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Iron bioaccessibility and sensory analysis of extruded cereals fortified with different Fe sources

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Abstract: To increase iron (Fe) intake in Fe deficiency-risk groups the combination of Fe source and food-vehicle must be chosen in order to minimize inhibitory effects of food matrix. Fe dialyzability and sensory properties were tested in six model systems (MS) made with extruded cereals fortified with different Fe sources such as FeNaEDTA, $FeSO_4$ and EDTA/$FeSO_4$ among others and with or without the addition of milk. Proximate composition and phytate content were also evaluated. Results showed that Fe dialyzability from samples fortified with FeNaEDTA was less affected by the presence of inhibitory factors such as phytates and milk. The addition of $FeSO_4$ to the extrudates showed sensory differences. Furthermore, fortification with EDTA/$FeSO_4$ or FeNaEDTA showed no sensory differences compared with unfortified or Fe^o (elemental iron) fortified matrix, with the advantage of increased iron bioaccessibility.

Keywords: Extruded Cereals, FeNaEDTA, Iron, Bioaccessibility, Sensory Analysis

1. Introduction

Iron (Fe) deficiency is the most prevalent nutritional deficiency worldwide. However, it is more severe and pervasive in developing countries. This is primarily because the diets are mainly based on cereals, legumes and vegetables that contain many Fe-absorption inhibitors. The difficulty that human beings have to absorb enough iron from their diets leads to Fe nutritional deficiencies. This fact prevents to achieve their body requirements [1]. Although food fortification represents a good strategy to increase Fe intake in at-risk groups, it is worth noting that, unfortunately, the factors affecting the intrinsic Fe in foods also affect the Fe salts added to such foods [1]. Several organizations promote the use of *ferric sodium ethylenediaminetetraacetic acid* (FeNaEDTA) as a food fortifier in developing countries. FeNaEDTA is a unique compound that allows a high Fe availability in the presence of diverse inhibitory factors. At the same time it can be incorporated in many foods without causing adverse effects on the sensory properties such as taste, aroma or colour [2]. Another useful and more economical strategy is the use of other EDTA salts, such as sodium or calcium salts, combined with FeSO4 to achieve similar results [3]. The Joint FAO/WHO Committee of Food Additives has established an Accepted Daily Intake (ADI) of 2.5mg/kg body weight/day for EDTA compounds [4]. Besides, there are no evidences that these compounds have carcinogenic, teratogenic or other toxic effects under physiological doses [5].

It is necessary to specify the factors that should be considered when selecting the proper Fe compound; these factors might include: bioavailability, sensory properties, technological compatibility and costs, among others. Regarding these factors, the texture of extruded cereals is well accepted as there are many extruded foods commercially available (snacks, breaskfast cereals). The advantage of the extrusion as a technological process is that is relativelly unexpensive and it produces foods that do not need special storage conditions (i.e: low temperatures).

Bioavailability refers to the degree to which a substance is absorbed into a living system and is available at the site of

physiological activity. It is affected by both, dietary factors and the physiological condition of the individual. Dietary factors refer to the presence of Fe absorption inhibitors and promoters in food, while consumer factors refer to the individual's nutritional status. The term bioaccessibility refers to the amount of a substance that is available for absorption. Strictly bioavailability includes bioactivity as well as bioaccessibility [6], but most of the times bioavailability and bioaccessibility terms are used indistinctly, as stated below.

To evaluate bioavailability human trials constitute an invaluable tool for obtaining absolute values of mineral absorption. Nevertheless, they require complex designs, the use of stable or radioactive isotopes (greatly increasing their cost and reducing their accessibility for some laboratories) and approval by ethics committees. On the other hand, *in vitro* techniques are fast, relatively simple and less expensive than *in vivo* tests [2] and they allow greater control of experimental variables [7,8].

Several *in vitro* methods have been developed to estimate the mineral fraction available for absorption or in other words its bioaccessibility. These methodologies include the assessment of mineral dialyzability and uptake by CaCo-2 cell culture. Despite the fact that *in vitro* digestion (dialyzability or techniques using CaCo-2 cell cultures) do not accurately reflect the complexity of natural systems, the information obtained from these techniques regarding the effects of enzymes and pH may be applied to *in vivo* situations [9].

