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Evaluation of the bioaccessible fractions of Fe, Zn, Cu and Mn in baby foods

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ABSTRACT

The bioaccessibility of four essential micronutrients (iron, zinc, copper and manganese) in some baby foods was evaluated using an *in vitro* gastrointestinal digestion model. For all of the flour-based foods evaluated, the bioaccessibility of Zn was low, while the bioaccessibility of Cu was above 50%. For these samples, the bioaccessibility of Mn was lower than 50%. Two samples composed of oat and rice flour and whole wheat flour demonstrated a lower bioaccessible fraction of Fe (less than 35%), while the sample made with wheat flour showed high Fe bioaccessibility (approximately 80%). For vegetable- and meat-based baby foods, the Fe bioaccessibility was greater than 80% in samples that contained meat and chicken and approximately 20% for the banana-based sample. The bioaccessibility of Zn was small for all of the foods studied, and in some cases, no Zn appeared to be released. The sample containing banana showed 100% Cu bioaccessibility, in contrast to meat and chicken-based samples, whose Cu bioaccessibility values were less than 50%. The opposite effect occurred for Mn, in which samples containing meat and chicken presented a bioaccessible fraction greater than 50% while the banana-based sample had a fraction less than 50%.

1. Introduction

The World Health Organization (WHO) recommends that breast milk should be the only nutrition source for children up to six months of age. However, less than 35% of children in the world at this age are in exclusive breastfeeding [1]. Infant formulas are very common breast milk substitutes in the USA and Europe [2–4], while the substitutes in developing countries are usually cow's milk, which may be powder or liquid and sweetened or unsweetened, and crushed solid food [5]. Additionally, a number of commercial infant foods are available for babies, such as instant milk flour, which can be mixed with milk, fruit or salty foods, or processed baby foods containing meat, vegetables, rice or pasta, whose favorable textures facilitate the ingestion by infants [4].

Food intended for children should be better controlled and special attention should be given to monitor amounts of protein, calories, vitamins and nutrients because dietary deficiencies of essential minerals and nutrients may lead to considerable social costs, the reduction of human potential and demands for large investments in health [4,6,7].

Information about inorganic constituents usually indicates the total concentrations present in the diet [8]. However, the total concentration of a nutrient in baby food is not sufficient information to determine whether the food will provide all of an infant's nutritional requirements. A very important factor is the bioaccessibility of the element, which is its soluble fraction that can be effectively absorbed by the body [8,9]. This fraction depends on the elemental species and the behavior of these species and organometallic complexes in the gastrointestinal tract, as well as the interaction with the food matrix. It is important to determine the fraction of nutrient that is potentially free to be absorbed, in addition to the factors that enhance absorption (e.g., ascorbic acid, bovine serum albumin, and meat proteins) or inhibit absorption (e.g., dietary fibers, polyphenols, acids, phosphates, calcium salts, and casein). This information is necessary to evaluate the real consumption of essential micronutrients, which is typically carried out using an *in vitro* sequential analysis with synthetic gastric and intestinal juices and analyzing the nutrients' soluble fractions [8].

Another important factor is the bioavailability of a nutrient, which is the amount that is effectively absorbed by the body to be used in physiological functions or stored for future use [10]. Many studies have quantified nutrients or toxic elements in baby foods, and some indicate the importance of bioaccessibility and/or bioavailability. Frontela et al. [11] studied the bioaccessibility of Fe, Ca and Zn using an *in vitro* procedure, in which five different cereals with low phytate contents complemented breastfeeding of children from 4 to 6 months of age. These cereals were mixed with water and follow-on formulas, and the results showed higher

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bioaccessibility of Fe and Zn in the presence of lower phytate contents. Eklund et al. [12] studied the *in vitro* bioaccessibility of Cd in infant foods by simulating the different means of digestion by children and adults, as well as the bioavailability of Cd by determining the amount absorbed by Caco-2 cells. A meal containing liver and vegetables showed the greatest Cd bioaccessibility, which was lower through means of digestion by children than by adults. The authors concluded that, in addition to this food matrix, age and digestion conditions may affect the bioavailability and the bioaccessibility of Cd.

