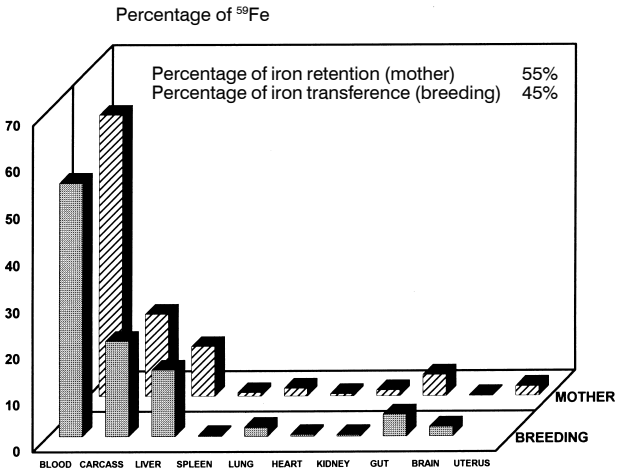

Figure 2. Biodistribution results and percentage of iron transferred from the mother to the offspring.

Toxicity Studies

Before we carried out experiments in human beings, we performed toxicity studies in rodents using a 50% oral lethal dose (LD_{50}) of SFE-171, with ferrous sulfate as the reference standard. We administered to two groups of 70 animals each different doses of the compounds under study, using the methodology of Litchfield and Wilcoxson[17] to analyze the results.

As can be observed in Table 6, the LD_{50} for the microencapsulated ferrous sulfate was significantly higher than that of the nonencapsulated compound. This result, which implies that the toxicity of SFE-171 is lower than that of nonencapsulated ferrous sulfate, is explained by the fact that in SFE-171 the liposomes surround the iron compound, precluding its abrupt release into the intestinal lumen.[13]

Absorption Studies in Human Beings

Following our experiments in rodents, we studied the absorption of iron from SFE-171-fortified fluid milk in human beings. The study was carried out on 29 physically and psychologically healthy volunteers. As shown in Table 7, the hematologic studies found that neither iron metabolism nor body iron deposits were altered.

At breakfast, the volunteers ingested 250 mL milk that contained 12 mg iron/L in the form of SFE-171. Iron was absorbed at the rate of 10.4% ±4.9%,[18] which correlates well with high-bioavailable nonheme iron absorption.[10] With

Table 6. Acute Oral Toxicity in Mice: Values of LD_{50} and Their Confidence Limits for Ferrous Sulfate and SFE-171, with Ferrous Sulfate as the Reference Standard

Iron Source	LD_{50}[a]	Lower Limit[a]	Upper Limit[a]	Animals (*n*)	Sex
Ferrous sulfate	680	572	808	70	Female
SFE-171	1200	956	1505	70	Female
Ferrous sulfate	670	565	816	70	Male
SFE-171	1230	967	1521	70	Male

[a] Expressed as mg ferrous sulfate/kg of body weight.

Table 7. Mean Values and Standard Deviations of the Parameters in Human Iron Absorption Study Carried Out in 29 Volunteers

Parameter	Mean Value	SD
Weight	73.5 kg	9.2 kg
Hematocrit	45.8%	3.1%
Hemoglobin	16.8 g/dL	1.4 g/dL
Serum ferritin	113 ng/mL	74 ng/mL

the objective of confirming this finding, another research group carried out an absorption study in human beings to evaluate the iron bioavailability of milk fortified with SFE-171. The study was carried out using a modified Eakins and Brown reference methodology[19] on a group of 15 healthy volunteers who ingested 250 mL milk fortified with 15 mg iron/L in the form of SFE-171 at breakfast. As can be seen in Table 8, iron metabolism was not altered in any of the subjects.

