Research Article

Received: 20 March 2013 Revised: 00 2013 Accepted article published: 21 June 2013 Published online in Wiley Online Library: 30 July 2013

(wileyonlinelibrary.com) DOI 10.1002/jsfa.6280

# Food matrix and cooking process affect mineral bioaccessibility of enteral nutrition formulas

**María Gimena Galán[a,b]* and Silvina Rosa Drago[a,b]**

## Abstract

**BACKGROUND: When enteral formulas (EF) are administered orally as a supplement to the normal diet, they are often mixed with conventional foods or included in recipes in order to seek new flavors and textures and avoid monotony. The aims of this work were to study the bioaccessibility of Fe, Zn and Ca from commercial EF and the impact upon their incorporation into sweet preparations. Twenty commercial EF, before and after inclusion in sweet food (rice pudding, RP; banana smoothie, BS; tea, T; chocolate dessert, CD) were evaluated regarding Fe, Zn and Ca dialyzability (%$D_{Fe}$, %$D_{Zn}$, %$D_{Ca}$) as an estimator of mineral bioaccessibility.**

**RESULTS: Fe, Zn and Ca dialyzability from EF was variable and generally low. Heating during EF–sweet food preparation (T and CD) lowered values to 44.1 %$D_{Fe}$, possibly due to degradation of vitamin C, and 52.7 %$D_{Zn}$ and 25.3 %$D_{Ca}$, due to the interaction with food components.**

**CONCLUSION: EF and EF–sweet foods did not represent a good supply of Fe, Zn and Ca as recommended. This study demonstrated how the bioaccessibility of these minerals is affected by the food matrix in which EF is included as well as heating during food preparation.**


**Keywords:** enteral formulas; bioaccessibility; minerals; iron; zinc; calcium

## INTRODUCTION

Enteral nutrition is a widely used technique for patients at nutritional risk and is a good alternative to parenteral nutrition for patients with functional digestive tract.[1] Nowadays, there is much evidence of the benefits of this practice.[2]

Enteral formulas (EF) are complex food systems which have all the necessary nutrients in their matrix for complete human nourishment. However, there are components in EF which can interact with minerals, reducing their absorption and therefore EF nutritional quality.[3]

Minerals are essential nutrients involved in more than 100 enzymatic reactions, which perform functions in macronutrient synthesis and physiological processes in the human organism. Mineral absorption depends not only on mineral content and the chemical form in a particular food, but also on other food components, individual physiological factors and interactions among elements.[4] Consequently, to estimate the supply of a trace element from the diet it is not sufficient to determine only its total content, but also to know how much is absorbed and used, i.e. what is known as bioavailability.[5]

Factors that influence the bioavailability of trace elements can be classified into two major groups: intrinsic or physiological, and extrinsic or dietary factors.[6] The physiological factors are complex. Among them can be mentioned the species, age, potential characteristics and genetic abnormalities, physiological and nutritional status (pregnancy and lactation), possible pathological conditions, intestinal flora, the pH of gastric and intestinal juices and, especially, the adaptability of the individual to variations in the nutrient supply, which affect the bioavailability of minerals for absorption and metabolism. Dietary factors include: amounts of the trace element in the diet or food, the chemical form and properties, physical properties (solubility, adsorption capacity on inert components of the food), capacity to react with other components of the food matrix or drug, biochemical properties and the ability to compete with other elements for organism active sites.[4] In this regard, the food matrix may contain substances which act as promoters or inhibitors of absorption. Phytates and polyphenols are potent inhibitors of Fe, Zn and Ca absorption.[7]

Dialyzability is an *in vitro* technique that can be used as an estimator of potential availability or bioaccessibility of a particular mineral. It is the ratio of an element that diffuses through a semipermeable membrane during simulated gastrointestinal digestion, after a period to allow equilibrium.[8] Although no *in vitro* method can reproduce the physiological conditions prevailing in *in vivo* studies, Fe dialyzability showed similar results to those obtained in human studies.[9] Even though it was only validated for iron, it was also used to measure the availability of other minerals such as Zn, Ca, Mg and Cu. Kennefick and Cashman[10] found that dialyzability can be a useful screening method for

* *Correspondence to: María Gimena Galán, Instituto de Tecnología de Alimentos, Facultad de Ingeniería Química, Universidad Nacional del Litoral, Santiago del Estero 2829 (CP 3000), Santa Fe, Argentina. E-mail: mggalan@fbcb.unl.edu.ar*

a *Instituto de Tecnología de Alimentos, Facultad de Ingeniería Química, Universidad Nacional del Litoral, Santa Fe, Argentina*

b *CONICET, Buenos Aires, Argentina*

assessing the effect of dietary factors such as phytate, oxalate, fiber and caseino-phospholipid lactose on calcium absorption, since it shows good correlation with the results of *in vivo* studies.

EF are sometimes used in prolonged treatment, which can take several months or even years. When they are administered orally as a supplement to the normal diet, they are often mixed with conventional foods or included in recipes in order to seek new flavors and textures and avoid monotony. However, there are no studies that analyze mineral bioaccessibility of EF when they are included in food matrices or undergo cooking processes. For the above mentioned, this paper aims to study the bioaccessibility of Fe, Zn and Ca from commercial EF and the impact upon their incorporation into sweet preparations.

## MATERIALS AND METHODS

### Reagents and materials

*Raw materials*

Distilled water was purified using an Easy Pure II RF System (Barnstead International, IA, USA), and this purified water was used for preparing all the solutions. AAS Titrisol standards containing 1000 mg Fe, Zn and Ca were used to prepare single-element stock standard solutions. PIPES (piperazine-*N*,*N*′-bis[2-ethanesulfonic acid] disodium salt) buffer, digestive enzymes and bile salts were purchased from Sigma Chemical Co. (St Louis, MO, USA). These reagents were used to prepare simulated gastric and intestinal juices. Spectra/Pore dialysis tubing (cut-off 6000–8000) was purchased from Fischer Scientific (Fairlawn, NJ, USA). All other chemicals were reagent grade. All glass materials and polyethylene bottles were washed with distilled and deionized water, kept for 24 h in 5 mol $L^{-1}$ nitric acid, and again washed with distilled–deionized water before use.

*Reagents*

EF were purchased from the market. Brands correspond entirely to international companies, both European and American.

