International Dairy Journal 110 (2020) 104808

Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj

Influence of various ingredients on mineral bioaccessibility in infant formula and whole milk

Maria Isabel Andrekowisk Fioravanti [a, b], Raquel Fernanda Milani [b], Esther Lima de Paiva [b, c], Marcelo Antônio Morgano [b, *]

[a] Adolfo Lutz Institute, Rua São Carlos, 720, Vila Industrial, Campinas, SP, 13035-420, Brazil
[b] Institute of Food Technology, Av. Brasil 2880, Jd. Chapadão, P.O. Box 139, Campinas, SP, 13070-178, Brazil
[c] School of Food Engineering, University of Campinas (UNICAMP), Rua Monteiro Lobato 80, Campinas, SP, 13083-862, Brazil

ARTICLE INFO

Article history:
Received 7 May 2020
Received in revised form
2 July 2020
Accepted 3 July 2020
Available online 15 July 2020

ABSTRACT

The mineral composition and bioaccessible fraction of different types of infant formula and milk samples were evaluated. Mineral content was higher in the infant formula than in the milk samples by 162, 63, 37 and 2-fold for Fe, Cu, Mn and Zn, respectively. The mineral bioaccessibility in milk products was found to be influenced by both the total content and the type of processing (pasteurised, powdered or UHT). For milk-based formulas, the addition of a prebiotic generally improved the dialysability of K, Fe, Mg, Cu and Zn, whereas the enzymatically hydrolysed protein had no effect on the bioaccessibility of most of the elements. Infant formulas containing soy protein showed results for dialysability similar to those for formulas containing whey protein. In general, infant formula results indicated that the high mineral absorption could be related to the protein type, presence of prebiotics and mineral concentrations.

1. Introduction

Breast milk is considered the ideal form of nutrition for babies up to six months of age, being a source of balanced nutrients and bioactive compounds for the growth and adequate development of the child. However, when breastfeeding is not adequate, possible or desirable, infant formula (IF) is the ideal nutritional alternative, and undoubtedly more adequate than the consumption of cows' milk (Vieira da Silva, Mattanna, Bizzi, Richards, & Barin, 2013; Zou, Pande, & Akoh, 2016). The nutritional safety and adequacy of an IF is important for the growth and development of the infant. Although the composition of IFs is being evolved to better approximate that of breast milk, some disparities still exist between breastfed infants and those fed on IF (Donovan, 2019; FAO/WHO, 2016).

To approximate breast milk, the composition of IFs are elaborated and classified according to the age range of the infant, the main classes being 0–6 months, 6–12 months and 0–12 months. There is also an IF category for specific therapeutic dietary needs (BRASIL, 2011a,b; EC, 2006). It is fundamental to determine the total contents of nutrients present in IF to guarantee the quality of the product according to the regulations. However, knowing only the total nutrient concentrations in the food is not sufficient to determine if the food adequately attends to the nutritional needs. It is also important to know the fraction effectively available for absorption (Do Nascimento da Silva, Leme, Cidade, & Cadore, 2013); bioaccessibility studies are a well-known tool that allows estimation of their absorption (Minekus et al., 2014).

Bioaccessibility is defined as the fraction of the element that is liberated from the food matrix after ingestion and solubilised in the intestinal lumen and intestinal tract, and this can be determined by in vitro solubility and dialysability trials (Barciela-Alonso & Bermejo-Barrera, 2015). Due to the extremely complex nature of the human gastrointestinal tract, the ability to recreate all its functions in one in vitro model is limited. Despite their limitations, in vitro digestion models are recognised alternatives to in vivo trials, with no ethical restrictions, providing precise results in a short time period (Hur, Lim, Decker, & McClements, 2011; Ménard et al., 2018).

Because of the lack of comprehensive information about the bioaccessibility of micronutrients in infant foods, effort is required

* Corresponding author. Tel.: +55 19 3743 1778.
E-mail address: morgano@ital.sp.gov.br (M.A. Morgano).

https://doi.org/10.1016/j.idairyj.2020.104808
0958-6946/

to understand the influence of the various ingredients on the nutrient absorption from these foods (Shani-Levi et al., 2017). Thus the objectives of the present study were to determine the total contents of Ca, Cu, Fe, K, Mg, Mn, Na, P and Zn in IF samples destined for infants in the 0–6, 0–12 and 6–12 months old age ranges and in whole milk samples (pasteurised, powdered and UHT), and to evaluate the influence of the ingredients on the bioaccessibility of these elements (soluble and dialysable fractions).

2. Material and methods

2.1. Equipment

An acid extraction was first carried out in an ultrasonic bath (model Easy 180H, Elma, Singen, Germany) and minerals were quantified using an inductively coupled plasma optical emission spectrophotometer (ICP OES 5100VDV, Agilent Technologies, Tokyo, Japan). The ICP OES operating conditions were as follows: power 1.2 kW; argon flow rates of 12, 1.0, and 0.7 L min^{-1} for plasma, auxiliary, and nebuliser, respectively. Selected emission lines were: Ca, 317.933; Na, 589.592; P, 213.618; Cu, 324.754; Fe, 259.940; Mn, 257.610; Zn, 206.200; K 766.491 and Mg, 279.553 nm. The linear ranges were 0.2–5 mg 100 mL^{-1} for Ca, Na and P; 0.5–200 μg 100 mL^{-1} for Cu, Fe, Mn and Zn; 0.2–10 mg 100 mL^{-1} for K and 0.2–2 mg 100 mL^{-1} for Mg. A Dubnoff type water bath (Nova Técnica, Piracicaba, Brazil), a pH meter equipped with a selective electrode (Ohaus, Parsippany, NJ, USA) and a refrigerated centrifuge (Eppendorf, Hamburg, Germany) were used for the bioaccessibility trials. Water used for solutions and samples was purified in a reverse osmosis system (Gehaka, São Paulo, Brazil).

2.2. Reagents and solutions

Nitric acid purified using a sub-boiling system (Distillacid, Berghof, Eningen, Germany); 30% hydrogen peroxide (v/v); 37% hydrochloric acid (Merck, Darmstadt, Germany); sodium bicarbonate, swine gastric mucous pepsin (250 U mg^{-1}), swine pancreas pancreatin (8 times the USP specifications), bovine and poultry bile salts and dialysis bags with porosity of 25 Å and cut-off of 12,000 Da (Sigma–Aldrich, St. Louis, MO, USA) were used. The enzyme solutions were prepared immediately before use (Fioravanti, Milani, de Paiva, & Morgano, 2020).

Analytical curves were prepared by successive dilutions of certified standards: multi-element 1000 mg L^{-1} solution (Cu, Fe, Mn and Zn) and 10,000 mg L^{-1} solution of P (Specsol, Quimlab, Jacareí, Brazil), Ca, Mg, K and Na (Merck) in a 5% HCl (v/v) solution.

2.3. Samples

A total of 18 IF samples were acquired from commercial establishments in Brazil, Chile, United States of America, New Zealand and Portugal, considering the following age groups: 0–6 months (n = 6), 0–12 months (n = 8) and 6–12 months (n = 4). The samples of pasteurised (n = 3), powdered (n = 3) and UHT (n = 3) whole cows' milk were acquired from commercial establishments in Campinas, Brazil. Table 1 shows the identification of the samples according to their coding, front panel labelling and main ingredients.

Sample coding was defined by a group of letters and numbers, where the first letter represents the matrix (F, IF; L, cows' whole milk); the second letter is the brand (A to I: different brands); the numbers 1, 2 and 3 represent the IF age ranges (0–6, 0–12 and 6–12 months, respectively); letters after the hyphen represent the IF country of origin (BR, Brazil; CH, Chile; PT, Portugal; NZ, New Zealand; USA, United States of America), and the cows' milk process (PAS, pasteurised; PO, powdered; UHT, ultra high temperature); letters SOY after the slash denoting IF soy protein-based.

Table 1
Identification and information on the label (front panel and main ingredients) of the infant formula (IF; n = 18) and milk (n = 9) samples.[a]

Sample	Labelling front panel: main ingredients
FA1-BR	IF for infants with prebiotics: demineralised whey; lactose; skimmed milk; galacto-oligosaccharides (prebiotics)
FA1-CH	Initial IF in powder form with Fe: whey, skimmed milk; lactose; ascorbic acid
FB1-BR	IF for infants: skimmed milk powder; lactose; whey protein concentrate; hydrolysed whey protein
FC1-BR	IF for infants: lactose; skimmed milk powder; whey protein concentrate
FD1-BR/SOY	Soy based IF for infants: maltodextrin; soy protein
FD1-NZ	Premium IF: milk solids; galacto-oligosaccharides (prebiotics); powdered omega; LCPUFAs
FA2-BR/SOY	Soy-based IF for infants and follow-up: maltodextrin; soy isolate protein
FA2-PT	Milk for infants; hypoallergenic: lactose; enzymatically hydrolysed whey proteins; *Lactobacillus reuteri* culture (DSM17938)
FA2-NZ	Starter premium IF: lactose (milk); enzymatically hydrolysed whey proteins; culture (*Bifidus*)
FB2-BR	IF for infants and follow up: maltodextrin; lactose; partially hydrolysed whey protein; ascorbic acid
FB2-USA	IF with Fe: skimmed milk; lactose; whey protein concentrate; galacto-oligosaccharides (prebiotics)
FC2-BR	IF for infants and follow up destined for specific diet-therapy: corn syrup solids; partially hydrolysed skimmed milk and whey proteins
FC2-USA	IF based on powdered milk with Fe: skimmed milk; lactose; whey protein concentrate; galacto-oligosaccharides (prebiotics)
FD2-BR	IF for infants and follow up and children in their early childhood: partially hydrolysed whey protein concentrate; galacto-oligosaccharides (prebiotics); lactose
FA3-BR	IF for the follow up of infants; prebiotics: demineralised whey; lactose; skimmed milk; galacto-oligosaccharides (prebiotics)
FB3-BR	IF for the follow up of infants: skimmed milk; lactose; whey protein concentrate
FC3-BR	IF for the follow up of infants: skimmed milk powder; lactose
FD3-BR/SOY	Soy based IF for infants and follow up and children in their early childhood: maltodextrin; soy protein
LA-PO	Instant whole milk powder - rich in Fe, Zn &Ca: whole milk; $CaCO_3$; Fe4(P2O7)3; ZnSO4; sodium ascorbate; cholecalciferol
LE-PO	Instant whole milk powder; +Fe: whole milk; Vitamins A, C & D; Fe
LF-PO	Instant whole milk powder: whole milk
LA-UHT	Whole UHT milk - rich in Fe, Zn: whole milk; ascorbic acid; Fe4(P2O7)3; ZnSO4; cholecalciferol
LF-UHT	Whole UHT milk: whole milk; sodium monophosphate, sodium diphosphate and sodium citrate
LJ-UHT	Whole UHT milk: whole milk; sodium triphosphate, monophosphate, diphosphate and sodium citrate
LG-PAS	Type A pasteurised whole milk
LH-PAS	Homogenised type A pasteurised whole milk
LI-PAS	Type A pasteurised whole milk

[a] Sample coding is as follows: first letter indicates infant formula (F) or milk (L); second letter, brand; numerals, age range; letters after hyphen, country of origin (BR, Brazil; CH, Chile; PT, Portugal; NZ, new Zealand; USA, united States of America) for infant formula or type of process (PAS, pasteurised; PO, powder; UHT, ultra high temperature) for milk; SOY after slash, soy protein-based infant formula. Milk samples presented a consumption recommendation in label (children over one year of age).