In many countries, including Brazil, few studies have focused on these factors, even though they are extremely important in the evaluation of the real nutritional value of food. Generally, the objective of food analysis is to determine the total concentration of the inorganic constituents. For example, Castro et al. [13] measured the concentrations of the toxic elements Pb and Cd and their respective antagonists, Ca and Zn, in infant formulas and cow's milk and found that 62% of the samples contained amounts of Pb higher than recommended levels established by the FAO/WHO, but they did not observe Cd contamination problems. In addition, the study also showed that the infant formulas can provide adequate amounts of Zn. Amorim et al. [14] have determined Mn in milk and infant foods. The measured Mn concentrations in soy-based infant formulas were greater than those in whole milk, dairy drinks and goat milk.

To address the importance and the small volume of nutritional information about infant foods in Brazil, the objective of this study was to evaluate the bioaccessibility of four essential micronutrients (Fe, Zn, Cu and Mn) in milk flour and baby foods. Such foods are used to supply the dietary needs of children during and after breastfeeding.

2. Experimental

2.1. Reagents

All polyethylene bottles and glassware used were previously decontaminated in 10% HNO_3 bath (v/v) for 24 h prior to rinsing with deionized water.

All reagents used were of analytical purity grade, and deionized water with conductivity of 18 MΩ cm was obtained by a Milli-Q system (Millipore, Bedford, MA, USA). Argon (White Martins, Sertãozinho, Brazil) used in the analysis was of 99.996% purity. Concentrated HNO_3 (Merck, Darmstadt, Germany) and 30% (v/v) H_2O_2 (Merck, Darmstadt, Germany) were also used.

Standard solutions for measuring the elements Fe, Zn, Cu and Mn by ICP-MS were prepared from monoelemental stock solutions of 1000 mg L^{-1} (Merck, Darmstadt, Germany). The stock solutions were diluted with deionized water to the following concentrations: 0.5, 1.0, 2.5, 5.0, 7.5, 10, 20, 40, 80 and 160 μg L^{-1}. Internal standard solutions of Sc and Y (1000 mg L^{-1}, Merck, Darmstadt, Germany) were included in both, calibration curves and analytical blank, in the same concentration (10 μg L^{-1}).

The following reagents were used for simulating the human digestive process occurring in the mouth, stomach and intestine: HCl, $NaHCO_3$ and NH_4Cl, (Merck, Darmstadt, Germany); KCl (Nuclear, São Paulo, Brazil); KSCN, NaH_2PO_4, $NaSO_4$, $CaCl_2$, $MgCl_2$, Urea and Glucose (Synth, Diadema, Brazil); NaCl (Carlo Erba, Milan, Italy); KH_2PO_4 (Cinética Química, São Paulo, Brazil); D-Glucuronic acid, Glucosamine, Amylase from *Aspergillus oryzae*, Uric acid, Mucin from porcine stomach, type II, Albumin from bovine serum, Pepsin from porcine gastric mucosa, Pancreatin from bovine pâncreas, Lipase from porcine pancreas, type II, and Bile extract from porcine (Sigma, St. Louis, U.S.A.).

2.2. Samples

Three of the food samples analyzed were based on mixtures of flour. The first sample contained rice flour and oatmeal (F1), the second sample contained wheat flour fortified with Fe and folic acid (F2), and the third sample contained whole wheat flour and wheat flour enriched with Fe and folic acid (F3). Vegetable- and meat-based baby food samples were also analyzed: meat with vegetables (P1), chicken breast with pasta and vegetables (P2) and banana with oats (P3).

All of the food samples were from the most commonly consumed brand and were acquired in the local trade of Campinas, SP, Brazil and they were analyzed in triplicate.