Another difficulty in Fe fortification is that Fe salts with high bioavailability such as ferrous sulfate ($FeSO_4$) catalyze oxidative changes in foods leading to off-flavors and colour changes. Ferrous sulfate is generally used as a metallic standard in applied sensory evaluation [10] and other Fe compounds can evoke this undesirable flavor [11]. Furthermore, Fe salts are characterized by a metallic retronasal smell in addition to astringency [12, 13]. Studies of divalent salts showed that they differ in the predominance of metallic, bitter and astringent sensations that they evoke. For example, ferrous salts evoke multiple sensory attributes and these sensory properties are generally unpleasant and may limit the use of Fe salts in food fortification. To alleviate this sensory problem, food scientists have tried various strategies, such as the use of chelated Fe [14].

Sensory properties of snack products are a key factor that guides the desire of consumers. The NaFeEDTA can become an alternative in food industries to provide fortified food with high Fe bioaccesibility but with less sensory impact. Thus, NaFeEDTA is attractive because of its chemical stability in long storage periods.

The main objectives of this paper were to evaluate Fe dialyzability (DFe%), proximate composition and inositol phosphates content from extruded cereals fortified with different iron sources, and to analyze the effect of various ferrous salts on sensory properties of 100% corn extruded cereals.

2. Method

2.1. Extrusion Conditions and Samples

Commercial corn, wheat and rice flours and rolled oat were purchase from the local market.

Cereals were extruded at pilot scale in the Institute of Food Technology, University of Litoral, using a Brabender 20DN single screw extruder. The extrusion process was carried out using a 4:1 compression ratio screw, a 3/20 mm (diameter/length) die and a screw speed of 175 rpm. While the extruder feeding section was maintained cool by circulating water through the jacketed device, the metering and die sections were both kept at 192ºC by using the heat control device of the extruder. The moisture content of the blends was 18%.

Extruded samples, later called "Model Systems", were the following:

M: corn flour *(Zea mays)* (100%)

MT: corn flour (75%) - wheat flour *(Triticum aestivum)* (25%)

MAv: corn flour (85%) – rolled oat (*Avena sativa*) (15%)

MAr: corn flour (75%) - rice flour *(Oryza sativa*) (25%)

MC: corn flour – commercial bitter cacao (5%)

MM: corn flour – commercial dry apple (15%)

Fat and fiber contents of the rolled oat limits its use in highly expanded extruded cereals [15]. For this reason MAv model systems were prepared with a mixture of 85% corn flour -15% rolled oat in order to obtain an acceptable expanded product.

Previous to the analysis of the different model systems, an optimum Fe: EDTA ratio was set. Thus, Fe dialyzability (DFe %) in model system M was evaluated using different Fe: EDTA ratios. Every 100g of extruded cereals, 7 mg of Zn as ZnO, 38 mg of ascorbic acid (AA) (AA:Fe 1:1) and 12 mg of Fe as $FeSO_4$ were added. Ascorbic acid was added to the formulation because all fortified commercial breaskfast cereals in Argentina contain this vitamin, and this compound is known to increase, in most cases, Fe bioavailability. In addition to this, Na_2EDTA was added in order to reach Fe: EDTA ratios of 1:0.3; 1:0.7 or 1:1. All model systems were tested with and without the addition of low fat milk (1.5% fat).

The lower Fe: EDTA ratio that provides the higher dializability was selected for the comparison of different Fe sources. Twenty four hours previous the dialyzability test, model systems were fortified with, 7 mg of Zn as ZnO, 38 mg of AA (AA:Fe 1:1) and 12 mg de Fe as NaFeEDTA, $FeSO_4$, $FeSO_4/Na_2EDTA$ or elemental Fe (electrolitic). All samples were tested with and without the addition of low fat milk.

Additionally M model system was fortified with 12 mg de Fe as ferrous fumarate or encapsulated ferrous sulphate.