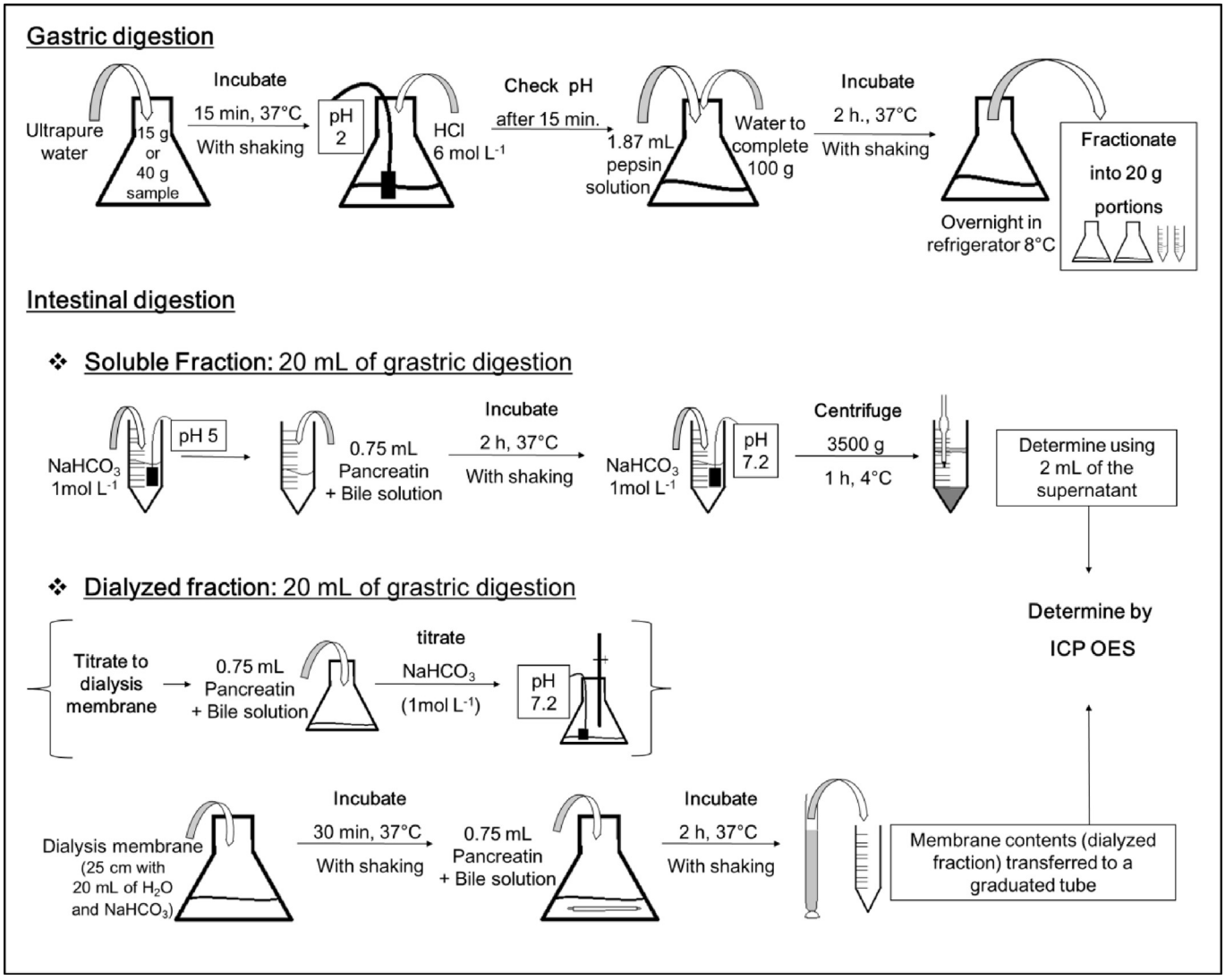

Fig. 1. Summary scheme of the methodology used for the mineral bioaccessibility determination.

2.4. Extraction

To determine the total contents of the minerals Ca, Cu, Fe, K, Mg, Mn, Na, P and Zn present in the IF and powdered milks, the samples were initially prepared according to the instructions on the labels: powdered IFs were reconstituted by weighing 15 g in 100 mL purified water. The samples of liquid milk (PAS and UHT) were weighed directly.

Ultrasonic assisted extraction was adapted from the methodologies of Bermejo-Barrera, Muñiz-Naveiro, Moreda-Piñeiro, and Bermejo-Barrera (2001), Cava-Montesinos, Cervera, Pastor, and de la Guardia (2005), and Machado, Bergmann, and Pistón (2016). Briefly, 2 g of liquid sample were weighed into a 50 mL graduated tube and 2 mL of HNO_3 and 1 mL of H_2O_2 added. The tube was closed and placed in an ultrasonic bath at 60 °C for 30 min. After cooling, the resulting solution was made up to 25 mL with purified water and filtered (0.45 μm PTFE filter, Agilent Technologies). The extractions were carried out in triplicate. For quality control, analytical blank experiments and certified reference materials Baby Food Composite (NIST 2383a) and Infant/Adult Nutritional Formula (NIST 1849a) were employed (Fioravanti et al., 2020).

2.5. Determination of the bioaccessible content

2.5.1. Gastric digestion

Gastric digestion was based on the methods of Fioravanti et al. (2020) and Perales, Barberá, Lagarda, and Farré (2005). Briefly, 15 g (powder) or 40 g (liquid) of sample were weighed into a 250 mL Erlenmeyer flask, 30 mL of ultrapure water added and the mix homogenised with shaking for 15 min in a water bath at 37 °C. The pH value was then adjusted to 2.0 ± 0.2 with 6 mol L^{-1} HCl and after 15 min rechecked and the pH readjusted, if necessary (Perales et al., 2005). A 1.87 mL aliquot of a 0.16 g mL^{-1} freshly pepsin solution prepared in 0.1 mol L^{-1} HCl (0.02 g of pepsin g^{-1} sample) was then added, then purified water added to make up to 100 g, and the mix was incubated in a shaken water bath at 37 °C for 2 h. The sample was maintained in a refrigerator at 8 °C overnight and the gastric digest then fractionated into 20 g portions, placing two into graduated 50 mL tubes (for the soluble fraction assay) and two into 250 mL Erlenmeyer flasks (for the dialysed fraction assay), giving a total of five 20 g fractions, with the fifth fraction remaining in the original Erlenmeyer (Fig. 1).

2.5.2. Intestinal digestion

Intestinal digestion was based on the methods of Fioravanti et al. (2020) and Perales et al. (2005). For the soluble fraction assay, the pH value of one of the digest fractions was adjusted to 5.0 ± 0.2 with 1 mol L^{-1} $NaHCO_3$, and 0.75 mL freshly made pancreatin + bile solution containing 0.004 g mL^{-1} pancreatin and 0.025 g mL^{-1} bile extract (providing 0.00015 g pancreatin and 0.00094 g bile salts g^{-1} sample) was added. The tube containing the sample, salts and enzymes was placed in a shaking water bath at 37 °C for 2 h, then the pH was adjusted to 7.2 ± 0.2 and the sample centrifuged at 3500× g for 1 h at 4 °C. The minerals present in the incubated extracts were determined using 2 mL of the supernatant (soluble fraction) according to the procedure described in section 2.4.

For the dialysed fraction, to prepare the dialysis membrane, 0.75 mL of freshly made pancreatin + bile solution were added to one of the gastric digest fractions (Erlenmeyer flasks) and titrated with 1 mol L^{-1} $NaHCO_3$ to pH 7.2 ± 0.2, so determining the titratable acidity. After preparing the dialysis membrane (25 cm in length containing 20 mL purified water and the volume of $NaHCO_3$ used in the titratable acidity), it was placed in the Erlenmeyer flasks containing the gastric digest fraction and incubated in a shaken water bath at 37 °C for 30 min. An aliquot of 0.75 mL of freshly made pancreatin + bile solution was then added and incubated for another 2 h. The membrane contents (dialysed fraction) were then transferred to a graduated tube and the mineral concentrations determined using ICP OES. A summary scheme of the methodology used for the mineral bioaccessibility determination is shown in Fig. 1.

2.5.3. Calculation of the bioaccessible mineral content

The soluble fraction (%S) was calculated as follows:

$$\% \text{ solubility} = 100 \times S/C$$

where S is the soluble mineral content (in mg or μg 100 mL^{-1} reconstituted sample) and C is the total mineral content of the sample (in mg or μg 100 mL^{-1} reconstituted sample). The dialysed fraction (%D) was calculated as follows:

$$\% \text{ dialysis} = 100 \times D/C$$

where D is the dialysed mineral content (in mg or μg 100 mL^{-1} reconstituted sample) and C is the total mineral content of the sample (in mg or μg 100 mL^{-1} reconstituted sample).

2.6. Statistical analyses

The results are expressed as the mean x ± SD of three analytical repetitions. The statistical (one-way ANOVA, considering a 95% confidence level) and multivariate analyses (principal components analysis, PCA and hierarchical cluster analysis, HCA) were carried out using XIstat (Addinsoft, Paris, France) and Pirouette (Infometrix, Woodinville, WA, USA) software, respectively.

3. Results

Tables 2 and 3 show the results obtained for the total, soluble (%S) and dialysed (%D) contents of micro (Cu, Fe, Mn and Zn) and macro-minerals (Ca, K, Mg, Na and P), respectively. The results show variations in the total content between the different types of IF, even between specific groups, such as the same brand, protein type and age range. The samples FA2-PT and FA2-NZ are the only types that show significantly similar results for the nine elements under study, a fact not observed for the other brands or samples.

From Tables 2 and 3, soluble fractions of the minerals show higher contents than the dialysed fractions, except for LF-UHT sample where the percentages of the soluble (%S) and dialysed (%D) fractions for Fe (57% and 61%, respectively) and Mn (22.5% and 23.5%, respectively) are the similar, showing that for this sample, the whole soluble Fe and Mn contents are dialysed. The Na bioaccessibility was not evaluated due to the amount of $NaHCO_3$ added during the trials. With respect to the total Na contents, they ranged from 17.6 (FC2-USA) to 64.9 (LF-UHT) mg 100 mL^{-1} and the highest concentrations were found in the UHT and reconstituted milk powders.

