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Nutritional and Technological Behavior of Stabilized Iron-Gluconate in Wheat Flour

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ABSTRACT

Food fortification has shown to be an effective strategy to overcome iron malnutrition. When a new iron compound is developed for this purpose, it must be evaluated from a nutritional and technological point of view before adding it into foods. In this way, we have evaluated ferrous gluconate stabilized by glycine as a new iron source to be used in wheat flour fortification. We performed biological studies in rats as well as sensory perceptions by human subjects in wheat flour fortified with this iron source. The productions of pentane as a rancidity indicator as well as the change of the sensorial properties of the biscuits made with stabilized ferrous gluconate-fortified wheat flour were negligible. Iron absorption in water from this iron source was similar to the reference standard ferrous sulfate. Nevertheless, because of the phytic acid content, iron absorption from fortified wheat flour decrease 40% for both iron sources. The addition of zinc from different sources did not modify iron absorption from ferrous sulfate and stabilized ferrous gluconate in water and wheat flour. The iron absorption mechanism as well as the biodistribution studies demonstrate that the biological behavior of this

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iron source does not differ significantly from the reference standard. These results demonstrate that the iron source under study has adequate properties to be used in wheat flour fortification. Nevertheless, more research is needed before considering this iron source for its massive use in food fortification.

Index Entries: Iron; zinc; bioavailability; fortification; wheat; flour; human.

INTRODUCTION

Zinc and iron malnutrition affects the world population in a large scale. Food fortification and supplementation with these micronutrients are effective strategies to prevent these deficiencies, but a potential problem of using iron and zinc together is that one of these ions might inhibit the absorption of the other *(1–10).*

On the other hand, any iron source developed for food fortification must comply with some important requirements. These are a high iron bioavailability, inertness in relation to the sensorial properties of the fortified food, absence of toxicity, resistance during storing or elaboration processes of the fortified food, and the absorption mechanism should follow the same absorption pattern as dietary iron. Because most iron sources used for food fortification do not comply with all of these requirements, multiple efforts have been undertaken to optimize their quality *(11–14).*

In general terms, those iron compounds that are highly soluble in water generally have high bioavailability, but, at the same time, they produce severe oxidative damage in the foods into which they are added, leading to the production of off-odors or off-flavors. To the contrary, those iron sources of low water solubility do not significantly affect the sensorial properties of the fortified foods, but their bioavailability is poorer than that of the soluble iron sources. Nevertheless, from a structural point of view, some iron compounds used in food fortification are stable enough to prevent the interaction with other nutrients, but, at the time, to release soluble iron in the intestinal tract. The production of sensorial damage in fortified food is consequently avoided while high bioavailable iron is provided. Examples of these "protected" iron compounds are NaFeEDTA, microencapsulated iron, and some amino acid–iron complexes. In the same way, currently, food fortification is made with other micronutrients as it is the case of zinc, and as iron–zinc interactions are not still defined, it is advisable to direct more efforts to clarify this point *(11–14).*

In this research, we performed biological studies in rats as well as sensory perceptions by human subjects in wheat flour fortified with this iron source.

MATERIALS AND METHODS

Sensory Evaluation (Triangular Test)

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Standardized protocols of sensory evaluation were carried out on biscuits made with iron-fortified wheat flour to determine if the ferrous gluconate fortified by glycine (SIG) or ferrous sulfate (reference standard) sources promote the development of off-odors or off-flavors after regular periods of storing. The iron-fortifying agents were added to approx 5 kg of wheat flour at a final concentration of 65 mg Fe/kg. The fortified biscuits as well as an equivalent amount of nonfortified biscuits were made, cooked, and packed in several bags under an industrial procedure and stored at room temperature for 3 mo. The sensorial characteristics of the fortified and nonfortified biscuits were determined by a trained panel by means of a triangular test that was performed in adequate environmental conditions.