The experimental results show that the geometric mean of iron absorption was 9.2%, which supports the use of SFE-171 in food fortification programs.[20]

Correction of Iron Deficiency with Fluid Milk Fortified with Iron

In a study performed at the Hospital Municipal del Niño de San Justo, variations in iron nutritional state were evaluated by replacing unfortified milk, which is usually consumed by children in Argentina, with fluid milk fortified with ferrous sulfate (15 mg elemental iron per liter).[21]

Sixteen children with an average age of 23.1 months ± 8.9 months and an average weight of 11.7 kg ± 1.6 kg were evaluated. All of them presented with a more or less severe iron depletion with low plasma-iron concentration < 60 µg/dL and ferritin < 15 ng/mL. Ten of them were anemic, with hematocrit and hemoglobin values lower than normal for this age group. All of the children received milk fortified with SFE-171 until they reached normal plasma-iron concentration values (minimum 60 days, maximum 120 days). The results are summarized in Table 9.

Table 8. Mean Values and Standard Deviations of the Parameters in Human Iron Absorption Study Carried Out in 15 Volunteers

Parameter	Mean Value	SD
Hemoglobin	15.0 g/dL	2.9 g/dL
Hematocrit	45.0%	1.0%
MCV	91.9	2.9
ZPP	22.1	5.2
Serum ferritin	155.7 µg/dL	51.1 µg/dL

MCV: mean corpuscular volume (μ^3).
ZPP: zinc protoporphyrin (µmol/mol hem).

Significant increases ($p<0.05$) in the hemoglobin, hematocrit, and plasma-iron concentration values were obtained. The consumption of fortified milk was effective in normalizing hemoglobin levels and plasma-iron concentration in 100% of the children, but ferritin reached normal values in only half of the cases.

The results obtained with the anemic patients in this study were compared with a control group of 52 anemic children of the same age and degree of anemia who were treated with ferrous sulfate in doses that ranged between 4 and 6 mg/kg/day. As can be seen in Table 10, the time required to reach normal hematocrit levels was lower in the group treated with ferrous sulfate, but the difference was not statistically significant ($p = 0.09$).

These results demonstrate that the high bioavailability of the iron in the milk fortified with microencapsulated fer-

Table 9. Variation in Nutritional State of Anemic Toddlers Fed SFE-171-fortified Milk

	Hematocrit[a]	Hemoglobin[a]	Plasma Iron[b]	Ferritin[b]
Initial (I)	(33.0 ± 1.9) %	(10.1 ± 0.9)g/dL	(37.2 ± 16.1)µg/dL	(12.7 ± 1.3)ng/mL
Final (F)	(38.5 ± 2.3) %	(12.6 ± 0.7)g/dL	(132.6 ± 47.3)µg/dL	(28.5 ± 26.4)ng/mL
Δ (I – F)	(5.5 ± 2.9) %	(2.6 ± 1.2)g/dL	(95.4 ± 48.1)µg/dL	(15.8 ± 26.8)ng/mL
T_n[c]	(51.1 ± 23.5)	(50.7 ± 19.2)	(46.6 ± 26.2)	(46.7 ± 16.8)
P_n[d]	10	10	16	8
$P_{\%}$[e]	100	100	100	50

[a] Performed on anemic patients.
[b] Performed on all patients.
[c] Time to reach normal values, given in days.
[d] Number of patients who reached normal values.
[e] Percentage of patients recovering normal values for each item.

Table 10. Variation of Hematocrit in Toddlers Who Received Ferrous Sulfate or SFE-171-fortified Milk

	Ferrous Sulfate ($n = 52$)	SFE-171 ($n = 10$)	p	Statistical Significance ($p < 0.01$)
Age (months)	20.0 ± 8.0	23.0 ± 9.2	0.22	Not significant
Initial hematocrit (%)	32.5 ± 1.9	33.0 ± 1.9	0.50	Not significant
Δ hematocrit (%)	6.1 ± 2.5	5.5 ± 2.9	0.66	Not significant
Time of normal hematocrit (days)	38.3 ± 15.0	51.1 ± 23.5	0.09	Not significant

rous sulfate allows an effective correction of iron-deficient states.