A total of 20 EF were analyzed. EF included 12 powder form (EFP, numbered from 1 to 12) and eight fluid form (EFL, numbered from 13 to 20). EFP were reconstituted according to label instructions. All EF were isocaloric and isoproteic.

The samples of EF declared ferrous sulfate and zinc sulfate as iron and zinc fortification sources. One exception was EFP3, where the iron source was ferric pyrophosphate. Calcium sources varied according to the different formulations, and a mixture of different calcium salts was used in some formulas.

### Procedures

*EF–sweet food preparation*

EF were included in desserts (rice pudding, chocolate dessert) or drinks (tea and banana smoothie), commonly consumed in our region, using the following recipes:

- *Rice pudding (RP)*: 40 g white rice was cooked for 20 min, drained and allowed to cool. Then, 100 mL EFL or reconstituted EFP, 40 g white sugar, 5 drops of vanilla extract and 3 g powdered cinnamon were added.
- *Chocolate dessert (CD)*: 250 mL reconstituted powder milk and 50 g chocolate dessert powder were mixed and heated. When this mixture boiled, it was removed from the heat and 55 g EFP were added slowly. It was then allowed to cool at 4 °C for 2 h. In the case of fluid formulas, 250 mL EFL was added to 32.5 g milk powder and 50 g chocolate dessert powder. The mixture was cooked until boiling and cooled in the same way.
- *Tea (T)*: 200 mL water was boiled and 50 g of EFP was added. A black tea bag (3 g) and 40 g white sugar were added, mixed and allowed to stand for 30 min. The tea bag was then removed. In the case of EFL, 200 mL boiled formula was used and the preparation proceeded in the same way as described for EFP.
- *Banana smoothie (BS)*: 200 mL reconstituted EFP or EFL, 70 g ripe banana and 20 g sugar were blended for 3 min using a blender.

These EF–sweet foods were divided into two categories: those which were heated by cooking (H), i.e. CD and T, and those which were not heated (NH), i.e. RP and BS.

*Total concentration of minerals*

To determine the concentration of minerals a an appropriate amount of weighed sample was carbonized and heated at 550 °C for 4 h. The ashes were lifted with 10 mL of 1.2 mol $L^{-1}$ HCl. The content of Fe, Ca and Zn was then measured using a single-beam flame atomic absorption spectrophotometer (Analyst 300, PerkinElmer, Norwalk, CT, USA).

Sample solutions were analyzed *versus* standard solutions with concentrations of 1.0–8.0 mg $L^{-1}$ (Ca and Fe) and 0.4–1.4 mg $L^{-1}$ (Zn) and suitable procedural reagent blanks. Precision, expressed as relative standard deviation (RSD) for repeated measurements of standard used for the calibration, was better than 5% (Ca, Zn) and 10% (Fe).

*Determination of bioaccessibility and potential supply of Fe, Zn and Ca*

A modification of the widespread *in vitro* Miller *et al.*[11] method, according to Drago *et al.*,[12] was followed. Aliquots (25 g) of homogenized samples were adjusted to pH 2.0 with 6 mol $L^{-1}$ HCl and, after addition of 0.8 mL pepsin digestion mixture (16 g 100 $mL^{-1}$ pepsin, solution in 0.1 mol $L^{-1}$ HCl), were incubated at 37 °C for 2 h in a shaking water bath. At the end of pepsin digestion, dialysis bags containing 20 mL of 0.19 mol $L^{-1}$ PIPES buffer were placed in each flask and were incubated for 50 min in a shaking water bath at 37 °C. Pancreatin–bile mixture (6.25 mL of 2.5 g 100 $mL^{-1}$ bile, 0.4 g 100 $mL^{-1}$ pancreatin, solution in 0.1 mol $L^{-1}$ $NaHCO_3$) was then added to each flask and the incubation continued for another 2 h. Bag contents were then weighed and analyzed for mineral content by flame atomic absorption spectrophotometry. Mineral dialyzability was calculated from the amount of each dialyzed mineral expressed as a percentage (%*D*) of the total amount present in each sample:

$$\%D = \left[D/\left(W \times M\right)\right] \times 100$$

where *D* is the total amount of dialyzed mineral (μg), *W* is the weight of sample (g) and *M* is the concentration of each mineral in the sample (μg $g^{-1}$).

Potential mineral supply (PS) was calculated considering a 200 g serving, using the following formula:

$$PS = M \times \%D \times \text{g serving}$$

### Statistical analysis

All analyses were performed in triplicate. Analysis of variance (ANOVA) was carried out using the software Statgraphics Plus 5.1, and the statistical differences among samples were determined



  