Certified reference materials (CRM) were also analyzed to evaluate the accuracy of the proposed method. The selected materials were Wheat Flour (NIST 1567a), Rice Flour (NIST 1568a), Infant Formula (NIST 1846) and Whole meal Flour (BCR 189). These CRMs were chosen based on similarity to the selected food samples.

2.3. Procedure for in vitro gastrointestinal digestion (flour and baby food samples)

The process of digestion by humans was simulated using in vitro digestion. The simplified process is based on mimicking the physiological conditions of the gastrointestinal tract, *i.e.*, chemical composition of digestive fluid, pH and typical residence time for each step of the digestion process. The procedure described by Versantvoort et al. [15,16] was used in this study with 3 g samples. The specific procedure for the gastrointestinal digestion simulation is presented in Table 1.

2.4. Mineralization using microwave-assisted digester

To measure the total analyte concentrations, triplicate of 0.8 g samples of flour, baby food or CRMs were weighed in Teflon® flasks and combined with 3 mL of concentrated HNO_3, 1 mL of 30% v/v H_2O_2 and 4 mL of deionized water. The samples were mineralized using the microwave heating procedure in Table 2. After mineralization, the volume was increased to 20 mL using deionized water.

To measure the bioaccessible fractions of analytes, triplicate samples of 5 mL of the supernatant from each gastrointestinal digestion were transferred to a mineralization flask, to which 3 mL of concentrated HNO_3 and 1 mL of 30% v/v H_2O_2 were added. The samples were mineralized using the microwave heating procedure in Table 2. After mineralization, the volume was increased to 15 mL with deionized water.

2.5. Total organic carbon content and residual acidity

Limitations of the ICP-MS used for these analyses include low tolerance to the amount of dissolved solids, which must not exceed 2% (and can be estimated as the total dissolved organic

Table 1
Gastrointestinal digestion simulation procedure (volume of each reagent used, pH and incubation time) for flour and baby food samples. $T_{incubation} = 37 \pm 2$ °C, with agitation; $t_{centrifugation} = 30$ min (3600 rpm).

Step	Digestion of flour/baby food samples			
	Juice	Volume (mL)	pH	Time
1	Saliva	6/3	6.8	5 min
2	Gastric Juice	12/6	2.0–3.0	2 h
	Duodenal Juice	12/6	6.5–7.0	2 h
3	Bile	6/3	6.5–7.0	2 h
	HCO_3^-	2/1	6.5–7.0	2 h

Table 2
Microwave program used for the mineralization of baby foods, infant flour and certified reference materials.

Time (min)	Temperature (°C)
6 (ramp)	80
2 (hold)	80
3 (ramp)	120
2 (hold)	120
10 (ramp)	180
10 (hold)	180

Table 3
Operational parameters for ICP-MS.

Characteristics	Parameters
Generator frequency (MHz)	40
RF applied power (kW)	1.4
Plasma gas flow rate (L min^{-1})	18
Auxiliary gas flow rate (L min^{-1})	1.8
Nebulizer gas flow rate (L min^{-1})	0.44
Sheath gas flow rate (L min^{-1})	0.17
Torch inner diameter (mm)	5.5
Points per peak	2
Scans/replicates	5
Replicates/Samples	5
Dwell time (ms)	100
Skimmer cone	Ni with CRI
Sampler cone	Ni without CRI
Nebulizer	Seaspray
Spray chamber	Scott-type
Spray chamber temperature	2 °C
Acquisition mode	Peak Hopping

carbon content), and the samples' final acidity, which must not exceed 2% [17].

These limitations were overcome by diluting the samples. For the total concentration analysis, 1.2 mL of mineralized material was transferred to a 10.0 mL flask, and the volume was completed with deionized water, resulting in a residual acidity of approximately 1.8%. For the bioaccessible fraction analysis, 1.0 mL of the mineralized material was transferred to and diluted in a 10 mL flask, and the resulting solution had a residual acidity of approximately 2%. The total organic content of each diluted solution was measured using a total organic carbon analyzer (SHIMADZU, TOC-5000 Model).