Conclusion

From the foregoing experiments, we can conclude that the recently developed SFE-171 for the iron fortification of food is effective and affordable in providing high-bioavailable iron for entire populations via milk and dairy products, thus significantly meeting the daily requirements for this element.

1. Hurrel RH. Iron fortification of infant cereals. Personal communication, 1994
2. Portela MLPM. Vitaminas y minerales en nutrición. Buenos Aires: Libreros López Editores, 1994
3. Dallman PR (1991) Hierro. In: Brown M, ed. Conocimientos actuales sobre nutrición. Publicación Científica no 532. Washington, DC: Organización Panamericana de la Salud/International Life Sciences Institute, 1991;277–88
4. Goodman GA. The pharmacological basis of therapeutics, 8th ed. New York: Pergamon Press, 1990
5. Atukorala MT, Silva L, Dechering W, et al. Evaluation of effectiveness of iron-folate supplementation and anthelminthic therapy against anemia in pregnancy: a study in the plantation sector of Sri Lanka. Am J Clin Nutr 1994;60:286–92
6. Enriching lives: overcoming vitamin and mineral malnutrition in developing countries. Washington, DC: International Bank for Reconstruction and Development/World Bank, 1994
7. Parr RM, Fjeld CR. Salud y nutrición humana: la ayuda de los radioisótopos para erradicar el "hambre escondida." Vienna: OIEA, 1994;36:18–27
8. Hurrell RF, Lynch SR, Trinidad PT, et al. Iron absorption in humans as influenced by bovine milk proteins. Am J Clin Nutr 1989;49:546–52
9. Minotti PL, Buchonski SM, Miller DD. Effects of calcium supplementation: calcium source and lactose on iron absorption in the rat. Nutr Res 1993;13:1173–81
10. Monsen E, Hallberg L, Layrisse M, et al. Estimation of available dietary iron. Am J Clin Nutr 1978;31:134–41
11. Boccio J, Zubillaga M, Caro R, et al. Bioavailability and stability of microencapsulated ferrous sulfate in fluid milk. J Nutr Sci Vitaminol 1996;42:233–9
12. Boccio J, Zubillaga M, Caro R, et al. New procedure to fortify fluid milk and derivatives with iron: comparative study in mice. J Nutr Sci Vitaminol 1995;41:619–26
13. Boccio J, Zubillaga M, Caro R, et al. Bioavailability, mechanism of absorption and toxicity of microencapsulated ferrous sulfate. Studies in mice. Biol Trace Elem Res 1997, in press
14. Boccio J, Zubillaga M, Caro R, et al. Microencapsulated ferrous sulfate in fluid milk. Metabolic and biochemical studies in mice. J Nutr Sci Vitaminol 1997, in press
15. Zubillaga M, Caro R, Boccio J, et al. New procedure to fortify fluid milk with iron: metabolic and biochemical study in rats. Nutr Res 1996;16:131–7
16. Boccio J, Zubillaga M, Caro R, et al. Mother-to-fetus iron transfer of fluid milk fortified by microencapsulated ferrous sulfate: study in mice. Unpublished observations
17. Litchfield JT, Wilcoxson F. A simplified method of evaluating dose-effect experiments. J Pharmacol Exp Ther 1949;96:99–113
18. Gotelli C, Gotelli M, Boccio J, et al. Bioavailability of microencapsulated ferrous sulfate in fluid milk: studies in human beings. Acta Physiol Pharmacol Ther Latinoam 1996;46:239–45
19. Eakins JD, Brown DA. An improved method for the simultaneous determination of iron-55 and iron-59 in blood by liquid scintillation counting. Int J Appl Radiat Isotope 1966;17:391–7
20. Uicich R, Pizarro F, Almeida C, et al. Absorción de hierro de leche de vaca fluida: fortificada con sulfato ferroso encapsulado. Medicina Infantil 1996;3:9–13
21. Rapetti MC, Donato H, De Galvani A, et al. Correction of iron deficiency with an iron-fortified, fluid, whole cow's milk, in children: results of a pilot study. Am J Pediatric Hemotol Oncol 1997, in press