In general, the K, Mg and P solubility in IF and milks were greater than 60% with respect to the total contents. The %S values found for K, Mg, P and Zn in the UHT milks were above 80%, comparable with the results found by Sanches, Peixoto, and Cadore (2019), where the %S values were above 60% for Zn and 70% for Ca, K, Mg, Na and P in whole, semi-skimmed and skimmed UHT milks. The %S ranged from 25% (LF-PO) to 94% (FC2-BR) for Ca, from 11% (FA2-BR/SOY) to 120% (LH-PAS) for Mn and from 4% (FD3-BR/SOY) to 111% (FD2-BR) for Zn. These variations can be associated with the mineral content, the technological process used and the sample composition. Cu showed a %S above 60% in the IFs, except for FC3-BR (46%). For the milk samples, only LA-PO and LE-PO presented %S above the quantification limit (LOQ) for Cu (40% and 63%, respectively).

Table 2
Mean concentration of the total, soluble and dialysed contents and bioaccessible fractions for Cu, Fe, Mn and Zn.[a]

Sample		Cu		Fe		Mn		Zn	
		$\bar{x}$ ± SD (μg 100 mL^{-1})	Bioaccessibility (% ± SD)	$\bar{x}$ ± SD (μg 100 mL^{-1})	Bioaccessibility (% ± SD)	$\bar{x}$ ± SD (μg 100 mL^{-1})	Bioaccessibility (% ± SD)	$\bar{x}$ ± SD (μg 100 mL^{-1})	Bioaccessibility (% ± SD)
FA1-BR	Total	63 ± 2	–	774 ± 15	–	14.2 ± 0.3	–	746 ± 10	–
	Soluble	58 ± 1	93 ± 2	560 ± 5	72 ± 1	12.4 ± 0.7	87 ± 5	495 ± 40	66 ± 5
	Dialysed	12 ± 1	20 ± 1	96 ± 1	12.4 ± 0.2	1.9 ± 0.1	13 ± 1	52 ± 3	7 ± 1
FA1-CH	Total	61 ± 1	–	968 ± 9	–	15.4 ± 0.1	–	720 ± 9	–
	Soluble	62 ± 2	101 ± 3	985 ± 16	102 ± 2	13.6 ± 0.2	89 ± 1	516 ± 46	72 ± 6
	Dialysed	13 ± 1	22 ± 1	331 ± 9	34 ± 1	3.1 ± 0.2	20 ± 1	79 ± 5	11 ± 1
FB1-BR	Total	63 ± 1	–	641 ± 17	–	21.8 ± 0.3	–	614 ± 11	–
	Soluble	70 ± 5	112 ± 8	968 ± 9	151 ± 1	17.0 ± 0.4	78 ± 2	326 ± 14	53 ± 2
	Dialysed	12 ± 2	19 ± 3	93 ± 5	15 ± 1	2.1 ± 0.2	10 ± 1	44 ± 3	7 ± 1
FC1-BR	Total	43 ± 1	–	828 ± 24	–	4.5 ± 0.1	–	811 ± 27	–
	Soluble	47.1 ± 0.2	109.9 ± 0.4	810 ± 21	98 ± 3	4.1 ± 0.2	92 ± 5	644 ± 22	79 ± 3
	Dialysed	10 ± 1	23 ± 3	83 ± 7	10 ± 1	0.61 ± 0.02	13.7 ± 0.4	45 ± 2	5.6 ± 0.2
FD1-BR/SOY	Total	36.0 ± 0.4	–	816 ± 8	–	33.7 ± 0.4	–	492 ± 7	–
	Soluble	32 ± 1	88 ± 3	142 ± 23	17 ± 3	9.4 ± 0.8	28 ± 2	50 ± 8	10 ± 2
	Dialysed	7.3 ± 0.4	20 ± 1	15.4 ± 0.3	1.9 ± 0.1	0.20 ± 0.04	0.6 ± 0.1	4 ± 1	0.8 ± 0.1

Table 2 (*continued*)

Sample		Cu		Fe		Mn		Zn	
		$\bar{x}$ ± SD (μg 100 mL^{-1})	Bioaccessibility (% ± SD)	$\bar{x}$ ± SD (μg 100 mL^{-1})	Bioaccessibility (% ± SD)	$\bar{x}$ ± SD (μg 100 mL^{-1})	Bioaccessibility (% ± SD)	$\bar{x}$ ± SD (μg 100 mL^{-1})	Bioaccessibility (% ± SD)
FD1-NZ	Total	68 ± 2	–	970 ± 23	–	6.3 ± 0.2	–	564 ± 15	–
	Soluble	73 ± 1	108 ± 2	1073 ± 22	111 ± 2	5.5 ± 0.4	87 ± 6	439 ± 10	78 ± 2
	Dialysed	13.8 ± 0.4	21 ± 1	105 ± 4	11 ± 1	0.70 ± 0.03	11 ± 1	31 ± 2	5.4 ± 0.4
FA2-BR/SOY	Total	75 ± 2	–	753 ± 17	–	41.8 ± 0.8	–	824 ± 16	–
	Soluble	53 ± 1	71 ± 2	785 ± 14	104 ± 2	4.6 ± 0.2	11 ± 1	512 ± 17	62 ± 2
	Dialysed	12 ± 1	16 ± 1	124 ± 4	17 ± 1	0.6 ± 0.2	1.4 ± 0.4	61 ± 8	7 ± 1
FA2-PT	Total	62 ± 2	–	782 ± 25	–	14.2 ± 0.9	–	663 ± 23	–
	Soluble	61 ± 1	99 ± 2	722 ± 14	92 ± 2	6.6 ± 0.3	47 ± 2	398 ± 31	60 ± 5
	Dialysed	14 ± 1	23 ± 1	65 ± 5	8 ± 1	<LOQ	<LOQ	22 ± 2	3.3 ± 0.2
FA2-NZ	Total	63 ± 1	–	790 ± 6	–	14.0 ± 0.3	–	671 ± 10	–
	Soluble	61 ± 3	98 ± 4	678 ± 27	86 ± 3	5.1 ± 0.5	37 ± 4	304 ± 12	45 ± 2
	Dialysed	13.8 ± 0.3	22 ± 1	51 ± 3	6.5 ± 0.4	<LOQ	<LOQ	20 ± 2	3.0 ± 0.3
FB2-BR	Total	56 ± 1	–	473 ± 3	–	18.8 ± 0.2	–	581 ± 6	–
	Soluble	54 ± 2	96 ± 3	454 ± 4	96 ± 1	8.2 ± 1.0	44 ± 5	322 ± 9	55 ± 2
	Dialysed	12.2 ± 0.1	21.6 ± 0.2	51 ± 5	11 ± 1	0.2 ± 0.1	1.3 ± 0.7	13 ± 1	2.2 ± 0.3
FB2-USA	Total	72 ± 1	–	1129 ± 14	–	8.4 ± 0.1	–	630 ± 7	–
	Soluble	74 ± 1	104 ± 2	1479 ± 44	131 ± 4	7.4 ± 0.4	88 ± 5	524 ± 18	83 ± 3
	Dialysed	15 ± 1	21 ± 1	312 ± 11	28 ± 1	0.4 ± 0.04	4 ± 1	13 ± 1	2.0 ± 0.2
FC2-BR	Total	58 ± 1	–	584 ± 10	–	20.0 ± 0.7	–	864 ± 25	–
	Soluble	55 ± 1	95 ± 1	717 ± 13	123 ± 2	17.6 ± 0.9	88 ± 4	762 ± 35	88 ± 4
	Dialysed	11.2 ± 0.4	19 ± 1	71 ± 6	12 ± 1	1.3 ± 0.2	6 ± 1	32 ± 4	4 ± 1
FC2-USA	Total	70 ± 1	–	1104 ± 6	–	14.6 ± 0.2	–	803 ± 6	–
	Soluble	65 ± 1	93 ± 2	1314 ± 44	119 ± 4	14.1 ± 0.3	96 ± 2	665 ± 21	83 ± 3
	Dialysed	15 ± 1	21 ± 1	149 ± 3	13.5 ± 0.3	1.6 ± 0.1	11 ± 1	36 ± 2	4.5 ± 0.2
FD2-BR	Total	43 ± 2	–	435 ± 11	–	7.0 ± 0.1	–	506 ± 13	–
	Soluble	44 ± 1	104 ± 2	472 ± 1	108.5 ± 0.2	4.5 ± 1.6	64 ± 23	564 ± 91	111 ± 18
	Dialysed	11 ± 1	26 ± 1	72 ± 1	16.6 ± 0.3	0.37 ± 0.03	5 ± 1	28 ± 1	5.5 ± 0.3
FA3-BR	Total	57 ± 2	–	1081 ± 25	–	4.8 ± 0.2	–	669 ± 18	–
	Soluble	51 ± 1	91 ± 3	119 ± 8	11 ± 1	1.34 ± 0.01	28.0 ± 0.2	33 ± 5	5 ± 1
	Dialysed	13 ± 1	24 ± 1	17 ± 3	1.6 ± 0.2	0.5 ± 0.1	10 ± 2	6 ± 1	0.9 ± 0.2
FB3-BR	Total	51 ± 1	–	1047 ± 7	–	18.1 ± 0.1	–	535 ± 6	–
	Soluble	50 ± 1	97 ± 2	1059 ± 13	101 ± 1	11.6 ± 0.1	64 ± 1	241 ± 1	45.1 ± 0.3
	Dialysed	11.9 ± 0.1	23.2 ± 0.3	82 ± 4	7.8 ± 0.4	0.6 ± 0.1	3 ± 1	12 ± 1	2.2 ± 0.3
FC3-BR	Total	93 ± 1	–	1142 ± 10	–	11.4 ± 0.2	–	690 ± 2	–
	Soluble	42 ± 4	46 ± 4	1142 ± 38	100 ± 3	8.7 ± 0.8	77 ± 7	463 ± 37	67 ± 5
	Dialysed	8 ± 2	9 ± 2	80 ± 7	7 ± 1	0.8 ± 0.1	7 ± 1	25 ± 5	4 ± 1
FD3-BR/SOY	Total	36.1 ± 0.3	–	1128 ± 7	–	31.0 ± 0.5	–	500 ± 2	–
	Soluble	28 ± 1	78 ± 3	114 ± 21	10 ± 2	6.7 ± 0.6	22 ± 2	18 ± 10	4 ± 2
	Dialysed	7.5 ± 0.4	21 ± 1	12 ± 1	1.1 ± 0.1	0.25 ± 0.03	0.8 ± 0.1	2.7 ± 0.4	0.5 ± 0.1
LG-PAS	Total	4 ± 1	–	8.9 ± 0.1	–	2.0 ± 0.9	–	365 ± 9	–
	Soluble	<LOQ	<LOQ	<LOQ	<LOQ	1.7 ± 0.2	86 ± 11	195 ± 9	53 ± 3
	Dialysed	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	17 ± 1	4.7 ± 0.4
LH-PAS	Total	1.5 ± 0.1	–	8 ± 1	–	1.3 ± 0.1	–	383 ± 8	–
	Soluble	<LOQ	<LOQ	5 ± 1	64 ± 12	1.7 ± 0.5	120 ± 35	225 ± 19	59 ± 5
	Dialysed	<LOQ	<LOQ	4 ± 1	49 ± 6	<LOQ	<LOQ	12 ± 2	3 ± 1
LI-PAS	Total	3.0 ± 0.2	–	7.0 ± 0.4	–	1.5 ± 1.5	–	364 ± 4	–
	Soluble	<LOQ	<LOQ	<LOQ	<LOQ	1.7 ± 0.1	113 ± 3	198 ± 17	55 ± 5
	Dialysed	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	11 ± 4	3 ± 1
LA-PO	Total	3.6 ± 0.1	–	2321 ± 295	–	14.5 ± 1.4	–	1124 ± 18	–
	Soluble	1.4 ± 0.2	40 ± 6	1000 ± 30	43 ± 1	11.6 ± 0.6	80 ± 4	938 ± 48	83 ± 4
	Dialysed	<LOQ	<LOQ	12 ± 2	0.5 ± 0.1	<LOQ	<LOQ	5 ± 1	0.4 ± 0.1
LE-PO	Total	4.4 ± 0.1	–	1327 ± 186	–	5.5 ± 0.7	–	459 ± 9	–
	Soluble	2.8 ± 0.5	62 ± 11	469 ± 68	35 ± 5	2.6 ± 0.1	47 ± 1	213 ± 19	46 ± 4
	Dialysed	0.7 ± 0.1	17 ± 2	7 ± 3	0.5 ± 0.3	<LOQ	<LOQ	3 ± 2	0.7 ± 0.5
LF-PO	Total	3.2 ± 0.3	–	17.7 ± 0.1	–	3.7 ± 0.2	–	423 ± 5	–
	Soluble	<LOQ	<LOQ	17.4 ± 0.3	99 ± 2	1.62 ± 0.04	44 ± 1	212 ± 16	50 ± 4
	Dialysed	<LOQ	<LOQ	3.5 ± 0.2	20 ± 1	0.41 ± 0.01	10.9 ± 0.1	31 ± 2	7.3 ± 0.5
LA-UHT	Total	2.8 ± 0.3	–	1177 ± 121	–	5.0 ± 0.2	–	1269 ± 21	–
	Soluble	<LOQ	<LOQ	1180 ± 27	100 ± 2	2.54 ± 0.02	50.9 ± 0.3	1014 ± 28	80 ± 2
	Dialysed	<LOQ	<LOQ	314 ± 8	27 ± 1	1.13 ± 0.01	22.5 ± 0.3	215 ± 5	17.0 ± 0.4
LJ-UHT	Total	2.0 ± 0.5	–	27 ± 4	–	1.1 ± 0.2	–	361 ± 12	–
	Soluble	<LOQ	<LOQ	23 ± 3	87 ± 9	0.88 ± 0.01	78 ± 1	318 ± 17	88 ± 5
	Dialysed	<LOQ	<LOQ	13.0 ± 0.3	48 ± 1	0.51 ± 0.01	44.6 ± 0.1	65 ± 2	18 ± 1
LF-UHT	Total	2.2 ± 0.6	–	21 ± 2	–	2.2 ± 0.4	–	358 ± 4	–
	Soluble	<LOQ	<LOQ	11.9 ± 0.3	57 ± 2	0.49 ± 0.01	22.5 ± 0.3	349 ± 6	97 ± 2
	Dialysed	<LOQ	<LOQ	12.8 ± 0.4	61 ± 1	0.51 ± 0.01	23.5 ± 0.2	67 ± 2	19 ± 1