**Table 1.** Dialyzability of Fe (%$D_{Fe}$), Zn (%$D_{Zn}$) and Ca (%$D_{Ca}$) from enteral formulas and EF–sweet foods

| Sample | %$D_{Fe}$ | %$D_{Zn}$ | %$D_{Ca}$ | Sample | %$D_{Fe}$ | %$D_{Zn}$ | %$D_{Ca}$ |
|---|---|---|---|---|---|---|---|
| EF1 | 4.67 ± 0.22c | 6.68 ± 0.23d | 5.22 ± 0.29a | EF11 | 4.58 ± 2.66c | 1.67 ± 2.93b | 13.17 ± 7.46d |
| EF1 + T | 2.48 ± 0.13b | 4.65 ± 0.17c | 8.21 ± 0.20b | EF11 + T | 1.24 ± 0.03b | 0.18 ± 0.00a | 9.22 ± 0.28c |
| EF1 + CD | 2.67 ± 0.01b | 1.20 ± 0.01a | 10.00 ± 0.55c | EF11 + CD | 0.55 ± 0.03a | 2.70 ± 0.11c | 6.48 ± 0.24a |
| EF1 + RP | 0.93 ± 0.05a | 1.55 ± 0.01b | 5.83 ± 0.23a | EF11 + RP | 5.94 ± 0.03d | 1.61 ± 0.07b | 7.78 ± 0.37b |
| EF1 + BS | 4.58 ± 0.11c | 4.68 ± 0.23c | 20.37 ± 0.90d | EF11 + BS | 6.74 ± 0.36e | 7.83 ± 0.44d | 18.10 ± 0.73e |
| EF2 | 2.06 ± 0.31b | 6.22 ± 0.27c | 4.21 ± 0.23a | EF12 | 3.28 ± 0.18b,c | 7.29 ± 0.29c | 16.50 ± 0.59c |
| EF2 + T | 1.18 ± 0.02a | 1.56 ± 0.08a | 8.68 ± 0.35c | EF12 + T | 0.63 ± 0.04a | 0.15 ± 0.01a | 8.39 ± 0.33b |
| EF2 + CD | 1.98 ± 0.01b | 1.36 ± 0.07a | 4.44 ± 0.17a | EF12 + CD | 0.46 ± 0.02a | 1.58 ± 0.03b | 4.67 ± 0.11a |
| EF2 + RP | 0.75 ± 0.04a | 2.29 ± 0.08b | 7.12 ± 0.02b | EF12 + RP | 3.06 ± 0.16b | 0.90 ± 0.04a,b | 7.69 ± 0.34b |
| EF2 + BS | 3.41 ± 0.01c | 10.84 ± 0.01d | 10.02 ± 0.29d | EF12 + BS | 3.52 ± 0.17c | 16.13 ± 0.59d | 17.03 ± 0.84c |
| EF3 | 1.70 ± 0.08b | 3.50 ± 0.21d | 5.13 ± 0.19b | EF13 | 1.42 ± 0.09b | 0.52 ± 0.02a | 11.89 ± 0.59b |
| EF3 + T | 1.80 ± 0.02b | 2.10 ± 0.10c | 9.87 ± 0.19d | EF13 + T | 0.86 ± 0.01a | 5.15 ± 0.30e | 12.39 ± 0.05b |
| EF3 + CD | 0.75 ± 0.01a | 0.67 ± 0.01a | 3.73 ± 0.17a | EF13 + CD | 1.44 ± 0.07b | 1.43 ± 0.07c | 5.92 ± 0.20a |
| EF3 + RP | 2.50 ± 0.02c | 2.09 ± 0.11c | 7.48 ± 0.29c | EF13 + RP | 8.93 ± 0.27d | 2.19 ± 0.11d | 27.06 ± 0.72d |
| EF3 + BS | 3.10 ± 0.03d | 1.36 ± 0.03b | 12.08 ± 0.66e | EF13 + BS | 5.40 ± 0.26c | 0.95 ± 0.03b | 14.62 ± 0.46c |
| EF4 | 2.41 ± 0.09d | 12.00 ± 0.77d | 4.81 ± 0.04b | EF14 | 2.28 ± 0.16b | 7.10 ± 0.31d | 10.49 ± 0.25b |
| EF4 + T | 0.63 ± 0.02a | 0.83 ± 0.03a | 7.93 ± 0.41c | EF14 + T | 2.28 ± 0.06b | 2.69 ± 0.09b | 9.23 ± 0.13b |
| EF4 + CD | 0.66 ± 0.04a | 0.52 ± 0.02a | 2.57 ± 0.11a | EF14 + CD | 0.97 ± 0.05a | 1.08 ± 0.03a | 4.44 ± 0.08a |
| EF4 + RP | 1.16 ± 0.00b | 2.31 ± 0.11b | 5.32 ± 0.04b | EF14 + RP | 8.90 ± 0.39d | 3.87 ± 0.15c | 23.82 ± 0.97d |
| EF4 + BS | 2.19 ± 0.01c | 7.94 ± 0.40c | 8.59 ± 0.20d | EF14 + BS | 3.82 ± 0.05c | 2.64 ± 0.05b | 18.40 ± 0.89c |
| EF5 | 1.65 ± 0.11b | 5.93 ± 0.29d | 14.45 ± 0.60d | EF15 | 1.22 ± 0.05a | 5.13 ± 0.02e | 3.82 ± 0.17a |
| EF5 + T | 1,44 ± 0.01a,b | 1.95 ± 0.05b | 12.42 ± 0.30c | EF15 + T | 1.41 ± 0.07a | 1.79 ± 0.08b | 8.78 ± 0.15c |
| EF5 + CD | 1.27 ± 0.03a | 0.80 ± 0.02a | 5.59 ± 0.19a | EF15 + CD | 1.37 ± 0.06a | 1.93 ± 0.05c | 6.03 ± 0.21b |
| EF5 + RP | 4.82 ± 0.21d | 2.36 ± 0.10c | 10.83 ± 0.45b | EF15 + RP | 6.04 ± 0.19c | 2.91 ± 0.10d | 28.44 ± 0.92e |
| EF5 + BS | 3.89 ± 0.19c | 14.35 ± 0.23e | 15.01 ± 0.88d | EF15 + BS | 2.92 ± 0.10b | 0.39 ± 0.00a | 16.28 ± 0.32d |
| EF6 | 1.07 ± 0.06a | 2.18 ± 0.11b | 11.30 ± 0.04d | EF16 | 1.28 ± 0.07a | 3.02 ± 0.17b | 9.36 ± 0.28b |
| EF6 + T | 1.31 ± 0.02a | 1.34 ± 0.07b | 6.88 ± 0.27c | EF16 + T | 3.25 ± 0.15b | 3.19 ± 0.12b | 9.81 ± 0.19b |
| EF6 + CD | 1.18 ± 0.07a | 0.47 ± 0.02a | 6.18 ± 0.16b | EF16 + CD | 1.06 ± 0.05a | 0.83 ± 0.01a | 4.29 ± 0.06a |
| EF6 + RP | 5.34 ± 0.31b | 1.64 ± 0.08a,b | 5.44 ± 0.19a | EF16 + RP | 4.53 ± 0.14c | 5.98 ± 0.26c | 29.85 ± 0.76d |
| EF6 + BS | 5.77 ± 0.19c | 14.85 ± 0.68c | 11.00 ± 0.59d | EF16 + BS | 4.44 ± 0.21c | 0.81 ± 0.00a | 15.48 ± 0.68c |
| EF7 | 2.89 ± 0.09c | 10.31 ± 0.60d | 15.12 ± 0.44c | EF17 | 1.58 ± 0.10a | 5.01 ± 0.02d | 18.09 ± 0.43d |
| EF7 + T | 1.88 ± 0.11b | 7.29 ± 0.26c | 15.84 ± 0.15c | EF17 + T | 1.35 ± 0.03a | 4.13 ± 0.16c | 11.15 ± 0.49b |
| EF7 + CD | 1.43 ± 0.03a | 0.77 ± 0.03a | 5.60 ± 0.32a | EF17 + CD | 1.54 ± 0.02a | 1.61 ± 0.02a | 6.28 ± 0.28a |
| EF7 + RP | 6.04 ± 0.09e | 1.71 ± 0.05b | 9.60 ± 0.33b | EF17 + RP | 10.24 ± 0.26c | 7.29 ± 0.33e | 19.46 ± 0.87e |
| EF7 + BS | 4.60 ± 0.14d | 10.37 ± 0.26d | 16.86 ± 0.44d | EF17 + BS | 4.03 ± 0.23b | 3.39 ± 0.12b | 15.60 ± 0.77c |
| EF8 | 0.36 ± 0.02a | 0.22 ± 0.01b | 5.76 ± 0.29c | EF18 | 2.44 ± 0.09c | 6.19 ± 0.32c | 14.07 ± 0.39d |
| EF8 + T | 0.24 ± 0.01a | 0.15 ± 0.01a | 3.38 ± 0.18b | EF18 + T | 1.96 ± 0.02b | 6.88 ± 0.32d | 11.36 ± 0.21c |
| EF8 + CD | 0.89 ± 0.02b | 0.35 ± 0.02c | 2.58 ± 0.12a | EF18 + CD | 1.09 ± 0.03a | 1.08 ± 0.05a | 4.97 ± 0.09a |
| EF8 + RP | 1.54 ± 0.05c | 0.64 ± 0.03d | 6.24 ± 0.31c | EF18 + RP | 10.27 ± 0.44d | 6.09 ± 0.37c | 20.15 ± 0.93e |
| EF8 + BS | 6.77 ± 0.09d | 1.35 ± 0.07e | 7.69 ± 0.32d | EF18 + BS | 5.24 ± 0.21e | 5.17 ± 0.16b | 9.51 ± 0.42b |
| EF9 | 5.48 ± 0.28b | 4.38 ± 0.21d | 8.67 ± 0.33d | EF19 | 1.19 ± 2.50a | 1.07 ± 2.83a | 6.17 ± 7.20a |
| EF9 + T | 1.06 ± 0.04a | 3.52 ± 0.18c | 7.80 ± 0.03c | EF19 + T | 1.10 ± 0.042a | 1.53 ± 0.06b | 6.25 ± 0.29a |
| EF9 + CD | 0.97 ± 0.03a | 2.47 ± 0.04b | 4.64 ± 0.28b | EF19 + CD | 1.50 ± 0.04b | 1.57 ± 0.06b | 6.20 ± 0.30a |
| EF9 + RP | 6.82 ± 0.10c | 1.49 ± 0.06a | 3.21 ± 0.14a | EF19 + RP | 5.35 ± 0.13c | 7.94 ± 0.35d | 26.43 ± 0.28c |
| EF9 + BS | 6.33 ± 0.36c | 4.43 ± 0.20d | 11.73 ± 0.46e | EF19 + BS | 9.58 ± 0.22d | 5.62 ± 0.21c | 13.83 ± 0.66b |
| EF10 | 3.84 ± 0.26d | 2.51 ± 0.12 b | 4.77 ± 0.23b | EF20 | 2.18 ± 0.12c | 5.75 ± 0.30b | 7.22 ± 0.27b |
| EF10 + T | 1.25 ± 0.07b | 3.23 ± 0.18 c | 10.06 ± 0.02c | EF20 + T | 1.83 ± 0.02b | 9.26 ± 0.22d | 8.71 ± 0.19c |
| EF10 + CD | 0.65 ± 0.01a | 3.14 ± 0.12 c | 3.71 ± 0.18a | EF20 + CD | 1.09 ± 0.02a | 1.77 ± 0.02a | 4.25 ± 0.23a |
| EF10 + RP | 3.32 ± 0.10c | 0.89 ± 0.05 a | 4.09 ± 0.08a | EF20 + RP | 8.54 ± 0.27e | 8.36 ± 0.39c | 19.69 ± 0.65d |
| EF10 + BS | 4.44 ± 0.13e | 11.54 ± 0.54d | 10.58 ± 0.35d | EF20 + BS | 7.80 ± 0.14d | 1.51 ± 0.06a | 33.20 ± 0.59e |