Physical and Chemical Stability

Parallel series of headspace gas chromatography (HSGC) were run on the headspace of sealed vials containing the fortified wheat flour (100 mgFe/kg) with SIG and ferrous sulfate, as the reference iron compound, to determine the quantity of accumulated pentane (rancidity indicator). The samples were placed in six different sealed vials, per iron source, and stored at room temperature (18–21°C). A sample of nonfortified wheat flour was also divided in the same way for control purposes. Pentane accumulation in the headspace of these vials was screened by HSGC at regular periods of time for 300 d.

Animals

We used inbred Wistar rats, 5 mo old, from the School of Pharmacy and Biochemistry, University of Buenos Aires. The rats were distributed in groups of 10 animals each. Each group was maintained in stainless-steel cages 315 mm × 445 mm × 240 mm high with a stainless-steel grated floor and collection trays of the same material, thus preventing the feces from coming into contact with the animals. They had free access to water and were nourished with a standard diet (Nutrimentos Diet no. 3). The animals were maintained with cycles of 12 h of light and 12 h of darkness throughout the experiment.

Administration of the Products

Each iron compound was intrinsically labeled with ^{59}Fe (NEN; Du Pont; catalog no. NEZ-037). The animals were food-deprived for 12 h before the administration of the preparations. The administration of the products was carried out through a syringe coupled to a plastic gastric

catheter, which allowed the standardization of the intake volume to 1 mL. Food was restored 6 h after the product intake. The iron dose was 73 μg/kg, and in the case of iron–zinc interaction, the zinc dose was 85 μg/kg (1 : 1 molar ratio) *(15).*

Radioactivity Measurement

The radioactivity retained by each rat was measured in a gamma spectrometer with a 5 × 5-cm NaI(Tl) well crystal in optimal electronic conditions. The ^{59}Fe radioactivity retained by each rat was measured using whole-body geometry, introducing each animal in a covered Lucite box, the size of which was similar to that of the animal and adequate to the detector geometry. In this way, detection errors attributed to eventual movements of the animal was minimized. The iron retention for each rat was determined. The retention percentage curve as a function of time was built for each rat. The iron absorption was determined by the extrapolation to the end of the iron percentage retention curve to the initial time by means of a linear regression analysis of the experimental data in order to eliminate the physical radioisotopic decay and the physiological loss *(15).*

Absorption Studies

The studies were performed to evaluate the iron bioavailability of SIG and its interaction with wheat flour in comparison with other iron sources like $^{59}FeSO_4$. Two groups of 10 animals each received the same iron compounds in water. In order to evaluate the effect of the nutritional matrix on iron absorption, other two groups of 10 rats each were administered wheat flour samples with ^{59}Fe–SIG and $^{59}FeSO_4$, respectively, added. In the case of the iron–zinc absorption, interaction we used similar groups of rats with the addition of zinc as zinc sulfate or stabilized zinc gluconate *(15,16).*

Absorption Mechanism

The studies were performed to determine if SIG follows the specific mechanism of intestinal regulation that protects the body from iron overload. For this purpose, we used a self-displacement study with increasing amounts of nonradioactive iron using $^{59}FeSO_4$ as the reference standard. Eight groups of 10 rats each received 10 μg of Fe^{2+} labeled with ^{59}Fe and different iron doses as ferrous sulfate or SIG *(17).*

Biological Distribution Studies

The studies were performed to determine whether the iron provided by SIG follows the same metabolic pathway as that provided by ferrous sulfate. These studies were performed in the same groups of rats used for the absorption ones. After the administration of the products, the rats were sacrificed 18 d later in order to determine the biodistribution pattern after

Table 1
Pentane Accumulation in the Headspace Determined by HSGC at Regular Periods of Time

Time (days)	0 day	60 days	150 days	210 days	300 days
SO_4Fe	Not detected	0.3 ppm	0.87 ppm	1.23 ppm	2.23 ppm
SIG	Not detected	Not detected	Not detected	Not detected	Not detected
Control	Not detected	Not detected	Not detected	Not detected	Not detected

Note: Pentane concentration in the headspace given as ppm (rancidity indicator). Iron concentration: 100 mg Fe/kg wheat flour. Samples stored at room temperature (18–21°C). Control: nonfortified wheat flour.