2.2. Proximate Composition

Proximate composition of each model system subjected to the dialyzability test was assessed using the AOAC methods,

moisture by AOAC Nº 925.09, ashes by AOAC Nº 923.03, proteins by AOAC Nº 984.13 and fats by AOAC Nº 954.02 [16]. The factors (f) used to transform %Nitrogen in % of proteins were:

f = 6.25 for M; MC y MM [17]

f = 6.11 for MT. This factor was the result of the addition of 75% of the corn factor with 25% of the factor for wheat [18].

f= 6.19 for MAv. This factor was the result of the addition of 85% of the corn factor with 15% of the factor for oat [19].

f= 6.13 for MAr [19]. This factor was the result of the addition of 75% of the corn factor with 25% of the factor for rice Total dietary fiber content was determinated over dryed and defatted samples using AOAC Nº 985.29 adopted by a Megazyme® commercial kit [20].

The carbohydrates percentage was calculated as follows: % Carbohydrates = 100 – (% moist + % ashes + % proteins + % fats + % total dietary fiber)

2.3. Dialyzability Determination

Mineral dialyzability (D%) as a predictor of potential bioaccessibility was determined using the method of Miller et. al [21], modified by Wolfgor et al. [22]. The procedure involves an enzymatic digestion simulating physiological conditions. Each sample was homogenized and two portions of 15 g of each cereal were incubated with 5mL of a 3% aqueous solution of ∝-amilase (SIGMA) and 45mL of ultrapure water (EASY pure RF, Barnstead) or low fat milk (fat content: 1.5mg/100mL) , stirring during 30 min at 37º C, adjusted to pH 2 with a 6N HCl and 1.6mL of pepsin (16g/100mL in 0,1N HCl) was added. The mixture was incubated during 2h at 37º C. After this procedure, two 15g portions of the pepsine digests were placed separately in erlenmeyers with a dialysis bag (Spectrapore Molecular Weight cut-off 6000-8000) containing 18.75mL of 0.15M PIPES buffer and variable pH inside. The buffer´s pH was calculated after previous assays of the food matrix in order to obtain a pH of 6.5 ± 0.2 after the pancreatine incubation. When the first hour of incubation was completed, 3.75 mL pancreatine-bile solution (2.5% bile and 0.4% of pancreatine in 0,1N $NaHCO_3$) were added and the samples were incubated for another 2h [23]. After that period, the dialysis bags were removed from the erlenmeyers, the outer part of the bag was cleaned, and the content of was placed in assay tubes and subsequently weighed.

The Fe content of the two replicated digested samples and dialyzed Fe in PIPES buffer were determined using absorption spectroscopy after mineralization of the samples with HNO_3 -$HClO_4$ (50:50) (Merk – Carlo Erba).

Dialyzability was calculated as the percentage of the mineral dialyzed with regard to the total concentration of the mineral in the sample

$$\text{Fe Dialyzability \% (DFe\%)} = \frac{\text{mg of Fe in the dialysate x 100}}{\text{mg of Fe in the pepsine digest}}$$

Dialyzability of ferrous fumarate and encapsulated $FeSO_4$ was also assessed. These two other Fe sources were chosen because they have higher bioavailability values or lower sensory impact than $FeSO_4$ [2].

2.4. Total Fe and Zn Content

The total Fe and Zn contents of the model systems were assessed using atomic spectroscopy after mineralizing the samples with HNO_3 - $HClO_4$ (50:50) (J.T. Baker- Carlo Erba).

2.5. Inositol Phosphates (IP) Determination

The methodology was developed by Dyner et al. [24], optimizing the conditions for the separation of the inositols hexa, penta, tetra and tri phosphates (IP6, IP5, IP4 e IP3) was applied. An HPLC system comprising a 515 Waters pump, a refraction index detector (temperature 30ºC), a Rheodyne inyector with a 50 µL loop, 0.9mL/min flow and a C18 column (XBridge®; C18; 5µm; 4.6 x 150mm; Waters) was used. The mobile phase consisted in methanol: aqueous solution (51:49) pH=4.30. Each 100 mL of the aqueous solution contained: 89.6mL of 0.05M formic acid; 4.5mL of 0.05M Na_2EDTA, 4.7mL of 20% tetrabutyl ammonium hidroxide and 0.2mL of phytic acid (0.6g/100mL hydrolized in an autoclave during 40min, 121ºC and 1 atm). Data adquisition was made using Cromatography Station CSW de DataApex Ltd. All the reagents used were HPLC quality (J.T. Baker) and ultrapure water (EASY pure RF, Barnstead).