Means ± SD. Different letters in each column indicate significant differences ($P < 0.05$). EF, enteral formula; EF + T, enteral formula included in tea; EF + CD, enteral formula included in chocolate dessert; EF + RP, enteral formula included in rice pudding; EF + BS, enteral formula included in banana smoothie.



  

**Table 2.** Multifactor ANOVA for the effect of food matrix on mineral dialyzability from enteral formulas and EF–sweet foods

| | Factor | | |
|---|---|---|---|
| Food matrix | $\%D_{Fe}$ | $\%D_{Zn}$ | $\%D_{Ca}$ |
| EF | 2.38 ± 1.36b | 4.80 ± 3.10c | 9.50 ± 4.60b |
| EF + T | 1.49 ± 0.71a | 3.10 ± 2.49b | 9.11 ± 2.53b |
| EF + CD | 1.19 ± 0.46a | 1.36 ± 0.73a | 5.16 ± 1.66a |
| EF + RP | 5.05 ± 3.04c | 3,29 ± 2.43b | 14.41 ± 9.36c |
| EF + BS | 4.98 ± 1.81c | 6.14 ± 4.91d | 14.88 ± 5.54c |

Means ± SD. Different letters in each column indicate significant differences ($P < 0.05$). EF, enteral formula; EF + T, enteral formula included in tea; EF + CD, enteral formula included in chocolate dessert; EF + RP, enteral formula included in rice pudding; EF + BS, enteral formula included in banana smoothie.

**Table 3.** Multifactor ANOVA for the effect of cooking heating on mineral dialyzability from enteral formulas and EF–sweet foods

| | Factor | | |
|---|---|---|---|
| Cooking heating | $\%D_{Fe}$ | $\%D_{Zn}$ | $\%D_{Ca}$ |
| EF | 2.38 ± 1.36b | 4.80 ± 3.10b | 9.50 ± 4.60b |
| H | 1.33 ± 0.61a | 2.27 ± 2.05a | 7.01 ± 2.87a |
| NH | 5.02 ± 2.43c | 4.68 ± 4.68b | 14.66 ± 7.48c |

Means ± SD. Different letters in each column indicate significant differences ($P < 0.05$). EF, enteral formula; H, cooking heating; NH, no cooking heating.

using the least significant difference (LSD) test. Multifactor ANOVA was used to analyze the effects of heating and food matrix factors on mineral dialyzability and PS.