[a] Abbreviations are: $\bar{x}$ ± SD, mean concentration (n = 3); F, infant formula; L, milk; A to J, brand; 1 to 3, age range; BR, Brazil; CH, Chile; NZ, New Zealand; PT, Portugal; USA, United States of America; PAS, pasteurised milk; PO, powdered milk; UHT, ultra high temperature treated milk; SOY, soy IF; LOQ, limit of quantification (Cu, Mn = 0.2 μg 100 mL^{-1}; Fe = 0.5 μg 100 mL^{-1}). FA1-CH, FB2-USA, FC2-USAand LE-PO were Fe-fortified; LA-PO &LA-UHT were Fe and Zn-fortified.

Table 3
Mean concentration of the total, soluble and dialysed contents and bioaccessible fractions for Ca, K, Mg, P and Na.[a]

Sample		Ca		K		Mg		P		Na
		$\bar{x}$ ± SD (μg 100 mL^{-1})	Bioaccessibility (% ± SD)	$\bar{x}$ ± SD (μg 100 mL^{-1})	Bioaccessibility (% ± SD)	$\bar{x}$ ± SD (μg 100 mL^{-1})	Bioaccessibility (% ± SD)	$\bar{x}$ ± SD (μg 100 mL^{-1})	Bioaccessibility (% ± SD)	$\bar{x}$ ± SD (μg 100 mL^{-1})
FA1-BR	Total	40.7 ± 0.8	–	77.6 ± 2.1	–	8.7 ± 0.2	–	23.6 ± 0.7	–	21.0 ± 0.7
	Soluble	11.2 ± 1.3	28 ± 3	91.9 ± 0.7	119 ± 1	8.6 ± 0.1	99 ± 1	21.7 ± 0.2	92 ± 1	–
	Dialysed	0.7 ± 0.2	1.7 ± 0.5	29.1 ± 0.4	38 ± 1	2.9 ± 0.1	33 ± 1	7.3 ± 0.1	31 ± 0.4	–
FA1-CH	Total	46.8 ± 1.1	–	115.3 ± 4.5	–	7.3 ± 0.2	–	30.0 ± 0.8	–	22.6 ± 0.9
	Soluble	12.5 ± 0.8	27 ± 2	121.4 ± 3.5	105 ± 3	7.1 ± 0.1	97 ± 1	29.6 ± 0.2	99 ± 0.5	–
	Dialysed	1.5 ± 0.5	3 ± 1	44.3 ± 0.8	38 ± 1	2.6 ± 0.1	35 ± 1	9.8 ± 0.2	33 ± 1	–
FB1-BR	Total	57.5 ± 0.7	–	81.8 ± 1.3	–	6.1 ± 0.1	–	32.2 ± 0.4	–	21.0 ± 0.3
	Soluble	30.1 ± 0.6	52 ± 1	98.5 ± 4.0	120 ± 5	5.8 ± 0.1	96 ± 2	31.3 ± 1.0	97 ± 3	–
	Dialysed	6.0 ± 0.4	10 ± 1	32.3 ± 0.8	40 ± 1	1.84 ± 0.04	30 ± 1	9.4 ± 0.3	29 ± 1	–
FC1-BR	Total	46.6 ± 1.3	–	99.1 ± 0.1	–	6.1 ± 0.2	–	35.8 ± 1.2	–	21.3 ± 0.7
	Soluble	23.5 ± 1.1	50 ± 2	106.4 ± 0.7	107 ± 1	6.1 ± 0.1	100 ± 2	35.9 ± 0.5	100 ± 1	–
	Dialysed	1.8 ± 0.4	4 ± 1	37.7 ± 0.7	38 ± 1	1.34 ± 0.01	22 ± 0.1	11.3 ± 0.2	32 ± 1	–
FD1-BR/SOY	Total	49.7 ± 0.4	–	72.0 ± 1.3	–	5.53 ± 0.04	–	29.7 ± 0.4	–	21.5 ± 0.4
	Soluble	19.4 ± 1.8	39 ± 4	80.6 ± 0.8	112 ± 1	5.1 ± 0.1	93 ± 1	20.2 ± 0.5	68 ± 2	–
	Dialysed	0.6 ± 0.2	1.2 ± 0.4	25.8 ± 0.7	36 ± 1	0.8 ± 0.1	15 ± 1	5.6 ± 0.2	19 ± 0.5	–
FD1-NZ	Total	56.8 ± 0.9	–	86.3 ± 1.4	–	6.3 ± 0.1	–	36.9 ± 0.7	–	23.1 ± 0.4
	Soluble	26.6 ± 4.9	48 ± 9	100.4 ± 1.5	116 ± 2	6.9 ± 0.2	110 ± 3	42.2 ± 0.8	114 ± 2	–
	Dialysed	1.7 ± 0.2	3.0 ± 0.3	36.1 ± 0.7	42 ± 1	1.60 ± 0.02	25.3 ± 0.3	13.8 ± 0.3	37 ± 1	–
FA2-BR/SOY	Total	67.5 ± 1.6	–	87.6 ± 1.6	–	8.1 ± 0.2	–	46.8 ± 1.0	–	25.9 ± 0.5
	Soluble	38.2 ± 1.5	57 ± 2	109.5 ± 0.2	125 ± 0.2	8.3 ± 0.2	104 ± 2	42.2 ± 0.8	90 ± 2	–
	Dialysed	5.7 ± 1.4	8 ± 2	35.3 ± 0.8	40 ± 1	2.9 ± 0.1	36 ± 1	14.0 ± 0.2	30 ± 0.5	–
FA2-PT	Total	47.9 ± 1.7	–	81.7 ± 2.6	–	7.2 ± 0.2	–	27.9 ± 1.1	–	28.5 ± 0.9
	Soluble	26.1 ± 1.5	54 ± 3	81.8 ± 2.0	100 ± 2	6.9 ± 0.2	96 ± 3	25.4 ± 0.8	91 ± 3	–
	Dialysed	0.6 ± 0.2	1 ± 0.4	30.8 ± 1.4	38 ± 2	1.2 ± 0.2	16 ± 2	9.1 ± 0.4	33 ± 2	–
FA2-NZ	Total	47.6 ± 0.6	–	79.8 ± 0.7	–	7.2 ± 0.1	–	26.7 ± 0.5	–	27.8 ± 0.8
	Soluble	19.4 ± 1.5	41 ± 3	78.9 ± 3.4	99 ± 4	6.9 ± 0.4	96 ± 5	23.9 ± 1.2	90 ± 4	–
	Dialysed	0.5 ± 0.1	1 ± 0.1	30.1 ± 0.4	38 ± 0.5	1.0 ± 0.1	14 ± 1	8.5 ± 0.3	32 ± 1	–
FB2-BR	Total	70.3 ± 0.2	–	106.7 ± 0.9	–	6.0 ± 0.04	–	55.5 ± 0.4	–	34.1 ± 0.4
	Soluble	37.3 ± 0.02	53 ± 0.1	108.0 ± 4.1	101 ± 4	3.8 ± 0.02	64 ± 0.4	50.0 ± 3.2	90 ± 6	–
	Dialysed	1.1 ± 0.3	2 ± 0.5	40.3 ± 1.0	38 ± 1	0.9 ± 0.1	15 ± 1	15.3 ± 0.5	28 ± 1	–
FB2-USA	Total	57.2 ± 0.2	–	113.2 ± 0.9	–	5.2 ± 0.04	–	37.3 ± 0.3	–	22.3 ± 0.3
	Soluble	46.3 ± 3.9	81 ± 7	129.8 ± 5.3	115 ± 5	5.3 ± 0.1	102 ± 3	39.2 ± 1.2	105 ± 3	–
	Dialysed	0.9 ± 0.2	2 ± 0.3	49.6 ± 0.8	44 ± 1	0.9 ± 0.04	18 ± 1	12.1 ± 0.1	33 ± 0.2	–
FC2-BR	Total	67.4 ± 1.0	–	101.0 ± 1.1	–	6.5 ± 0.1	–	36.1 ± 0.5	–	24.9 ± 0.5
	Soluble	63.5 ± 4.7	94 ± 7	113.1 ± 1.1	112 ± 1	6.4 ± 0.1	99 ± 1	34.9 ± 0.6	97 ± 2	–
	Dialysed	0.2 ± 0.2	0.4 ± 0.3	40.1 ± 0.3	40 ± 0.3	1.0 ± 0.1	15 ± 1	10.8 ± 0.1	30 ± 0.3	–
FC2-USA	Total	42.5 ± 0.2	–	81.9 ± 0.9	–	6.9 ± 0.04	–	31.9 ± 0.3	–	17.6 ± 0.3
	Soluble	37.8 ± 3.6	89 ± 9	88.8 ± 0.8	108 ± 1	6.6 ± 0.2	96 ± 3	31.7 ± 0.4	100 ± 1	–
	Dialysed	0.8 ± 0.2	2 ± 0.5	35.2 ± 0.8	43 ± 1	1.3 ± 0.02	20 ± 0.3	9.7 ± 0.2	30 ± 0.5	–
FD2-BR	Total	44.4 ± 1.0	–	74.8 ± 1.7	–	5.2 ± 0.1	–	25.4 ± 0.6	–	19.4 ± 0.5