For IP`s extraction, 1g of the sample was mixed with 20mL of an aqueous solution of 0.5M HCl stirring during 2h at room temperature. The mixture was centrifuged for 20 min at 2000 rpm and the supernatant was filter through a 0.22 µm nylon membrane. The filtrated was dried and the residue was reconstituted with 15 mL of 25mM HCl.

Then, IPs were purified and concentrated using a 0,70g anion Exchange resin column (AG® 1-X4, 100-200 mesh, cloride form, BIO-RAD®) washed with 25mL of a 25mM aqueous solution of HCl. For the elution of IPs, 15 mL of a 2M aqueous solution of HCl was used and the collected fraction was dried out. Finally the sample was reconstituted with 1mL of ultrapure water (EASY pure RF, Barnstead) and it was ready to inject into the chromatographic system.

Many authors relate the total content of phytic acid (IP6) with mineral bioavailability. Thus, IPs were converted in IP6 adding the mols of phosphorous contributed by each of the different IPs and transforming them in IP6 using the molecular weight.

2.6. Sensory Analysis

2.6.1. Samples

Extruded Samples were 100% corn and extrusion conditions were the same described above. In this case samples were fortified before the extrusion process and were classified as follows: Controls: C1: extruded corn without addition of iron,

C2: extruded corn with addition of 120 mg/kg of Fe as

electrolitic Fe (elemental Fe). Test samples: S1: extruded corn with addition of 120 mg/kg of Fe as FeNaEDTA, S2: extruded corn with addition of 120 mg/kg of Fe as $FeSO_4$: EDTA (molar ratio Fe: EDTA, 1:0.7), S3: extruded corn with addition of 120 mg/kg of Fe as $FeSO_4$.

2.6.2 .Sensory Analysis

Sensory analysis was performed 2-3 month after the sample production using triangle test (discriminative technique). Thirty three potential candidates gave their written informed consent at the beginning of the sensory task.

Participants had apparent good health and reported no problems in olfactory or gustatory functions. Judges were selected and trained for the test during 3 sessions. The objective of the first session was to familiarize with the metallic taste of $FeSO_4$ solutions. For doing that, different concentrations (0 to 32 mg/L of $FeSO_4$) were presented to the judges and they were asked to order the different solutions from the one that presented null or the least metallic taste to the one that presented the strongest metallic taste. In the second stage of selection 3 triangles were presented to the judge using the same solutions than in the first stage of selection. The objective of this stage was to familiarize the judges with the type of test they had to perform. Finally, the judges received 3 triangles with the samples C1 vs. S3 and 3 triangles of the samples C2 vs. S3. They had to be able to pick odd sample correctly 2 out of 3 times for all combinations.

2.7. Statistical Analysis

The statistic analysis was performed using ANOVA and post-hoc Tuckey test. Significant differences were established at $p < 0.05$.

3. Results and Discussion

3.1. Proximate Composition of Model Systems

Proximate composition was determined before the addition of Fe, Zn and ascorbic acid in order to obtain the macronutrient characterization of the model systems. Results are summarized in table 1.