## RESULTS

### Mineral dialyzability from enteral formulas and EF–sweet foods

Table 1 shows the percentages of mineral dialyzability from EF and EF included in sweet foods. It can be seen that mineral bioaccessibility was low and different among the evaluated EF. The mean value and range of mineral dialyzability for EF were: $\%D_{Fe}$, 2.38 ± 1.36% (0.36–5.48%); $\%D_{Zn}$, 4.8 ± 3.1% (0.2–12.0%); and $\%D_{Ca}$, 9.5 ± 4.6% (3.8–18.1%). EFL showed higher $\%D_{Fe}$ than EFP, and no significant differences were observed in the cases of $\%D_{Zn}$ and $\%D_{Ca}$.

### Effect of food matrix on mineral dialyzability from enteral formulas

To evaluate the effect of the food matrix on mineral dialyzability, multifactor ANOVA was performed, taking into account the type of food.

Table 2 shows the multifactor ANOVA for the effect of food matrix on mineral dialyzability from EF. For the three minerals analyzed, there was a decrease of mineral dialyzability when EF were incorporated into CD. Chocolate has polyphenols which can interact with minerals, decreasing its solubility and bioaccessibility.

In the cases of $\%D_{Fe}$ and $\%D_{Zn}$, the same effect was observed by incorporating the EF in T, probably due to tannins, which reduce mineral bioaccessibility. However, no statistically significant difference was observed for $\%D_{Ca}$.

An increase of mineral dialyzability for the three minerals analyzed was observed when EF were included in BS. Also, $\%D_{Fe}$ and $\%D_{Ca}$ increased when EF were incorporate into RP, while $\%D_{Zn}$ decreased, with respect to the EF.

### Effect of heating on mineral dialyzability from enteral formulas

Table 3 shows the multifactor ANOVA for the effect of heating on EF mineral dialyzability. For the three minerals analyzed, a decrease in mineral dialyzability was observed when the EF were included in foods subjected to heating during the preparation (T and CD). However, an increase was observed for $\%D_{Fe}$ and $\%D_{Ca}$ when EF were included in preparations where no heating was necessary (RP and BS).

### Mineral potential contribution of enteral formulas and sweet preparations

Table 4 shows the PS of Fe, Zn and Ca of sweet foods. The serving corresponds to 200 g, equivalent to a cup. The mean and range of $PS_{Fe}$ for EF and EF–sweet foods were: EF, 50.41 ± 20.50 µg (13.05–106.35 µg); EF + T, 28.25 ± 19.25 µg (7.14–91.95 µg); EF + CD, 44.19 ± 15.40 µg (19.92–75.94 µg); EF + RP, 59.09 ± 33.92 µg (10.14–144.61 µg); EF + BS, 89.10 ± 43.98 µg (42.63–215.40 µg).

Regarding $PS_{Zn}$: EF, 101.05 ± 66.63 µg (6.92–225.54 µg); EF + T, 62.50 ± 64.37 µg (5.57–297.47 µg); EF + CD, 25.27 ± 13.26 µg (9.25–54.45 µg); EF + RP, 35.44 ± 26.23 µg (13.22–125.80 µg); EF + BS, 82.00 ± 56.93 µg (11.45–210.77 µg).

Finally, $PS_{Ca}$: EF, 13.57 ± 7.10 mg (4.60–28.40 mg); EF + T, 11.92 ± 3.60 mg (4.94–16.96 mg); EF + CD, 12.92 ± 3.81 mg (6.16–21.65 mg); EF + RP, 8.29 ± 4.09 mg (2.89–15.78 mg); EF + BS, 15.53 ± 7.24 mg (5.719–29.93 mg).

Table 5 shows the multifactor ANOVA for the effects of food matrix and cooking heating on the PS of Fe, Zn and Ca from EF and EF included in sweet foods. It is observed that $PS_{Fe}$ increased when EF were included in the BS and decreased when they were included in T; $PS_{Zn}$ decreased in all cases except when EF were included in BS and $PS_{Ca}$ decreased when EF were included in the RP.

## DISCUSSION

Dialyzability of Fe, Zn and Ca from commercial EF was low and variable among EF. Higher values of mineral dialyzability were found by Drago *et al.*[12] for infant formulas made from milk proteins. However, EF not only contain milk protein (casein and whey proteins) but may also contain soy proteins, which has an inhibitory effect on Fe absorption.[13]

EFL (EF13–20) had better $\%D_{Fe}$ than EFP (EF 1–12). Similar results were observed by Drago *et al.*[12] for commercial infant formulas. This may be related to drying processes carried out during the elaboration of EF. Mineral–nutrient interactions could be produced during food processing, rendering minerals unavailable for absorption, and decreasing their bioavailability.[14]

The main objective of enteral nutrition is to provide all the necessary nutrients to contribute to the improvement of the patient. However, some minerals of the EF may interact with different components of the matrix and modify its bioavailability, in some cases impairing the nutritional quality of the enteral diet.[15]



  