	Soluble	40.4 ± 18.0	91 ± 41	84.3 ± 0.5	113 ± 1	5.6 ± 0.9	109 ± 18	21.9 ± 1.4	86 ± 5	–
	Dialysed	1.0 ± 0.2	2 ± 0.3	31.3 ± 0.5	42 ± 1	1.0 ± 0.02	19 ± 0.4	7.9 ± 0.2	31 ± 1	–
FA3-BR	Total	70.9 ± 2.0	–	94.1 ± 0.9	–	8.1 ± 0.3	–	42.3 ± 1.2	–	29.1 ± 0.7
	Soluble	39.0 ± 0.6	55 ± 1	104.7 ± 0.5	111 ± 0.5	7.7 ± 0.04	95 ± 0.5	33.9 ± 0.1	80 ± 0.2	–
	Dialysed	1.8 ± 0.4	3 ± 1	30.7 ± 1.6	33 ± 2	1.3 ± 0.03	16 ± 0.3	9.1 ± 0.3	22 ± 1	–
FB3-BR	Total	78.7 ± 0.4	–	80.7 ± 0.4	–	5.7 ± 0.02	–	44.8 ± 0.1	–	20.6 ± 0.1
	Soluble	36.3 ± 1.3	46 ± 2	92.8 ± 1.3	115 ± 2	5.8 ± 0.04	102 ± 1	45.8 ± 0.9	102 ± 2	–
	Dialysed	2.7 ± 0.2	3 ± 0.3	32.8 ± 0.9	41 ± 1	1.1 ± 0.02	20 ± 0.3	14.0 ± 0.5	31 ± 1	–
FC3-BR	Total	73.3 ± 0.6	–	82.5 ± 1.2	–	6.8 ± 0.02	–	49.6 ± 0.6	–	29.9 ± 0.3
	Soluble	45.4 ± 3.8	62 ± 5	87.8 ± 1.8	106 ± 2	6.9 ± 0.2	101 ± 3	50.4 ± 1.6	102 ± 3	–
	Dialysed	3.0 ± 0.2	4 ± 0.2	29.8 ± 0.4	36 ± 0.5	1.3 ± 0.02	19 ± 0.4	14.4 ± 0.2	29 ± 0.4	–
FD3-BR/SOY	Total	54.3 ± 1.0	–	71.0 ± 0.1	–	5.0 ± 0.05	–	32.3 ± 0.4	–	25.7 ± 0.3
	Soluble	19.8 ± 2.4	36 ± 4	80.2 ± 0.9	113 ± 1	4.5 ± 0.1	91 ± 2	21.0 ± 0.4	65 ± 1	–
	Dialysed	1.4 ± 0.1	3 ± 0.2	22.4 ± 1.0	32 ± 1	0.8 ± 0.01	17 ± 0.2	5.4 ± 0.2	17 ± 1	–
LG-PAS	Total	105.1 ± 0.2	–	146.6 ± 0.4	–	8.5 ± 0.1	–	77.8 ± 0.3	–	33.5 ± 0.1
	Soluble	36.3 ± 2.1	35 ± 2	137.8 ± 5.1	94 ± 3	8.2 ± 0.4	97 ± 4	79.3 ± 3.3	102 ± 4	–
	Dialysed	7.8 ± 0.2	8 ± 0.1	60.3 ± 1.5	41 ± 1	2.3 ± 0.02	27 ± 0.3	28.3 ± 0.9	36 ± 1	–
LH-PAS	Total	107.5 ± 4.2	–	167.5 ± 1.5	–	9.2 ± 0.2	–	80.8 ± 0.9	–	34.7 ± 0.9
	Soluble	59.2 ± 5.4	55 ± 5	153.8 ± 3.3	92 ± 2	9.3 ± 0.2	100 ± 2	80.9 ± 1.9	100 ± 2	–
	Dialysed	7.6 ± 0.4	7 ± 0.3	63.0 ± 0.9	38 ± 1	2.5 ± 0.1	27 ± 1	25.8 ± 1.1	32 ± 1	–
LI-PAS	Total	103.0 ± 2.7	–	155.6 ± 2.0	–	8.8 ± 0.04	–	77.3 ± 0.7	–	34.1 ± 0.3
	Soluble	30.4 ± 0.9	30 ± 1	156.7 ± 0.1	101 ± 0.1	9.2 ± 0.02	104 ± 0.2	82.4 ± 0.6	107 ± 1	–
	Dialysed	7.6 ± 1.0	7 ± 1	62.2 ± 0.9	40 ± 1	2.3 ± 0.1	26 ± 1	24.3 ± 2.2	31 ± 3	–
LA-PO	Total	184.8 ± 11.2	–	180.6 ± 2.0	–	10.6 ± 0.1	–	98.9 ± 3.2	–	49.4 ± 0.2
	Soluble	130.1 ± 2.2	70 ± 1	179.7 ± 6.4	100 ± 4	10.7 ± 0.4	100 ± 3	93.4 ± 3.2	94 ± 3	–
	Dialysed	15.5 ± 1.7	8 ± 1	70.3 ± 2.0	39 ± 1	1.6 ± 0.1	15 ± 1	11.9 ± 1.7	12 ± 2	–
LE-PO	Total	130.2 ± 1.3	–	183.8 ± 1.5	–	11.1 ± 0.2	–	98.4 ± 0.3	–	49.3 ± 0.7
	Soluble	61.5 ± 1.0	47 ± 1	188.7 ± 4.7	103 ± 3	11.0 ± 0.3	99 ± 2	97.4 ± 2.7	99 ± 3	–
	Dialysed	11.8 ± 2.8	9 ± 2	61.1 ± 2.6	33 ± 1	2.7 ± 0.2	25 ± 2	17.6 ± 2.0	18 ± 2	–
LF-PO	Total	134.9 ± 2.4	–	178.0 ± 2.4	–	11.1 ± 0.2	–	93.6 ± 1.4	–	50.5 ± 0.9
	Soluble	33.4 ± 3.3	25 ± 2	153.1 ± 7.7	86 ± 4	9.5 ± 0.4	86 ± 4	73.6 ± 4.5	79 ± 5	–
	Dialysed	3.3 ± 0.2	2 ± 0.1	56.4 ± 0.8	32 ± 0.4	3.2 ± 0.1	29 ± 0.4	26.7 ± 0.5	29 ± 0.5	–

Table 3 (*continued*)

Sample		Ca		K		Mg		P		Na
		$\bar{x}$ ± SD (μg 100 mL^{-1})	Bioaccessibility (% ± SD)	$\bar{x}$ ± SD (μg 100 mL^{-1})	Bioaccessibility (% ± SD)	$\bar{x}$ ± SD (μg 100 mL^{-1})	Bioaccessibility (% ± SD)	$\bar{x}$ ± SD (μg 100 mL^{-1})	Bioaccessibility (% ± SD)	$\bar{x}$ ± SD (μg 100 mL^{-1})
LA-UHT	Total	115.3 ± 1.0	–	162.5 ± 0.6	–	9.9 ± 0.1	–	103.2 ± 3.3	–	57.4 ± 1.3
	Soluble	44.9 ± 3.6	39 ± 3	166.3 ± 5.5	102 ± 3	10.1 ± 0.2	103 ± 2	96.3 ± 2.9	93 ± 3	–
	Dialysed	1.7 ± 0.3	2 ± 0.2	65.5 ± 1.5	40 ± 0.9	2.7 ± 0.02	27 ± 0.2	36.0 ± 0.8	35 ± 1	–
LJ-UHT	Total	109.6 ± 2.9	–	159.8 ± 3.3	–	9.5 ± 0.2	–	94.4 ± 7.4	–	57.9 ± 1.6
	Soluble	52.7 ± 0.0	48 ± 0.1	163.4 ± 0.9	102 ± 1	10.1 ± 0.3	106 ± 3	92.8 ± 3.6	98 ± 4	–
	Dialysed	1.3 ± 0.3	1 ± 0.2	67.2 ± 1.6	42 ± 1	2.6 ± 0.02	27 ± 0.2	34.8 ± 0.9	37 ± 1	–
LF-UHT	Total	110.1 ± 4.9	–	153.6 ± 6.9	–	9.2 ± 0.2	–	89.9 ± 6.3	–	64.9 ± 1.9
	Soluble	88.1 ± 5.7	80 ± 5	150.8 ± 6.5	98 ± 4	9.1 ± 0.3	99 ± 3	83.1 ± 2.7	93 ± 3	–
	Dialysed	1.4 ± 0.3	1 ± 0.3	66.1 ± 1.9	43 ± 1	2.5 ± 0.03	27 ± 0.4	33.6 ± 0.8	37 ± 1	–

[a] Na bioaccessibility was not evaluated due to the amount of $NaHCO_3$ added during the trials. Abbreviations are: $\bar{x}$ ± SD, mean concentration (n = 3); F, infant formula; L, milk; A to J, brand; 1 to 3, age range; BR, Brazil; CH, Chile; NZ, New Zealand; PT, Portugal; USA, United States of America; PAS, pasteurised milk; PO, powdered milk; UHT, ultra high temperature treated milk; SOY, soy IF; LA-PO was Ca-fortified.