Table 1. *Proximate composition and mineral content of the different model systems (dry base).*

	Protein	Fat	Fibre	Ash	Fe	Zn	Ca
M	8.7^{c}	1.2^{a}	5.0^{c}	0.2^{c}	1.0^{d}	0.7^{c}	1.7^{d}
MT	9.3^{b}	1.9^{a}	5.4^{c}	0.5^{b}	1.2^{c}	0.8^{b}	1.9^{d}
MAv	9.7^{a}	1.7^{a}	5.9^{b}	0.7^{a}	1. 6^{b}	1.2^{a}	7.8^{a}
MAr	8.6^{c}	1.6^{a}	3.3^{d}	0.5^{b}	0.5^{e}	0. 6^{d}	3.3^{c}
MC	9.5^{a}	1.8^{a}	5.0^{c}	0.7^{a}	2.0^{a}	0.7^{cd}	4.9^{b}
MM	7.7^{d}	1.2^{a}	6.7^{a}	0.7^{a}	0.7^{e}	0.4^{e}	1.4^{e}

Proximate composition (g/100g). Mineral content (mg/100g). Different letters in each column indicate significant differences between samples ($p<0,05$).

Even though statistically significant differences among the samples were observed in Table 1, these differences are not of nutritional significance.

3.2. IPs Content of the Model Systems

The IP contents are shown in table 2.

Table 2. *IPs content of the different model systems and molar ratio between phytic acid and Fe with and without the addition of milk*

	M	MT	Mav	MAr	MC	MM
IP3 (mg/100g)	11.6^{b}	14.7^{a}	9.2^{cd}	8.1^{d}	10.3^{bc}	9.1^{cd}
IP4 (mg/100g)	33.0^{b}	41.3^{a}	25.4^{cd}	23.2^{d}	29.2^{bc}	26.0^{cd}
IP5 (mg/100g)	65.3^{ac}	65.7^{b}	110.5^{a}	49.2^{e}	59.2^{cd}	52.9^{d}
IP6 (mg/100g)	76.9^{b}	65.9^{bc}	250.0^{a}	54.1^{c}	63.5^{bc}	56.5^{c}
[phytic acid]*:[Fe] without milk	15	12	21	19	7	17
[phytic acid]*:[Fe] with milk	13	11	19	16	6	14

IP3: inositol triphosphate, IP4: inositol tetraphosphate; IP5: inositol pentaphosphate and IP6: inositol hexaphosphate. Different letters in each column show significant statistical differences ($p<0.05$).*obtained calculating the content of phosphorous of IP3, IP4, IP5 e IP6 and transforming everything in IP6 using the molecular weight [24], M: corn flour (Zea mays) (100%); MT: corn flour (75%) + wheat flour (Triticum aestivum) (25%); MAv: corn flour (85%) + rolled oat (Avena sativa) (15%); MAr: corn flour (75%) + rice flour (Oryza sativa) (25%); MC: corn flour + commercial cacao (5%); MM: corn flour + commercial dry apple (15%)

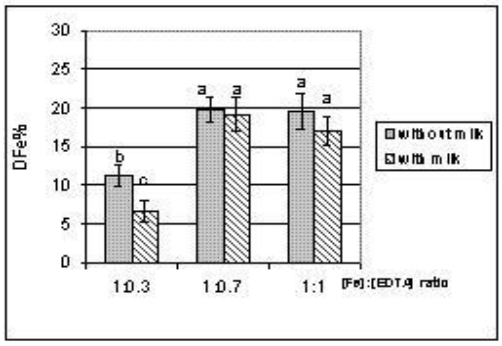
The higher proportion of IP5 and IP6 in MAv in relation to the other model systems becomes evident in table 2. It is important to notice that these two types of IPs are the ones that showed the main negative effects over Fe bioavailability [24].

The last two rows of Table 2 depict the molar ratios between phytic acid (PA) and Fe with and without the addition of milk. Some authors have proposed that the: PA:Fe ratio should not be higher than 6, although others have suggested that it should be lower than 1 to avoid compromising Fe absorption [25].These proposed ratios have limited utility because they do not consider the presence of other Fe absorption enhancers or inhibitors present in the diet.

Comparing these proposed PA:Fe molar ratios with the ones obtained in the present model systems it is suggested that Fe bioaccessibility would be compromised by the presence of PA in all cases.

3.3. Iron Dialyzability from Samples with Different Fe: EDTA Molar Ratios

The results of DFe% of M samples (100% corn flour) fortified with $FeSO_4$/Na_2EDTA using different [Fe]:[EDTA] molar ratios (1:0.3; 1:0.7 y 1:1) are shown in Figure 1.