2.6. Instrumentation

The total concentrations and bioaccessible fractions were measured by a mass spectrometer with inductively coupled plasma, ICP-MS (Varian, Mulgrave, Australia, model 820-MS), equipped with a CRI (collisional/reaction interface) and a Varian SPS3 auto sampler. The operating conditions of the equipment were optimized by evaluating the recovery values of certified reference materials. Table 3 shows the conditions used to measure the analytes Fe, Zn, Cu and Mn. This work monitored the following isotopes: $^{45}Sc^+$, $^{54}Fe^+$, $^{55}Mn^+$, $^{56}Fe^+$, $^{63}Cu^+$, $^{64}Zn^+$, $^{65}Cu^+$, $^{66}Zn^+$, $^{68}Zn^+$ and $^{89}Y^+$.

3. Results and discussion

The total organic carbon concentration after mineralization was approximately 100 mg L^{-1} for all of the total concentration and bioaccessible fraction samples. This result indicated that the dilutions used were suitable for this ICP-MS technique.

ICP-MS was selected for this analysis because of its high sensitivity, which allows for the determination of low analyte concentrations, and its multielementar characteristic [18]. This is important for bioaccessible fraction studies because the final analyte concentrations are usually very low, which other multi-elemental techniques, such as ICP OES, cannot adequately detect.

The CRI is an instrumental device that can improve ICP-MS results by decreasing isobaric interferences. The CRI was evaluated by adding H_2 (60 mL min^{-1} as a reaction gas) and He (80 mL min^{-1} as a collisional gas) [19]. It was also studied the use of an internal standard for the measurements, as well as the linearity of the standard analytical curves (r^2).

The experimental conditions for evaluating the analytes in the flour and baby food samples were optimized by recovery studies of the four certified reference materials. Table 4 shows the recoveries obtained for $^{68}Zn^+$, $^{55}Mn^+$, $^{65}Cu^+$ and $^{57}Fe^+$ isotopes, that were the best considering the evaluated isotopes. Recovery values were between 93% and 113%, for all the elements in the four certified reference materials studied. All of the analytes, except Fe, were quantified without using CRI, but in the presence of internal standard. The use of $^{89}Y^+$ provided adequate corrections for the quantified analytes. Considering the results obtained, the method showed good accuracy and precision. The limit of detection and quantification for bioaccessibility determination are, respectively (μg L^{-1}): 0.24 and 0.82 for Cu; 3.46 and 11.52 for Fe; 0.13 and 0.45 for Mn and 0.58 and 1.95 for Zn.

Figs. 1 and 2 and Table 5 show the results obtained for the three flour samples. Note the bioaccessibility of an analyte depends on the type of food. This is plausible because these samples have complex compositions, which may interfere with the digestion efficiency. From these data, it can be concluded that some of the foods studied have low bioavailability for some elements; that is, they have a low nutritional value for the elements studied.

The measured Zn bioaccessibility in the flour was fairly low in all of the samples, while the Cu bioaccessibility was greater than 50%. Conversely, the Mn bioaccessibility was less than 50% for all of the foods evaluated. The F1 and F3 samples demonstrated a lower bioaccessible fraction of Fe (less than 35%) than the F2 sample.

Although most of the population's average total dietary iron exceeds the daily recommended amount, a major global health problem is the lack of Fe, which causes anemia. According to the literature, iron can be found in two different forms in food

Table 4
Determination of Fe, Zn, Cu and Mn in certified reference materials; $n=3$.