Principal components analysis (PCA) was used to evaluate the similarity in mineral composition (Cu, Fe, Mn, Zn, Ca, K, Mg, P and Na) in IF and milk samples. The results were organised in a 27 × 9 matrix, the lines corresponding to the samples (IF and milk) and the columns to the mean values obtained for the nine elements. The pre-processing used was autoscaling and the principal components (PC) 1 and 2 were selected to classify the samples. Fig. 2 shows the scores (samples) and loadings (elements) plots. PC 1 and PC 2 explained 61.1% and 20.1%, respectively, of the total variance in the data (total of 81.2%). A two group classification was found from the PCA results: group 1 (IF samples) had high Cu and Mn values and low Ca, Mg, K, Na and P contents; group 2 (milk samples) had high Ca, Mg, K, Na and P values and low Cu and Mn values. A sub-group was also observed: Fe-fortified samples (LA-PO, LA-UHT and LE-PO) and Zn-fortified samples (LA-PO and LA-UHT).

Hierarchical cluster analysis is presented in Fig. 3. In this analysis, autoscaling and euclidean distance were employed. From the dendogram (similarity of 0.598), it is possible to notice five groups, according to samples mineral compositions: (i) non-fortified milk; (ii) fortified milk from brand E (LE-PO); (iii) fortified from brand A (LA-UHT and LA-PO); (iv) IF containing soy protein and (v) IF containing milk protein.

It is important to point out that HCA and PCA are complementary and allow the classification of IF according to their protein source (soy or milk). Amongst the IF soy based, there was also a clear difference between samples from brand A (FA2-BR/SOY) and brand D (FD1-BR/SOY and FD3-BR/SOY).

4. Discussion

The total contents found for the 9 elements in the IFs were within the ranges recommended for IF by the Brazilian regulations and by Codex (BRASIL, 2011a,b; FAO/WHO, 2016) and also agreed with the concentrations informed on the labels (±20 deviation) (BRASIL, 2003).

In comparison, the total contents of Fe, Cu, Mn and Zn found in the IFs were higher than those found in the unfortified milks by 162, 63, 37 and 2-fold, respectively, whereas for P–Na, Ca–K and Mg the values were up to 4, 3 and 2 times higher, respectively, than in the milk samples and in agreement with the study of Sager, McCulloch, and Schoder (2018). IF are formulated to resemble the constitution of breast milk, which allows for a better equilibrium of the amounts of nutrients for the development of the infant, according to Lönnerdal and Hernell (2016), Martin, Ling, and Blackburn (2016), Vieira da Silva et al. (2013), and Zou et al. (2016).

The total contents of Ca, Fe and Zn are higher in the fortified milk samples than in the IFs and other milk samples. For Na, the IF samples for the 0–6 months age present the smallest contents and the UHT milks the highest contents. The types and amounts of additives added to the milks could have contributed to the Na levels and the content increased according to the addition of the stabilisers (Table 1). Mineral levels in milk products (and in IFs) are a result of the dynamic equilibrium of the minerals between the aqueous and colloidal milk phases, the technological treatment (reverse osmosis, membrane ultrafiltration or spraying) and of any mineral fortification applied during the manufacturing process (Martin et al., 2016; Poitevin, 2016).

The type of process, the physical state of the sample (solid or liquid), the way in which the element binds to the milk components and their total contents could also influence the bioaccessible fractions of the elements (Drago & Valencia, 2004; Perales et al., 2005). In the present study, the %D values of the Mn and Zn were higher in UHT than in the other milks, while a lower %D was found for Ca in UHT than in the other milk samples (Tables 2 and 3). It is well known that bioaccessible fraction of these minerals in the UHT milk could be affected due to the formation of soluble complexes with milk sugars and proteins (Sanches et al., 2019), heat processes applied, or ingredients added (Drago & Valencia, 2004).

In general, the %D values obtained for K, Mg and P were similar between liquid milks and IFs. However, for Ca the %D was higher in the milk samples and this difference could be related to the ratio of whey protein: casein, which in cows' milk is approximately 20:80, whereas in IFs the ratio of whey protein:casein is similar to mature human breast milk, i.e., or 60:40 (Donovan, 2019). The phosphopeptides present in the milk casein interact with Ca, forming a complex that allows the Ca to remain in the soluble form in the gastrointestinal tract, so favouring Ca absorption (Bosscher et al., 2001; Perales et al., 2005; Sanches et al., 2019).

With respect to the IF age ranges, differences were found between the total contents of the elements studied: a tendency for high contents of Ca, Fe, P and Na was observed in the IF destined for the 6–12 month age range (Tables 2 and 3), in agreement with the work of Martínez et al. (2018), who studied IF samples from Spain. The composition of IFs considering different ages is important to reach nutrient requirements and to avoid high levels during periods of less need (Lönnerdal & Hernell, 2016).

The Fe total contents in Fe-fortified IF samples from the USA for 0–12-month age (FB2-USA and FC2-USA) are similar to the samples destined for the 6–12-month age. According to the studies of Aly, López-Nicolás, Darwish, Frontela-Saseta, and Ros-Berruezo (2016) and Lönnerdal and Hernell (2016), full-term normal weight babies are born with relatively large Fe-reserves, sufficient to provide the Fe requisites during the first 6 months of life. After the sixth months, fortification is recommended, as also the introduction of Fe-rich foods in diet.

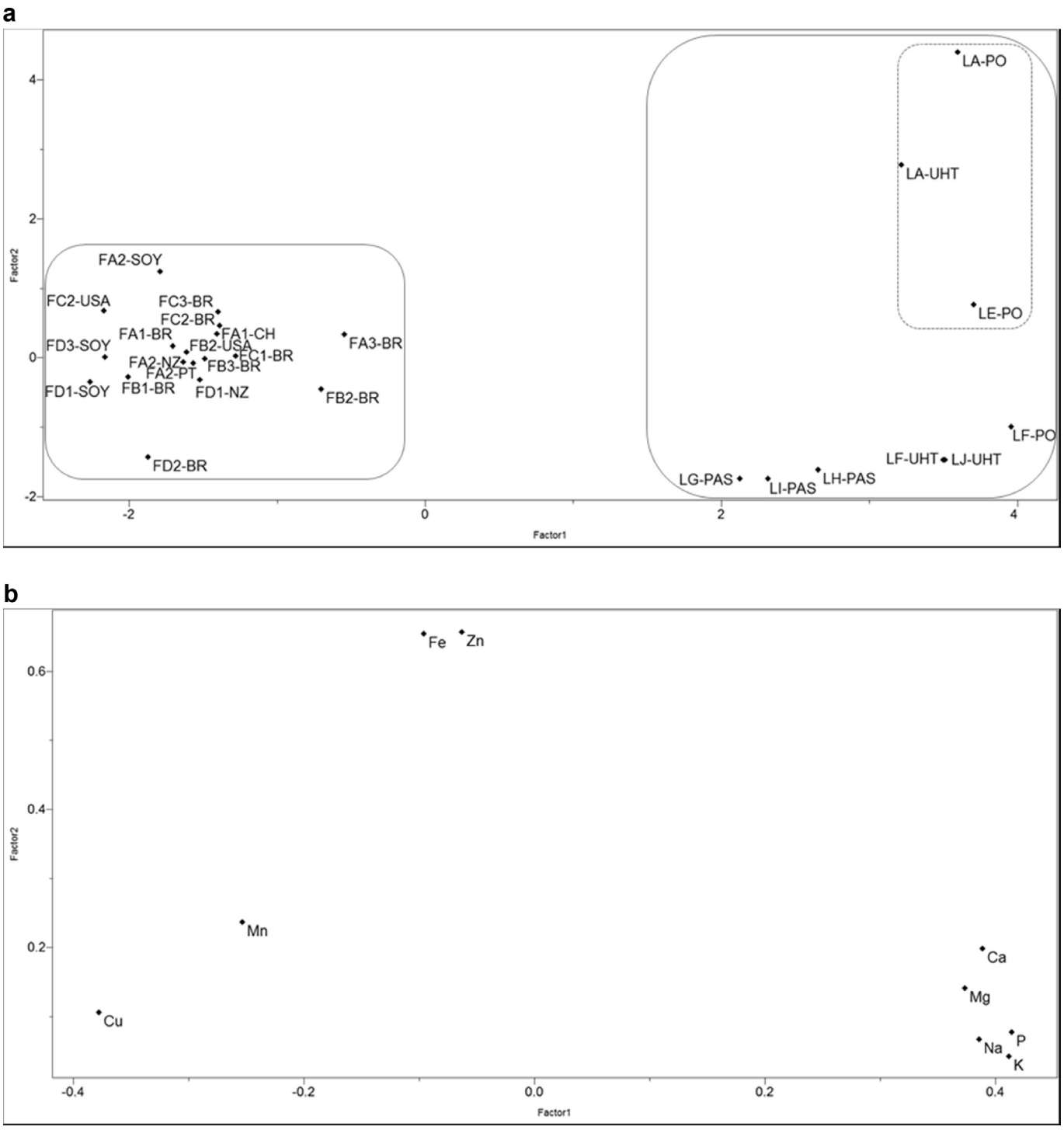

Fig. 2. Principal components analysis of the mineral content of the infant formula and milk samples: (a) score and (b) loadings plots. F, infant formula; L, milk; A to J, brand; 1 to 3, age ranges; BR, Brazil; CH, Chile; NZ, New Zealand; PT, Portugal.

For the soy protein based IF, brand A (FA2-BR/SOY), which was labelled as soy isolate protein being the source, presented higher results for the total content, %D and %S than brand D (FD1-BR/SOY & FD3-BR/SOY), which was labelled as soy protein being the source. The bioaccessibility results found for the FA2-BR/SOY sample are also comparable with the results obtained for the milk-based IF and may be related to the presence of soy isoflavones or phytase. They also agree with the results of Devaraju, Thatte, Prakash, and Lakshmi (2016) and Theodoropoulos, Turatti, Greiner, Macedo, and Pallone (2018), who evaluated the beneficial effects of using the enzyme phytase and enzymatic hydrolysis in the bioaccessibility of Ca, Fe and Zn in soy products.