**Table 4.** Mineral potential supply from enteral formulas and EF–sweet foods corresponding to 200 g serving

| Sample | $PS_{Fe}$ (μg) | $PS_{Zn}$ (μg) | $PS_{Ca}$ (mg) | Sample | $PS_{Fe}$ (μg) | $PS_{Zn}$ (μg) | $PS_{Ca}$ (mg) |
|---|---|---|---|---|---|---|---|
| EF1 | 106.35 | 193.38 | 7.87 | EF11 | 53.53 | 33.35 | 28.40 |
| EF1 + T | 91.95 | 87.64 | 13.80 | EF11 + T | 14.92 | 4.08 | 16.84 |
| EF1 + CD | 75.94 | 29.22 | 21.18 | EF11 + CD | 19.92 | 54.45 | 21.65 |
| EF1 + RP | 10.14 | 14.46 | 2.89 | EF11 + RP | 78.02 | 30.65 | 15.44 |
| EF1 + BS | 88.10 | 70.19 | 15.45 | EF11 + BS | 65.65 | 105.24 | 25.19 |
| EF2 | 59.42 | 101.46 | 6.98 | EF12 | 88.27 | 91.54 | 26.40 |
| EF2 + T | 32.38 | 29.95 | 14.60 | EF12 + T | 15.30 | 3.57 | 14.01 |
| EF2 + CD | 68.13 | 20.20 | 12.17 | EF12 + CD | 21.97 | 35.54 | 15.91 |
| EF2 + RP | 12.01 | 18.42 | 5.26 | EF12 + RP | 72.45 | 16.44 | 10.66 |
| EF2 + BS | 62.23 | 77.74 | 8.91 | EF12 + BS | 71.38 | 210.77 | 18.66 |
| EF3 | 40.76 | 44.37 | 6.96 | EF13 | 39.10 | 21.54 | 21.05 |
| EF3 + T | 50.62 | 34.06 | 15.54 | EF13 + T | 11.84 | 130.49 | 10.43 |
| EF3 + CD | 23.65 | 9.25 | 9.30 | EF13 + CD | 52.47 | 25.60 | 14.01 |
| EF3 + RP | 44.52 | 22.06 | 5.68 | EF13 + RP | 62.08 | 27.28 | 7.72 |
| EF3 + BS | 54.21 | 16.33 | 10.42 | EF13 + BS | 88.70 | 20.46 | 13.13 |
| EF4 | 54.57 | 135.38 | 7.27 | EF14 | 64.90 | 220.10 | 16.04 |
| EF4 + T | 17.92 | 17.54 | 14.45 | EF14 + T | 36.82 | 71.60 | 12.67 |
| EF4 + CD | 26.43 | 10.07 | 6.16 | EF14 + CD | 43.93 | 22.26 | 10.95 |
| EF4 + RP | 20.92 | 28.67 | 4.43 | EF14 + RP | 69.26 | 54.70 | 9.27 |
| EF4 + BS | 42.63 | 89.97 | 8.88 | EF14 + BS | 59.85 | 64.44 | 29.93 |
| EF5 | 35.75 | 80.41 | 23.41 | EF15 | 45.23 | 225.54 | 7.10 |
| EF5 + T | 27.01 | 33.66 | 16.17 | EF15 + T | 26.62 | 51.31 | 12.96 |
| EF5 + CD | 48.12 | 14.40 | 13.05 | EF15 + CD | 51.15 | 29.43 | 13.54 |
| EF5 + RP | 97.30 | 39.58 | 9.62 | EF15 + RP | 51.39 | 35.52 | 15.78 |
| EF5 + BS | 77.16 | 204.67 | 15.50 | EF15 + BS | 66.73 | 11.45 | 25.33 |
| EF6 | 26.78 | 43.08 | 17.65 | EF16 | 39.29 | 105.13 | 14.89 |
| EF6 + T | 26.93 | 27.51 | 10.44 | EF16 + T | 55.10 | 73.29 | 13.29 |
| EF6 + CD | 49.20 | 9.85 | 14.88 | EF16 + CD | 50.58 | 24.35 | 12.24 |
| EF6 + RP | 144.61 | 37.74 | 7.58 | EF16 + RP | 28.52 | 76.12 | 12.21 |
| EF6 + BS | 109.26 | 151.32 | 11.71 | EF16 + BS | 83.98 | 27.53 | 18.23 |
| EF7 | 38.03 | 150.49 | 16.44 | EF17 | 49.16 | 105.72 | 10.02 |
| EF7 + T | 20.41 | 69.59 | 16.96 | EF17 + T | 22.28 | 68.56 | 5.81 |
| EF7 + CD | 49.53 | 12.14 | 12.10 | EF17 + CD | 55.56 | 28.37 | 14.81 |
| EF7 + RP | 120.31 | 21.43 | 10.39 | EF17 + RP | 56.42 | 34.89 | 6.37 |
| EF7 + BS | 60.50 | 93.61 | 13.99 | EF17 + BS | 82.94 | 64.03 | 6.36 |
| EF8 | 13.05 | 6.92 | 12.30 | EF18 | 64.73 | 132.99 | 14.62 |
| EF8 + T | 7.14 | 4.94 | 7.11 | EF18 + T | 27.07 | 94.60 | 8.52 |
| EF8 + CD | 39.07 | 9.88 | 6.84 | EF18 + CD | 50.54 | 21.81 | 12.39 |
| EF8 + RP | 49.69 | 15.59 | 10.48 | EF18 + RP | 37.27 | 38.95 | 4.36 |
| EF8 + BS | 187.19 | 29.66 | 11.67 | EF18 + BS | 117.89 | 108.62 | 16.52 |
| EF9 | 43.01 | 60.79 | 5.57 | EF19 | 46.87 | 53.73 | 14.60 |
| EF9 + T | 12.42 | 46.82 | 4.94 | EF19 + T | 21.69 | 61.29 | 10.86 |
| EF9 + CD | 29.06 | 44.02 | 10.39 | EF19 + CD | 52.97 | 25.43 | 13.96 |
| EF9 + RP | 79.32 | 17.08 | 3.03 | EF19 + RP | 41.94 | 40.18 | 14.97 |
| EF9 + BS | 65.72 | 52.62 | 5.71 | EF19 + BS | 215.40 | 88.64 | 27.48 |
| EF10 | 41.53 | 34.54 | 4.60 | EF20 | 57.89 | 180.61 | 8.30 |
| EF10 + T | 18.08 | 41.99 | 8.15 | EF20 + T | 28.52 | 297.47 | 10.89 |
| EF10 + CD | 25.63 | 51.40 | 10.88 | EF20 + CD | 50.03 | 27.69 | 12.01 |
| EF10 + RP | 58.58 | 13.22 | 3.59 | EF20 + RP | 47.02 | 125.80 | 6.17 |
| EF10 + BS | 59.22 | 116.09 | 6.64 | EF20 + BS | 123.22 | 36.63 | 20.85 |

$PS_{Fe}$, potential supply of Fe; $PS_{Zn}$, potential supply of Zn; $PS_{Ca}$, potential supply of Ca. EF, enteral formula; EF + T, enteral formula included in tea; EF + CD, enteral formula included in chocolate dessert; EF + RP, enteral formula included in rice pudding; EF + BS, enteral formula included in banana smoothie.