Different letters mean significant differences ($p<0.05$)

Figure 1. *DFe% of samples M fortified with different ratios $FeSO_4/Na_2EDTA$, (n=6, mean ± sd). DFe%: Iron dializability, M: corn flour (Zea mays) (100%).*

Figure 1 show that DFe% increased significantly as the ratio of $FeSO_4/Na_2EDTA$ was increased from 1:0.3 to 1:0.7, but not when the ratio was increased from 1:0.7 to 1:1. It was mentioned previously that EDTA has an ADI value of 2.5 mg/kg/person/day.In order to keep the potential daily intake of this compound as low as possible, the 1:0.7 ratio with an intermediate concentration of EDTA but allowing the highest DFe%, was chosen.

3.4. Iron Dialyzability from Different Model Systems

The results of DFe% of the different model systems fortified with NaFeEDTA, $FeSO_4$, $FeSO_4$/EDTA or Feº, with or without the addition of milk are presented in the Figure 2A and B.

In model systems fortified with different Fe sources but without milk (Figure 2A), the effect of the food matrix was not as evident as expected. In general, there were no significant differences among M, MT and MAr. The negative effect of cacao´s polyhenols [26] expected in the case of MC was more evident when the samples were fortified with NaFeEDTA or Feº without the addition of milk.

On the contrary, the negative effect of apple polyphenols expected for MM was not observed. The organic acids present in the dried apple could enhance DFe% overcoming the negative effect of other inhibitors of this sample. Regarding that, malic acid occurring in apple could complex the Fe avoiding its bonding with inhibitory ligands.

It is interesting to notice that at acidic pH (range between 2 and 4), the apparent constants of complex formation for EDTA-Fe are low, appearing a competition for the Fe between the EDTA moiety and the ascorbic acid [27].

However, in the case of the samples that contained both, EDTA and apple, a combination of the positive effects of EDTA and the malic acid could be considered.

All the samples that contained oat presented an evident highest viscosity throughout the entire dialyzability assay; this fact could interfere with the DFe% in addition to the presence of inhibitory components such as phytic acid.

As table 2 shows, sample MAv is the one that presented the higher content of IP6 and IP5, both related to the inhibitory effect of phytates. Again, this sample presented a higher [Phytic acid]:[Fe] ratio so it would be expected that the DFe% would be lower than the rest of the model systems.

It is important to remember that the method used here to evaluate the Fe availability is an *in vitro* method that simulates the process of human digestion. The last stage of the assay is the dialysis that corresponds to the small intestine absorption stage of human digestion. Recently it had been described the possibility that, under special situations, absorption of minerals in the large intestine could take place. Among the factors under study, the presence of fermentable fiber, the consistent production of short chain fatty acids and the lowering of the large intestine´s pH could promote the absorption of minerals. This affirmation has been scientifically proved for calcium [28,29]. This is why, it could be expected (if the hypothesis for Fe is confirmed) that the presence of fermentable fibre would enhance Fe absorption in the large intestine. However, this positive effect would have an impact over all the different fortificants improving the bioavailability of Fe independently of the Fe source used. This affirmation must be proved in further investigations.