With respect to the milk proteins, the benefits that some ingredients bring for the absorption of the IF nutrients are well established, such as the addition of lactose and ascorbic acid, the protein fraction, the total mineral content and particle size, amongst others (Aly et al., 2016; Bosscher et al., 2001; Brodkorb et al., 2019; Devaraju et al., 2016; Donavan, 2019; Drago &

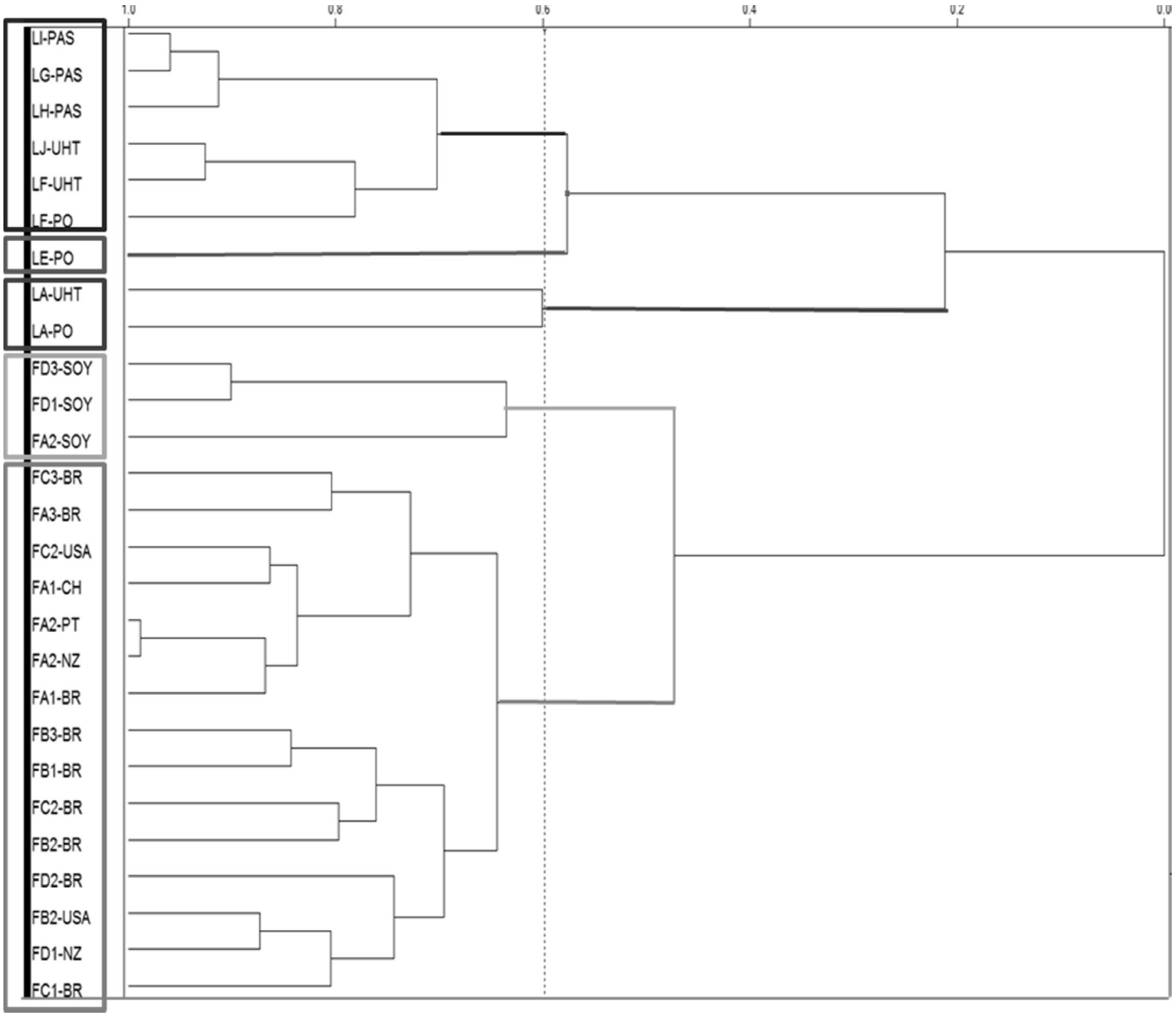

Fig. 3. Dendrogram obtained from the cluster analysis by hierarchal methods (HCA) from the mineral contents of the infant formula and milk samples.

Valencia, 2004; Gomez, Perez-Corona, & Madrid, 2016; Peña et al., 2004; Perales et al., 2005). In the present study, these benefits were shown by the high percentages of soluble fractions (%S values) for the minerals in the IF, in general, above 60% (Tables 2 and 3). In few cases, values above 100% of %S were observed.

To perform real bioaccessibility experiments, samples were analysed unaltered and, even with strict controls and analytical blanks in all experiments, there is an inherent variation due to the amount of solution used in the pH adjustment steps.

The effect of the different ingredients on the mineral bioaccessibility from IF and milk are exemplified by their dialysed fractions, as shown in Figs. 4 and 5. These data demonstrate the mineral patterns, considering the matrixes, the IF recommended age, the types of milk processing and mineral content (total and dialysed fractions).

For Ca, the highest %S (94%) and lowest %D (0.4%) were found for FC2-BR sample, which is declared as dietetic-therapeutic and composed of partially hydrolysed protein and 2.2 g 100 kcal-1 lactose; whereas FD1-NZ sample, which declared no lactose in labelling, presented a %S of 48% and %D of 3%. The %D values may be related to the use of prebiotics (galacto-oligosaccharides) present in FD1-NZ. The same type of prebiotic present in FD1-NZ sample is declared in another 5 IFs (FA1-BR, FB2-USA, FC2-USA, FD2-BR and FA3-BR samples) and a correlation between the use of this prebiotic and the bioaccessible fractions of the elements was shown, as observed for the %D for Fe above 11% and for K above 38%, except for the FA3-BR sample. This sample also presents the lowest %D for Fe, K, Mg, P and Zn, which could be associated with the recommend age (6–12 months) and the type of protein used (demineralised whey).

The use of prebiotics in IF was studied by Aly et al. (2016), who evaluated the effect of lactoferrin and galacto-oligosaccharide addition on Fe solubility in a standard IF, obtaining an increase in solubility from 66% (initial) to 96% in relation to its total content. Although the work of Aly et al. (2016) does not evaluate the dialysability or other minerals, in our study, samples with addition of prebiotics presents higher %D values for Cu, Fe, K, P and Zn than the samples containing only powdered skimmed milk and lactose

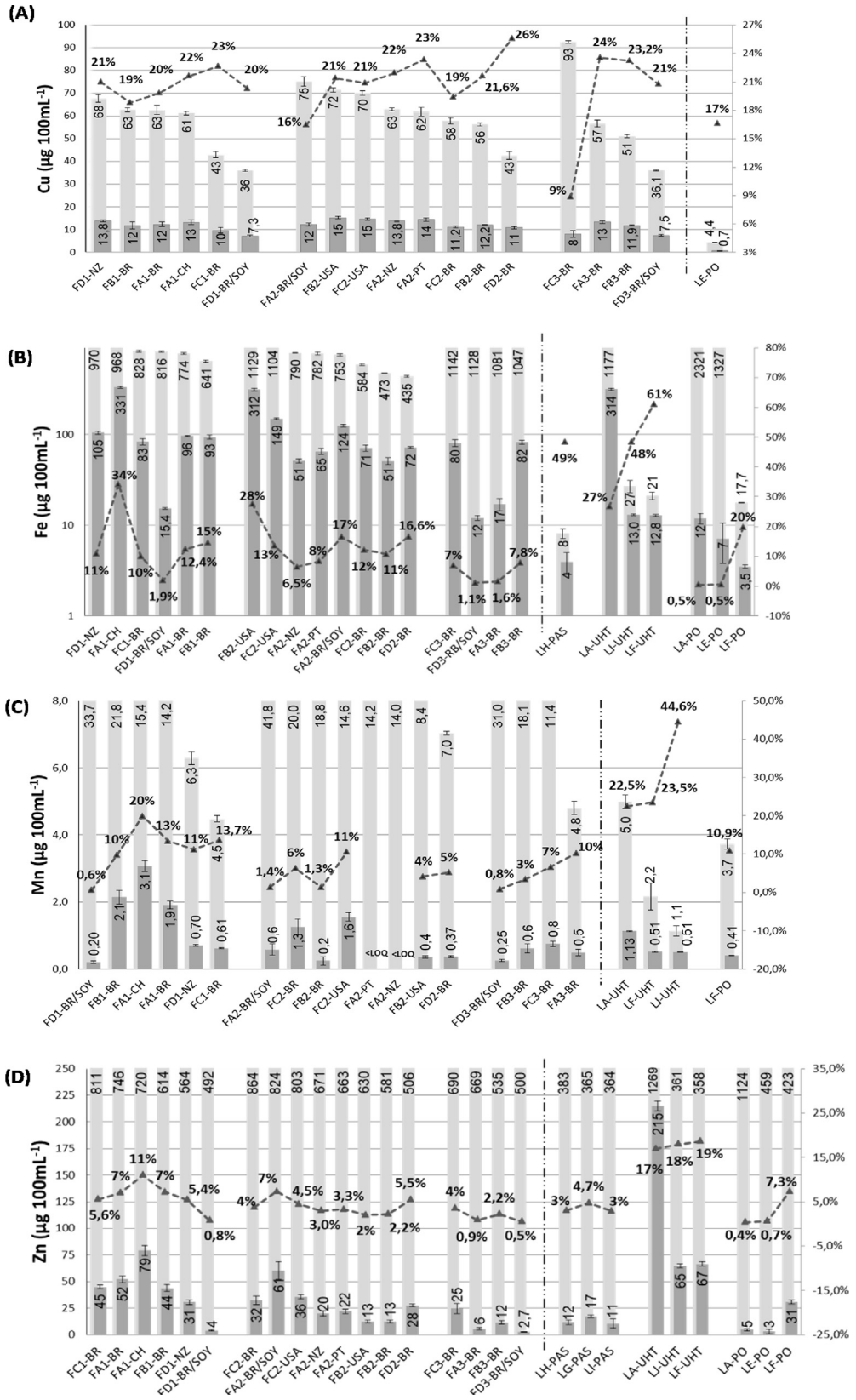

Fig. 4. Total content (▒, µg 100 mL-1) and dialysed fraction (■, µg 100 mL-1; ▲, %) of micro-minerals in infant formula and milk samples: A, Cu; B, Fe; C, Mn; D, Zn. Samples with mineral concentrations below the LOQ are not presented.