  

**Table 5.** Multifactor ANOVA for the effect of food matrix and cooking heating on mineral potential supply from enteral formulas and EF–sweet foods

| | $PS_{Fe}$ (μg) | $PS_{Zn}$ (μg) | $PS_{Ca}$ (mg) |
|---|---|---|---|
| Food matrix | | | |
| EF | 50.41 ± 20.50 b | 101.05 ± 66.63d | 13.52 ± 7.10b,c |
| EF + T | 28.25 ± 19.25a | 62.50 ± 64.37b,c | 11.92 ± 3.60b |
| EF + CD | 44.19 ± 15.40a,b | 25.27 ± 13.26a | 12.92 ± 3.81b,c |
| EF + RP | 59.09 ± 33.92b | 35.44 ± 26.23a,b | 8.29 ± 4.09a |
| EF + BS | 89.10 ± 43.98c | 82.00 ± 56.93c,d | 15.53 ± 7.24c |
| Cooking heating | | | |
| H | 36.22 ± 19.01a | 43.88 ± 49.59a | 12.42 ± 3.69a |
| NH | 74.09 ± 41.64b | 59.72 ± 49.70a | 11.91 ± 6.87a |

Means ± SD. Different letters in each column indicate significant differences ($P < 0.05$). $PS_{Fe}$, potential supply of Fe; $PS_{Zn}$, potential supply of Zn; $PS_{Ca}$, potential supply of Ca; EF, enteral formula; EF + T, enteral formula included in tea; EF + CD, enteral formula included in chocolate dessert; EF + RP, enteral formula included in rice pudding; EF + BS, enteral formula included in banana smoothie. H, cooking heating; NH: no cooking heating.

Regarding the low bioavailability of some minerals, like Fe and Zn, the mineral level used in formulas is sufficient to supply the mineral requirement. However, mineral bioaccessibility of EF when they are included in food matrices or undergo cooking processes is not taken into account.

Food matrix affected mineral dialyzability differently from EF. Both food matrix and EF have mineral absorption enhancers, such as ascorbic acid, and inhibitors: mainly phytic acid, tannins and polyphenols.[4]

Hexaphosphate and pentaphosphate derivatives from phytic acid form insoluble complexes at pH near neutrality, thereby preventing Fe absorption[16] and dialyzability.

The action of ascorbic acid involves the reduction of ferric ion to ferrous form, which is better absorbed; the formation of soluble and stable chelates with Fe occurs in the stomach, maintaining their solubility when food enters the neutral–alkaline environment of the duodenum.[17] This latter effect can be explained by the fact that ascorbic acid forms soluble complexes with food Fe at pH values lower than inhibitory ligands, i.e. at stomach level where the pH conditions are unfavorable for the formation of complexes with other ligands.[4] This would counteract the inhibitory effects of phytates.

CD was the food matrix that most affected mineral dialyzability, followed by T. The inhibitory effect of chocolate, milk and tea on mineral dialyzability was also described by Binaghi *et al.*,[18] who analyzed the effects of these foods on complementary infant foods.

In the case of CD, the inhibitory effect could be due to the presence of chocolate polyphenols, milk proteins and milk Ca, which are inhibitors of Fe absorption.[19] The presence of phosphoserine groups in casein subunits may explain the binding of Fe to insoluble casein peptides. thus decreasing $\%D_{Fe}$ and bioavailability. Ca salts impair Fe absorption when they are present in the same foods. The inhibitory effect of Ca occurs as a result of physicochemical interactions in the gastrointestinal tract, which also influence Fe dialyzability, as well as at the site of absorption.[20] Furthermore, cocoa polyphenols also act as inhibitors, forming insoluble complexes which prevent absorption.

In the case of T, the inhibitory effect on mineral dialyzability would be caused by tannins depressing absorption ligands, inhibitors of non-heme Fe and Zn absorption. Studies in rats have shown that the polyphenols form insoluble complexes with Fe and Zn, which precipitate and therefore cannot be absorbed[21] or dialyzed.

Another very important factor to consider is that CD and T involve heating of EF during preparation. Heating can degrade ascorbic acid, which is an enhancer of Fe absorption, and would also generate mineral–nutrient interaction.

On the other hand, mineral dialyzability was increased when EF were included in BS, for the three analyzed minerals, while RP increased $\%D_{Fe}$ and $\%D_{Ca}$. Both banana and polished white rice contain low levels of phytic acid[22] and these food preparations do not involve EF heating. Both banana and white rice should have no major effect on Zn and Ca bioaccessibility. However, the ascorbic acid in banana could contribute to the increased $\%D_{Fe}$. According to Wall,[23] banana has an ascorbic acid content of about 9.7 mg 100 $g^{-1}$ of fresh fruit.

Mineral PS was highly variable between different EF and EF–sweet foods and was also low. The different food matrices had variable effects on mineral PS, which were related to the mineral concentrations as well as the dialyzability of each mineral, other than the size of serving. Regarding the effect of heating on mineral PS, only $PS_{Fe}$ was affected.

Recommended daily intake (RDA) of a nutrient is always above the actual need, as the nutritional recommendation is calculated using factors related to environmental factors, individual variability and bioavailability of nutrients in the diet.[24] As the PS value takes into account the bioaccessibility/availability, the daily requirement of a particular mineral and not its RDA was considered in calculating the mineral contribution from a particular EF or EF–sweet food.

In this regard, it is estimated that it is necessary to absorb 1.8 mg Fe daily to meet the needs of 80–90% of adult women and adolescents of both sexes.[25] EF and EF–sweet food servings (200 g) would cover the following percentages of the daily requirement: EF, 2.8%; EF + T, 1.6%; EF + CD, 2.5%; EF + RP, 3.3%; BS, 4.9%.

The inevitable losses of Ca in the adult are about 300 mg per day.[26] Those losses could be covered by the following percentages: EF, 4.5%, EF + T, 3.9%; EF + CD, 4.3%; EF + RP, 2.8%; BS, 5.2%.

Finally, Zn requirements are 2.2 mg per day.[26] EF and EF–sweet food servings would cover the following percentages of this requirement: EF, 4.6%; EF + T, 2.8%; EF + CD, 1.1%; EF + RP, 2.6%; BS, 3.7%.