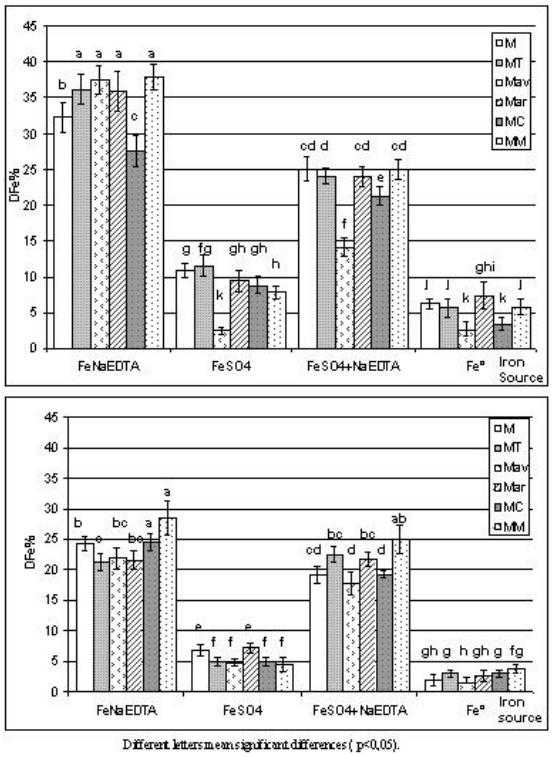


Figure 2. *Top DFe% of the different model systems fortified with NaFeEDTA, FeSO4, FeSO4/EDTA or Feº, without the addition of milk, n=6, (mean + sd)). Bottom DFe% of the different model systems fortified with NaFeEDTA, FeSO4, FeSO4/EDTA or Feº, with the addition of milk. DFe%: Iron dializability, M: corn flour (Zea mays) (100%); MT: corn flour (75%) + wheat flour (Triticum aestivum) (25%); MAv: corn flour (85%) + rolled oat (Avena sativa) (15%); MAr: corn flour (75%) + rice flour (Oryza sativa) (25%); MC: corn flour + commercial cacao (5%); MM: corn flour + commercial dry apple (15%)*

The negative effect of milk over the DFe% is obvious when comparing figures 2A and B. Nevertheless, this effect is less pronounced in the model systems that contained EDTA (FeNaEDTA or Na2EDTA). For example, when Fe is added as NaFeEDTA, DFe% ranged between 27 and 38% without addition of milk and between 21 and 28% with the addition of milk (a dialyzability reduction of 25%). On the other hand, when the Fe is added as FeSO4 this value ranged between 8 and 11.5% without the addition of milk (with the exception of Mav) and between 5 and 7% with addition of milk (a dialyzability reduction of 40%).

The DFe% obtained for samples fortified with $FeSO_4$/EDTA could not reach the values obtained for FeNaEDTA. This may be due to the heterogeneity of the different components (Fe and EDTA) throughout the sample and the difficulty to form the Fe-EDTA complex. In the case of FeNaEDTA the complex is already formed but when EDTA and Fe are added separately both parts of the complex have to interact with each other to avoid the negative effects of the food matrix.

3.5. DFe% of Six Different Fe Sources

Results of DFe% of model system M fortified with NaFeEDTA, $FeSO_4$, $FeSO_4$/EDTA, Feº, ferrous fumarate and $FeSO_4$ encapsulated are shown in Figure 3. Results of the first 4 sources were presented previously in Figure 2 but they were also included here to compare the results.

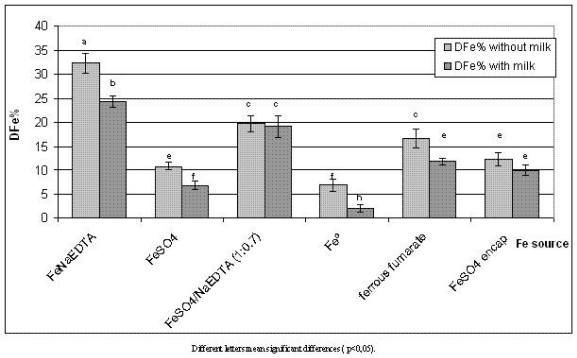


Figure 3. *DFe% from six different Fe sources (FeNaEDTA, $FeSO_4$, $FeSO_4$/EDTA, Feº, ferrous fumarate and $FeSO_4$ encapsulated) with or without the addition of milk, n=6, (mean ± sd). DFe%: Iron dializability*

The results of DFe% from samples added with ferrous fumarate or encapsulated FeSO4 are higher than with FeSO4 or Feº. Nevertheless, these values are still lower than those obtained with NaFeEDTA or FeSO4/EDTA. This increase in Fe availability may give the opportunity to lower the level of Fe added to the samples and still obtain satisfactory results from a Fe-bioaccessibility point of view.