the end of the iron metabolic cycle. The ^{59}Fe activity was determined in blood and different organs such as the liver, spleen, carcass, gastrointestinal system, and kidneys. The results are given as percentage of the radioactivity concentration, *C*% [i.e., *(A/w)*%, where *A* is the radioactivity and *w* is the weight of each organ or tissue] *(18,19).*

Statistical Analysis

The data are given as mean ± SD. The results were evaluated by a two-way analysis of variance (ANOVA). To test the differences among the means, we used the Tukey method, considering statistically significant the probability levels <0.01 *(20,21).*

RESULTS

Sensory Evaluation (Triangular Test)

The sensory characteristics of the iron-fortified biscuits with SIG and nonfortified biscuits were not different for time 0, 1 mo, 2 mo, and 3 mo. In the case of fortified biscuits with the reference standard, ferrous sulfate, the sensorial characteristics were different for all the times. In the case of initial time (time 0) the color of ferrous sulfate-fortified biscuits was a little grayish compared to nonfortified biscuits. The taste of these fortified biscuits was also different for all the times.

Physical and Chemical Stability

In Table 1, we can see the results of pentane accumulation in the headspace (as rancidity indicator) determined by HSGC at regular periods of time. In the case of SIG and nonfortified wheat flour, we could not detect pentane accumulation in the headspace. In the case of ferrous sulfate-fortified flour, pentane accumulation was detected after 60 d of storage in our experimental condition.

Table 2
Iron Absorption and Zinc Interaction in Water and Wheat Flour

SO_4Fe in water	SIG in water	SO_4Fe + SO_4 Zn in water	SIG − SZG in water	SO_4Fe in flour	SIG in flour	SO_4Fe + SO_4 Zn in flour	SIG + SZG in flour
55.2±12.9%	52.6±12.3%	51.5±11.2%	54.6±12.3%	35.5±11.3%	34.9±10.4%	32.4±12.4%	33.4±13.1%

SZG: stabilized zinc gluconate *(22)*.

Table 3
Absorption Values of In Vivo Self-displacement Studies

	SO_4Fe + Zero	SO_4Fe + 100 ug Fe	SO_4Fe + 500 ug Fe	SO_4Fe + 1000 ug Fe
Ferrous Sulphate	41.6 ± 13.6%	21.3 ± 8.2%	6.7 ± 1.8%	5.8 ± 1.3%
SIG	41.8 ± 14.3%	19.5 ± 6.4%	7.1 ± 2.1%	5.6 ± 1.7%

Note: Displacement of $^{59}Fe^{2+}$ by increasing doses of iron from ferrous sulfate as the reference iron compound or SIG.

Absorption Studies and Iron–Zinc Absorption Interaction

Table 2 shows the results of iron absorption and zinc interaction in water or wheat flour. No differences on iron absorption were found among the groups that received ferrous sulfate or SIG in water with or without zinc. No differences on iron absorption were found among the groups that received ferrous sulfate or SIG in wheat flour with or without zinc. Nevertheless, the differences on iron absorption were significant ($p<0.01$) among the groups that received both iron compounds in water compared to the groups that received each iron source in wheat flour.

Absorption Mechanism

Table 3 shows that when the amount of administered nonradioactive iron increased, whether from SIG or from ferrous sulfate, the absorption of radiolabeled iron ($^{59}Fe^{2+}$) decreases. These findings demonstrate that the iron from either compound competes for the same specific binding sites and that this absorption mechanism is saturable. In other words, iron from SIG is transported through the intestinal wall in the same way as iron from the reference standard, ferrous sulfate, and is subject to the same regulatory mechanisms, which protect the body from iron overload.