CRM/SRM	Element	Certified value (mg kg^{-1} ± s)	Found value (mg kg^{-1} ± s)	Rec. (%)
Wheat Flour (NIST)	Fe	14.1 ± 0.5	13.1 ± 0.4	93
	Zn	11.6 ± 0.4	11.3 ± 0.7	98
	Cu	2.1 ± 0.2	2.0 ± 0.1	94
	Mn	9.4 ± 0.9	9.0 ± 0.6	95
Rice Flour (NIST)	Fe	7.4 ± 0.9	8.3 ± 1.2	113
	Zn	19.4 ± 0.5	18.7 ± 0.6	97
	Cu	2.4 ± 0.3	2.4 ± 0.1	99
	Mn	20.0 ± 1.6	18.8 ± 0.5	94
Infant Formula (NIST)	Fe	63.1 ± 4.0	68.5 ± 6.8	109
	Zn	60 ± 3.2	65 ± 69	108
	Cu	5.04 ± 0.27	5.1 ± 0.5	101
	Mn	–	–	–
Wholemeal Flour (BCR)	Fe	68.3 ± 1.9	63.3 ± 1.1	93
	Zn	56.5 ± 1.7	60.1 ± 1.2	106
	Cu	6.4 ± 0.2	6.2 ± 0.1	97
	Mn	63 ± 1.6	63 ± 4	100

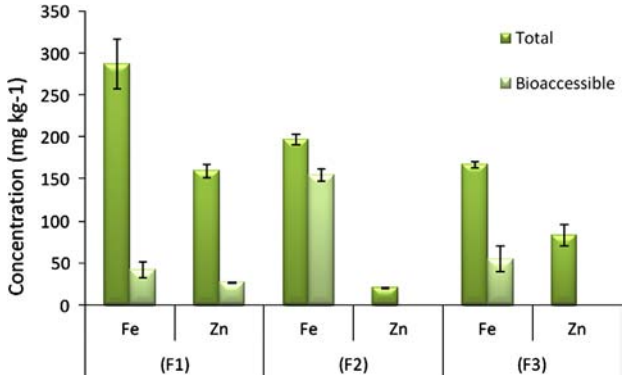

Fig. 1. Total concentrations and bioaccessible fractions of Fe and Zn in flour samples (F1: Oat and rice flour; F2: wheat flour+Fe+folic acid; F3: whole wheat flour+wheat flour+Fe+folic acid); $n=3$.

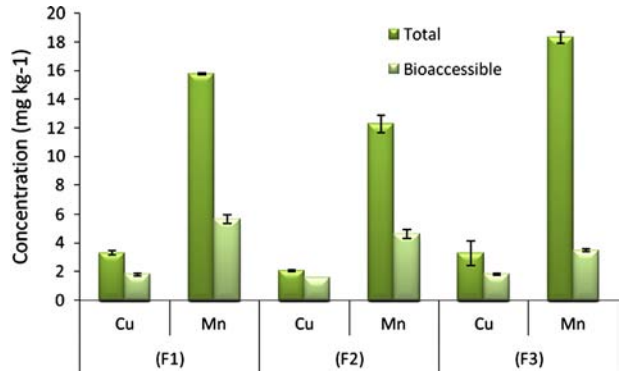

Fig. 2. Total concentrations and bioaccessible fractions of Cu and Mn in flour samples (F1: Oat and rice flour; F2: wheat flour+Fe+folic acid; F3: whole wheat flour+wheat flour+Fe+folic acid); $n=3$.

Table 5
Total concentrations and bioaccessible fractions of the analytes in the flour samples; $n=3$.