## CONCLUSIONS

Fe, Zn and Ca bioaccessibility from commercial EF was variable and generally low. This could be due to EF, which are complex



  

mixtures of nutrients that may interact with each other, reducing mineral dialyzability and absorption. Using enhancers of mineral absorption could be a way to increase mineral bioaccessibility.

The different food matrices where EF were included affected differently their mineral dialyzability, because they have promoters (vitamin C) and inhibitors (phytic acid, tannins and polyphenols) of mineral absorption. However, the clearest effect on mineral dialyzability was given by heating during EF–sweet food preparation (T and CD), lowering *D* Fe%, possibly due to degradation of vitamin C, and $\%D_{Zn}$ and $\%D_{Ca}$, due to interaction with food components. Furthermore, the preparations that did not involve EF heating (RP and BS) showed increased mineral bioaccessibility. For this reason it could be important to recommend no heating when an EF is included in a food.

When mineral potential supply of 200 g serving was analyzed, EF and EF–sweet foods did not show a good supply of Fe, Zn and Ca, as recommended.

This study demonstrated the low Fe, Zn and Ca availability in commercial EF and analyzed how the bioaccessibility of these minerals is affected by the food matrix in which EF is included, as well as the effect of heating during food preparation.

## REFERENCES


1 Suchner U, Senftleben U, Eckaw T, Scholz M, Beck K, Murr R, *et al,* Enteral versus parenteral nutrition: effects on gastrointestinal function and metabolism. *Nutrition* **12**: 76–81 (1996).
2 Lochs H, Pichard C and Alison SP, Evidence supports nutritional support. *Clin Nutr* **25**:177–179 (2006).
3 Harvey L, Mineral bioavailability. *Nutr Food Sci* **31**:179–182 (2001).
4 Hurrell RF and Egli I, Iron bioavailability and dietary reference values. *Am J Clin Nutr* **91**:1461–1467 (2010).
5 O'Dell BL, Bioavailability and interactions among trace elements. *J Nutr* **102**:653–660 (1985).
6 Southon S, Fairweater-Tait J and Hazell T, Trace element availability from the human diet. *Proc Nutr Soc* **47**:25–35 (1998).
7 Wiesstaub AR, Menéndez AM, Montemerlo H, Pastene H, Piñeiro A and Guidoni ME, Zinc plastmático, cobre sérico y zinc y cobre eritrocitarios en adultos sanos de Buenos Aires. *Acta Bioq Clin Latinoam* **42**:315–323 (2008).
8 Ummadi P, Chenoweth WL and Uebersax MA. The influence of extrusion processing on iron dialyzability, phytates and tannins in legumes. *J Food Process Pres* **19**:119–131 (1995).
9 Schricker BR, Miller DD, Rasmussen RR and Van Campen D. A comparison of in vivo and in vitro methods for determining availability of iron from meals. *Am J Clin Nutr* **34**:2257–2263 (1981).
10 Kennefick S and Cashman KD, Investigation of an in vitro model for predicting the effect of food components on calcium availability from meals. *Int J Food Sci Nutr* **51**:45–54 (2000).
11 Miller DD, Schricker BR, Rasmussen RR and Van Campen D, An in vitro method for estimation of iron availability from meals. *Am J Clin Nutr* **34**:2248–2256 (1981).
12 Drago SR, Binaghi MJ, Ronayne de Ferrer PA and Valencia ME, Assessment of iron, zinc and calcium dialyzability in infant formulas and iron fortified milks, in *Food Research, Safety and Policies*, ed. by Riley AP. Nova, Hauppauge, NY, pp. 113–132 (2005).
13 Lynch SR, Dasenko SA, Cook JD, Juillerat MA and Hurrell RF, Inhibitory effect of a soybean-protein-related moiety on iron absorption in humans. *Am J Clin Nutr* **60**:567–572 (1994).
14 Watzke HJ, Impact of processing on bioavailability examples of minerals in foods. *Trends Food Sci Technol* **9**:320–327 (1998).
15 Winichagoon P, McKenzie JE, Chavasit V, Pongcharoen T, Gowachirapant S, Boonpraderm A, *et al,* A multimicronutrient-fortified seasoning powder enhances the hemoglobin, zinc, and iodine status of primary school children in north east Thailand: a randomized controlled trial of efficacy. *J Nutr* **136**:1617–1623 (2006).
16 Fairweather-Tait SJ, Bioavailability of trace elements. *Food Chem* **43**:213–217 (1992).
17 Lee K and Clydesdale FM, Effect of thermal processing on endogenous and added iron in canned spinach. *J Food Sci* **46**:1064–1068 (1981).
18 Binaghi MJ, López LB, Ronayne de Ferrer PA and Valencia ME, Evaluación de la influencia de distintos componentes de la dieta sobre la biodisponibilidad de minerales en alimentos complementarios. *Rev Chil Nutr* **34**:1–4 (2007).
19 Hurrell RF, Bioavailability of iron, in Assessment of the Bioavailability of Micronutrients: Proceedings of an ILSI Europe Workshop. *Eur J Clin Nutr* **51**:54–58 (1997).
20 Hallberg L, Rossanser-Hultén L, Brune M and Gleerup A, Calcium and iron absorption: mechanism of action and nutritional importance. *Eur J Clin Nutr* **46**:317–327 (1992).
21 Brown RC, Klein A, Simmons W and Hurrell R, The influence of Jamaican herb teas and other polyphenol-containing beverages on iron absorption in the rat. *Nutr Res* **10**: 343–353 (1990).
22 Cúneo F, Farfan JA and Carraro F, Distribuição dos fitatos em farelo de arroz estabilizado e tratado com fitase exógena. *CiêncTecnol Aliment* **20**:94–98 (2000).
23 Wall MM, Ascorbic acid, vitamin A, and mineral composition of banana (*Musa* sp.) and papaya (*Carica papaya*) cultivars grown in Hawaii. *J Food Compos Anal* **19**:434–445 (2006).
24 Ziegler EE and Filer LJ, *Conocimientos Actuales Sobre Nutrición*. OMS, Washington, DC (1990).
25 Monsen ER, Estimation of available dietary iron. *Am J Clin Nutr* **31**:134–142 (1978).
26 Martín de Portela ML, *Vitaminas y Minerales en Nutrición*. Libreros López Editores, Buenos Aires (1993).




  