Biological Distribution Studies

Table 4 shows that the highest concentration of iron was found in blood where iron forms a vital component of hemoglobin, the oxygen-carrying protein present in all red blood cells. A smaller amount of iron was found in the liver, where it is deposited as ferritin and hemosiderin (both iron-containing proteins). No significant differences were noted for iron distribution when the two experimental groups were compared. This

Table 4
Percentage of Radioactivity Concentration (*C*%) in Different Organs for Each Iron Compound

	Blood	Spleen	Liver	Kidneys	Heard	Lungs	Intestine	Carcass
SO_4Fe	64.4 ± 3.1	11.4 ± 2.4	6.1 ± 1.1	5.5 ± 0.8	4.8 ± 0.9	8.0 ± 3.0	0.5 ± 0.1	0.1 ± 0.01
SIG	63.7 ± 4.0	11.5 ± 2.9	6.0 ± 1.5	5.9 ± 1.0	5.4 ± 1.5	7.6 ± 2.6	0.5 ± 0.1	0.1 ± 0.04

Note: All values are given as mean ± SD. *C*% = *(A/w)*%, where *A* is the radioactivity measured of each tissue or organ and *w* is the weight; in all cases, the sum of the whole organs' radioactivity concentration was considered equal to 100% for each animal.

result suggests that the distribution and metabolism of iron was similar whether administered as SIG or as standard forms of ferrous sulfate. Thus, the iron provided by SIG has been shown to follow conventional physiological, metabolic, and biochemical pathways in the mammalian body.

DISCUSSION

One of the most important parameters to be taken into account in food fortification is the no change of the sensorial properties in fortified food. Several iron compounds with high bioavailability, in general, produce modifications on the taste and color of the fortified foods or they might produce rancidity because of fat oxidation. In this case, we observed that the sensorial properties of the fortified biscuits by SIG were not affected even 3 mo after the addition of the stabilized iron compound. In addition, fat oxidation was not detected by means of gas chromatography, demonstrating that under our experimental conditions, wheat flour fortified with SIG was more stable than in the case of ferrous sulfate (Table 1) *(11–14,23).*

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The absorption studies (Table 2) show that in water the bioavailability of SIG is similar to the reference standard, ferrous sulfate. In wheat flour, iron absorption values for both compounds are lower than in the case of water. This effect can be explained taking into account the composition of the nutritional matrix that in the case of wheat flour, the content of phytic acid, even in refined flour, is high enough to inhibit iron absorption *(14,24).* In none of the cases, under our experimental conditions, did we observe any negative interaction on iron absorption by the addition of zinc as stabilized zinc gluconate by glycine or zinc sulfate. On the other hand these results are interesting because they demonstrate that both iron compounds have the same absorption behavior. In this way, absorption mechanism of SIG demonstrated that it is similar to the reference standard, ferrous sulfate (Table 3). This fact is important because iron homeostasis is controlled at the level of the enterocites by a specific transport mechanism. These cells determine iron absorption according to the physiologic and metabolic needs of the body. For this reason, it is important that a compound used in

food fortification follow the specific mechanism of intestinal regulation that protects the body from iron overload *(25).*

Even though bioavailability is an important parameter for the study of a new iron source used for food fortification, it is also important to determine that this iron has a metabolic and physiologic behavior similar to the iron provided naturally by foods. With this purpose, the biodistribution studies of iron were carried out. Table 4 shows the radioactivity concentration percentage (*C*%) for each iron source and tissue. Because only the used iron source was labeled with ^{59}Fe, the measured radioactivity indicates the metabolic behavior of the iron provided by this source. The highest percentage was found in the tissues related to iron metabolism; in blood, nearly 64% values were found. Even though in the remaining tissues we found a smaller radioactivity concentration, their iron content has important functions because it is included in biomolecules, which are metabolically essential for these tissues. In no case were we able to observe any statistically significant difference in the radioactivity concentration percentage of any organ with regard to the iron sources, demonstrating that SIG has the same metabolic behavior as the reference standard, ferrous sulfate *(25).*

From a toxicological point of view, Lysionek et al. found that the acute oral toxicity in rats for SIG was LD_{50}-1775 and 1831 mg SIG/kg body wt for female and male rats. These results are significantly higher than the LD_{50} reported for ferrous sulfate, evidencing that SIG can be considered as a safe compound from a toxicological point of view *(23).*

These results demonstrate that the iron source under study has adequate properties to be used in wheat flour fortification. Nevertheless, more research is needed before considering this iron source for massive use in food fortification.

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