Sample	Element	Total (mg kg^{-1} ± s)	Bioaccessibility (%)
F1	Fe	287 ± 30	14
	Zn	159 ± 8	17
	Cu	3.31 ± 0.13	54
	Mn	15.8 ± 0.1	36
F2	Fe	196 ± 6	78
	Zn	20.1 ± 0.2	–
	Cu	2.06 ± 0.06	75
	Mn	12.3 ± 0.6	38
F3	Fe	166 ± 3	33
	Zn	82.7 ± 12.7	–
	Cu	3.3 ± 0.8	56
	Mn	18.3 ± 0.4	19

samples: heme and non-heme. Most of the iron from foods of animal origin is heme-Fe, which is more bioavailable. Bovine meat has approximately 50% of its iron content in the heme-Fe form, for which the bioavailability varies from 15% to 35%. Heme-Fe absorption is relatively independent of the composition of the food. The absorption of non-heme iron is increased when it is ingested with ascorbic acid. However, proteins in milk significantly decrease iron absorption; this effect is due to high concentrations of calcium and phytates, which reduce iron absorption by forming insoluble complexes in the intestinal lumen. Some flours studied contained milk powder in their compositions [20].

Therefore, the flour samples studied should have low Fe bioaccessibility because they do not contain large amounts of the more favorably absorbed form of iron, heme-Fe. Despite this, the manufacturer affirms that this flour sample improves absorption of Fe [20–22]. The low bioaccessibility of this element is due to the high phytate content, which forms insoluble compounds with Fe and is present in components of the samples, such as grains, cereals and wheat [8,23,24]. Iron bioavailability in foods of vegetal origin is low because of the presence of relatively low concentrations of amino acids, especially cysteine, and the absence of proteins from meat, which usually increase the bioavailability of this analyte [8,24,25].

The results obtained for copper were expected because seeds and grains, such as rice and wheat, are known sources of Cu. Additionally, Cu-phytate complexes are soluble at the gastrointestinal tract pH, in contrast to complexes with Fe and Zn [8,21,24,26,27]. Copper absorption inhibitors are sugars, animal proteins, S-amino acids and histidine, instead of fibers, as with Fe and Zn [8,28].

The low bioaccessibility of zinc could be attributed to the strong binding of phytates with zinc. Thus, processed cereal-based foods typically present low Zn bioaccessibility [21,24,29]. Phytates in grains and seeds can bind Zn^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Cu^{2+} and other divalent cations. Previous reports indicate that the bioaccessibility of Zn^{2+} is the most impaired of these cations due to the formation of insoluble salts or co-precipitation as a Zn–Ca-phytate complex, which is emphasized in the presence of Ca from milk [30,31]. As commented before some of the flours studied contained milk powder in their compositions.

Oat, wheat and rice flours are usually good sources of Mn; however, in this study, their bioaccessibility was less than 50%. According to Bergner [21], Mn bioaccessibility is inversely correlated with the amount of phytates. Other authors, however, have demonstrated that the Mn bioaccessibility is low in foods that contain wheat flour only but improved upon the addition of other raw materials, such as oats, because of decreased phytate effects. Although high levels of Mn are present in this type of food, its release in the gastrointestinal tract is hindered such that most of the element will only be present in a low accessible form to the human body [8,28].

Figs. 3 and 4 and Table 6 show the results obtained for the baby food samples. The Fe bioaccessibility in P1 and P2 was greater than 80%, which is greater than the P3 sample, for which the Fe bioaccessibility was approximately 20%. This difference is most likely due to the presence of meat in the composition of P1 and P2 because Fe is more bioaccessible when originating from this type of matrix [8,21,22].

The bioaccessibility of Zn was low for all of the foods studied. In some cases, this element did not appear to be released during the gastrointestinal digestion. P1 was the only sample that showed

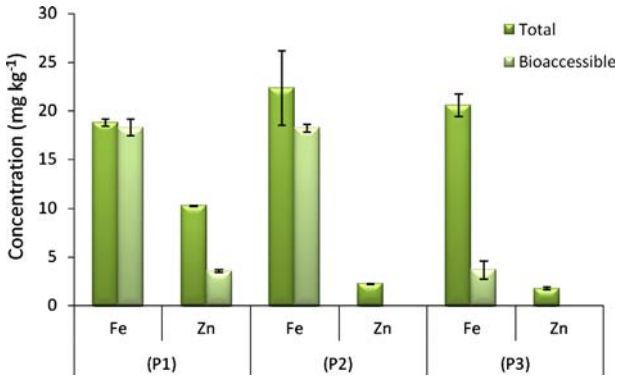

Fig. 3. Total concentrations and bioaccessible fractions of Fe and Zn in baby food samples (P1: meat+vegetables; F2: chiken+macaroni+vegetables; F3: banana+oat flour); $n=3$.