This would reduce the production costs and the negative effects of iron over other nutrients (for example lipid oxidation). A previous research showed that consuming every day 7,1 mg of Fe as $FeSO_4$, (equivalent to 7,1 mg of Fe as ferrous fumarate; 4,6 mg of Fe as NaFeEDTA or 10 mg of electrolitic Fe) through fortified flour could improve the level of Fe in women at childbearing age [30].

3.6. Sensory Analysis of Model Systems

The individuals that could not discriminate at least water and 32 mg/L of $FeSo_4$ were rejected and the remaining passed to the next selection stage.

The judges had to identify correctly 2 out of 3 triangles in order to continue training.

As a result of the selection/training steps 18 judges were selected out of 33.

A minimum number of 18 correct responses were required for significance at the stated α-level (0.05) for a total of 36 obtained judgments [31]. The rejection of the assumption of "no difference" was stated when the number of correct responses was greater than or equal to this critical number of 18 correct responses.

Figure 4 depicts the hits to discriminate fortified extrudates. Judges noticed significant sensory differences between extruded corn with the addition of 120mg/Kg of Fe as $FeSO_4$ (S3) and the control of extruded corn without the addition of iron (C1). The others iron fortified extrudates were not significantly different from C1 and C2 ($p > 0.05$), i.e. judges did not achieve the minimum number of hits to identify the odd sample.

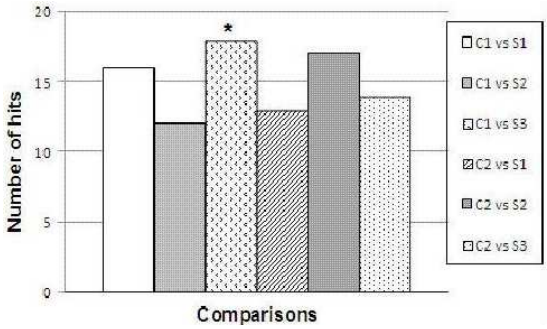


Figure 4*. Sensory analysis of fortified extrudates. Number of hits in the sensory discrimination of fortified cereals. C1 and C2 are controls without and with addition of iron. S1, S2 and S3 are the different model systems fortified with NaFeEDTA, FeSO4/EDTA (1:0.7) and FeSO4, respectively. n=36,* significant difference at p < 0.05.*

The addition of FeSO4 to the extrudates evoked differences in sensory properties and therefore, if the objective is to develop a food fortified with this salt, it should be addressed how to avoid the unpleasant flavor that will give this compound to food, for example by adding other ingredients or additives which improve its taste [32]. However, since this is a very simple food matrix, i.e. without addition of flavorings or other ingredients, it is encouraging to find that the use of NaFeEDTA or EDTA/FeSO4 does not impact significantly from the sensory viewpoint.

4. Conclusions

Fortification of extruded cereals with NaFeEDTA would increase Fe availability in relation to other Fe sources. This fact is independent of the model system evaluated. The increase in Fe availability could lead to decrease the level of Fe added to the samples and still obtain satisfactory results

from a Fe-bioavaility or Fe-bioaccessibility point of view. This would reduce the production costs and the negative effects of iron over other nutrients (for example lipid oxidation).

A less expensive strategy would be the use of $FeSO_4$/EDTA, but this should be considered carefully. A key point of adding two different compounds that need to interact to obtain the searched results is the homogeneity of each compound in the mixture. On the contrary, when using NaFeEDTA the complex is already formed.

Sensory results showed that all but one of the iron-combinations bypass unpleasant metallic notes. This contributes to reinforce specifically the use of FeNaEDTA as it was previously mentioned.

The results obtained here could be used for the design of fortified extruded cereals using NaFeEDTA o EDTA/$FeSO_4$ as no negative impact was noticed from a sensory point of view.

Nevertheless, at the time of developing other food products using these extruded model systems it must be taken into account the impact of Fe over other ingredients. In a near future, if the aims are to increase the unsaturated fat in a healthy extruded product or if these extrudates are added to a soup to increase its nutritional value or if they will be added to dried fruit to be eaten as breakfast cereals it will be imperative to perform a new sensory analysis to verify the acceptability of each new product.

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