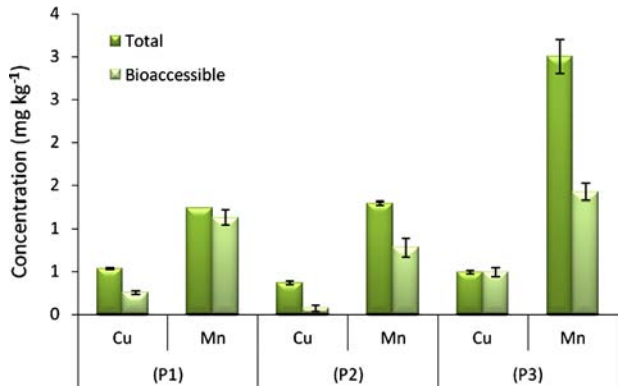

Fig. 4. Total concentrations and bioaccessible fractions of Cu and Mn in baby food samples (P1: meat+vegetables; F2: chiken+macaroni+vegetables; F3: banana+oat flour); $n=3$.

Table 6
Total concentrations and bioaccessible fractions of the analytes in the baby food samples; $n=3$.

Sample	Element	Total (mg $kg^{-1} \pm s$)	Bioaccessibility (%)
(P1)	Fe	18.8 ± 0.3	97
	Zn	10.2 ± 0.1	34
	Cu	0.53 ± 0.01	47
	Mn	1.24 ± 0.01	91
(P2)	Fe	22.3 ± 3.9	82
	Zn	2.21 ± 0.03	–
	Cu	0.37 ± 0.02	19
	Mn	1.29 ± 0.02	60
(P3)	Fe	20.6 ± 1.2	18
	Zn	1.72 ± 0.14	–
	Cu	0.49 ± 0.02	100
	Mn	3.00 ± 0.20	47

some effect from the presence of meat because the proteins may increase the bioaccessibility of this element [8,21,24].

Sample P3 showed 100% Cu bioaccessibility, in contrast to the P1 and P2 samples, for which the bioaccessibilities were less than 50%. Phytate-Cu complexes are soluble at the pH of the gastrointestinal tract; additionally, animal proteins inhibit the bioaccessibility of Cu. This is supported by low Cu bioaccessibility values in the samples containing meat and the high bioaccessibility in the sample containing banana [24,26–28].

The opposite effect occurs for Mn, for which the samples P1 and P2 presented a bioaccessible fraction greater than 50%, while the P3 sample fraction was less than 50%. This may occur because the P1 and P2 samples contain vegetables, such as potatoes and rice flour (P1 sample) and potato and wheat flour (in the macaroni of the P2 sample), which are good sources of Mn [8,21,28].

4. Conclusion

To ensure the predictive value of the *in vitro* approaches, further research is required to establish reliable methods coupled with validation studies. However, the application of an *in vitro* digestion method with the ICP-MS technique allowed for the evaluation of Cu, Fe, Mn and Zn bioaccessibility in some foods used as supplements for children and for adults.

The bioaccessibility of these elements varies according to the sample composition. Thus, it is important to complement the determination of total mineral content in food samples with this type of study to accurately assess the amount of a particular essential element that is truly bioavailable to the human body.

This study showed that no food presented 100% bioaccessibility for all of the nutrients evaluated, clearly indicating the need to study the amount of nutrients released during gastrointestinal digestion.

The inclusion of nutritional contents on food labels offers valuable information for consumers, who can select different types of food to fulfill requirements for key dietary nutrients and to maintain health.

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