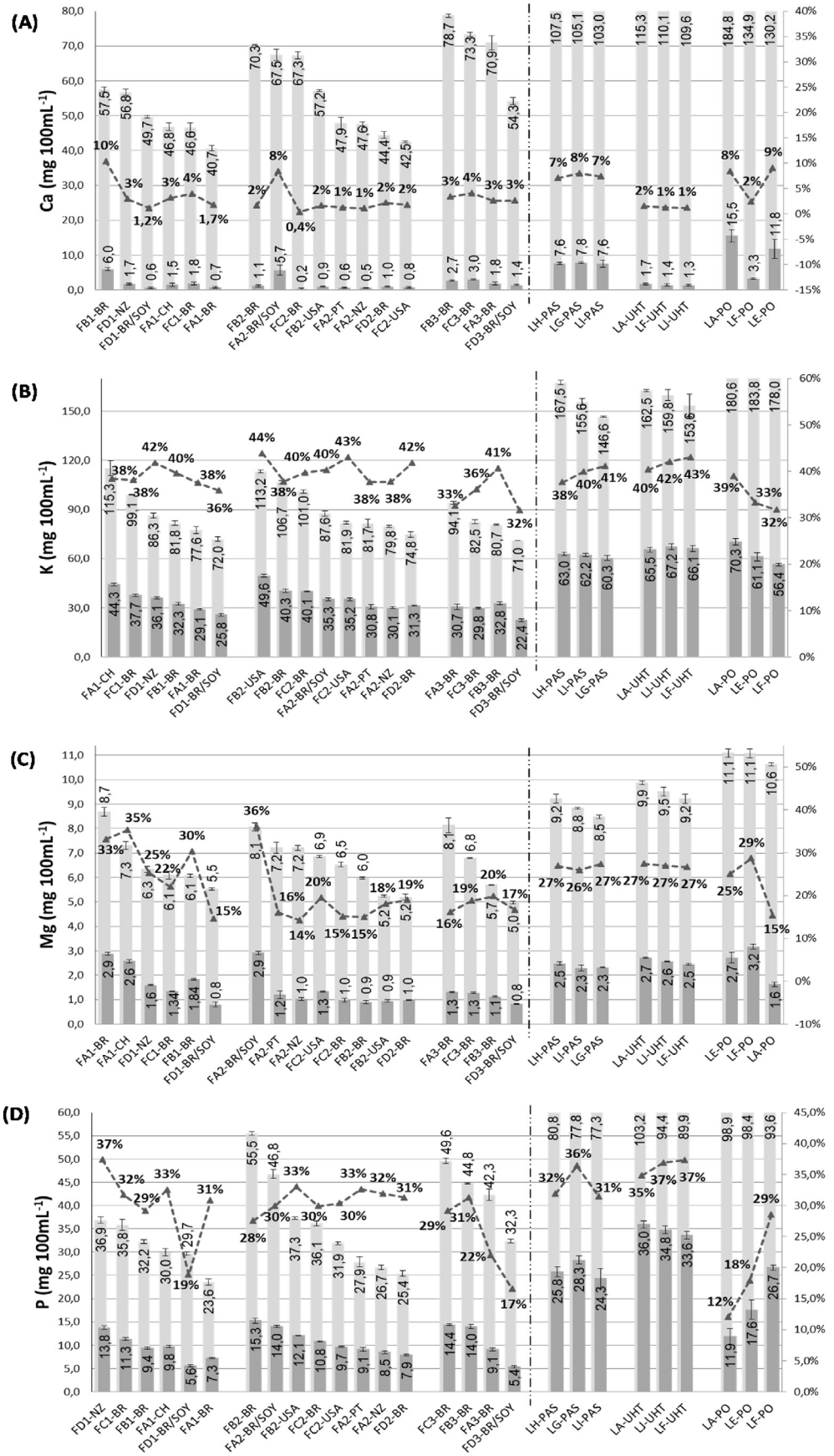

Fig. 5. Total content (▒, mg 100 mL-1) and dialysed fraction (■, mg 100 mL-1; ▲, %) and dialysed fractions of macro-minerals in infant formula and milk samples: A, Ca; B, K; C, Mg; D, P.

(FC3-BR) and similar or higher values than IF that contained concentrated protein (FC1-BR and FB3-BR) or hydrolysed protein (FB1-BR).

Hydrolysed protein (chemically or enzymatically) is incorporated into IF to make lower molecular weight peptides available, which are associated with high bioavailability and solubility of the nutrients (Devaraju et al., 2016). The results obtained for samples that contained hydrolysed whey protein (FB1-BR) presented higher values for the %D of the minerals Ca, Fe, Mg, Mn and Zn than that in IF samples with enzymatically hydrolysed protein (FA2-PT: *Lactobacillus reuteri* and FA2-NZ: *Bifidus* samples) and the partially hydrolysed (FB2-BR and FC2-BR) samples. It is interesting to note that the two samples containing enzymatically hydrolysed proteins, in addition to presenting the same total contents for the 9 elements, also presented dialysed fractions for Mn below the LOQ and are the only samples with %D significantly similar ($p < 0.05$) for all elements, except for Fe.

Thus, the use of prebiotics appears to favour the minerals dialysability more than enzymatic hydrolysis, and the combination of prebiotics with partially hydrolysed protein (FD2-BR sample), shows no differences for %D in relation to the other IF containing prebiotics.

The evaluation of the total and bioaccessible mineral contents (% S and %D) in IF with different composition and whole milk samples allows for verification that the bioaccessibility of the elements can be affected by various factors including the ingredients and the total contents.

5. Conclusions

The in vitro bioaccessibility method applied for milk and IF samples (available commercially and unaltered in the laboratory) allowed estimation of their mineral fraction available for absorption. This study showed that for pasteurised whole milk, reconstituted powdered milk and UHT milk, mineral bioaccessibility was mainly affected by their total content and the milk processing (heating, drying, fortification and/or addition of stabilisers). UHT milk samples presented the highest bioaccessible fractions.

For IF, mineral bioaccessibility appeared to be affected by the protein type, presence of prebiotics, Fe-fortification and/or mineral concentrations. IF with soy isolate protein presented similar levels as the whey protein-based IF. The addition of prebiotics and whey protein concentrate improved mineral bioaccessibility, whereas the addition of enzymatically hydrolysed protein showed no effect on the bioaccessibility of the elements under study.

Acknowledgements

The authors acknowledge the São Paulo Research Foundation (FAPESP) [Grant number 2017/11334-8]; the Brazilian National Council for Scientific and Technological Development (CNPq) [Grant number 303142/2017-0] and the Brazilian Federal Agency for the Support and Evaluation of Graduate Education (CAPES) [Finance code 001].

References

Aly, E., López-Nicolás, R., Darwish, A. A., Frontela-Saseta, C., & Ros-Berruezo, G. (2016). Supplementation of infant formula with recombinant human lactoferrin and/or galactooligosaccharides increases iron bioaccessibility as measured by ferritin formed in Caco-2 cell model. *Food Research International, 89*, 1048–1055.

Barciela-Alonso, M. C., & Bermejo-Barrera, P. (2015). Variation of food mineral content during industrial and culinary processing. In M. de la Guardia, & S. Garrigues (Eds.), *Handbook of mineral elements in food* (pp. 163–176). Valencia, Spain: John Wiley & Sons.

Bermejo-Barrera, P., Muñiz-Naveiro, Ó., Moreda-Piñeiro, A., & Bermejo-Barrera, A. (2001). The multivariate optimisation of ultrasonic bath-induced acid leaching for the determination of trace elements in seafood products by atomic absorption spectrometry. *Analytica Chimica Acta, 439*, 211–227.

Bosscher, D., Van Caillie-Bertrand, M., Robberecht, H., Van Dyck, K., Van Cauwenbergh, R., & Deelstra, H. (2001). In vitro availability of calcium, iron, and zinc from first-age infant formulae and human milk. *Journal of Pediatric Gastroenterology and Nutrition, 32*, 54–58.

BRASIL. (2003). *Resolução RDC n° 360, de 23/12/2003. The Brazilian Health Regulatory Agency. Diário Oficial da União*. Rio de Janeiro, Brasil: Brazilian National Press.

BRASIL. (2011a). *Resolução RDC n° 43, de 19/07/2011. The Brazilian Health Regulatory Agency. Diário Oficial da União*. Rio de Janeiro, Brasil: Brazilian National Press.

BRASIL. (2011b). *Resolução RDC n° 44, de 19/07/2011. The Brazilian Health Regulatory Agency. Diário Oficial da União*. Rio de Janeiro, Brasil: Brazilian National Press.

Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assunção, R., Balance, S., et al. (2019). INFOGEST static in vitro simulation of gastrointestinal food digestion. *Nature Protocols, 14*, 991–1014.

Cava-Montesinos, P., Cervera, M. L., Pastor, A., & de la Guardia, M. (2005). Room temperature acid sonication ICP-MS multielemental analysis of milk. *Analytica Chimica Acta, 531*, 111–123.

Devaraju, S. K., Thatte, P., Prakash, J., & Lakshmi, J. A. (2016). Bioaccessible iron and zinc in native and fortified enzyme hydrolyzed casein and soya protein matrices. *Food Biotechnology, 30*, 233–248.

Do Nascimento da Silva, E., Leme, A. B. P., Cidade, M., & Cadore, S. (2013). Evaluation of the bioaccessible fractions of Fe, Zn, Cu and Mn in baby foods. *Talanta, 117*, 184–188.

Donovan, S. M. (2019). Human milk proteins: Composition and physiological significance. In S. M. Donovan, J. B. German, B. Lonnerdal, & A. Lucas (Eds.), *Vol. 90. Human milk: Composition, clinical benefits and future opportunities. Nestle nutrition institute workshop series* (pp. 93–101). Basel, Switzerland: S. Karger AG.

Drago, S. R., & Valencia, M. E. (2004). Influence of components of infant formulas on in vitro iron, zinc, and calcium availability. *Journal of Agricultural and Food Chemistry, 52*, 3202–3207.

EC. (2006). Directiva 2006/141/CE. *Journal Oficial da União Europeia, L401*, 3–33.

FAO/WHO. (2016). *Standard for infant formula and formulas for special medical purposes intended for infants. Codex Stan 72 – 1981*. Rome, Italy: FAO/WHO.

Fioravanti, M. I. A., Milani, R. F., de Paiva, E. L., & Morgano, M. A. (2020). Simple and fast ultrasound-assisted method for mineral content and bioaccessibility study in infant formula by ICP OES. *Analytical Methods, 12*, 3225–3254.

Gomez, B. G., Perez-Corona, M. T., & Madrid, Y. (2016). Availability of zinc from infant formula by in vitro methods (solubility and dialyzability) and size-exclusion chromatography coupled to inductively coupled plasma-mass spectrometry. *Journal of Dairy Science, 99*, 9405–9414.

Hur, S. J., Lim, B. O., Decker, E. A., & McClements, D. J. (2011). In vitro human digestion models for food applications. *Food Chemistry, 125*, 1–12.

Lönnerdal, B., & Hernell, O. (2016). An opinion on "Staging" of infant formula: A developmental perspective on infant feeding. *Journal of Pediatric Gastroenterology and Nutrition, 62*, 9–21.

Machado, I., Bergmann, G., & Pistón, M. (2016). A simple and fast ultrasound-assisted extraction procedure for Fe and Zn determination in milk-based infant formulas using flame atomic absorption spectrometry (FAAS). *Food Chemistry, 194*, 373–376.

Martínez, M. A., Castro, I., Rovira, J., Ares, S., Rodríguez, J. M., Cunha, S. C., et al. (2018). Early-life intake of major trace elements, bisphenol A, tetrabromobisphenol A and fatty acids: Comparing human milk and commercial infant formulas. *Environmental Research, 169*, 246–255.

Martin, C. R., Ling, P. R., & Blackburn, G. L. (2016). Review of infant feeding: Key features of breast milk and infant formula. *Nutrients, 8*, 1–11.

Ménard, O., Bourlieu, C., De Oliveira, S. C., Dellarosa, N., Laghi, L., Carrière, F., et al. (2018). A first step towards a consensus static in vitro model for simulating full-term infant digestion. *Food Chemistry, 240*, 338–345.

Minekus, M., Alminger, M., Alvito, P., Balance, S., Bohn, T., Bourlieu, C., et al. (2014). A standardised static in vitro digestion method suitable for food – an international consensus. *Food Function, 5*, 1113–1124.

Peña, E., Domínguez, R., Bermejo, A., Cocho, J. A., Fraga, J. M., & Bermejo, P. (2004). Enzymolysis approach to compare cu availability from human milk and infant formulas. *Journal of Agricultural and Food Chemistry, 52*, 4887–4892.

Perales, S., Barberá, R., Lagarda, M. J., & Farré, R. (2005). Bioavailability of calcium from milk-based formulas and fruit juices containing milk and cereals estimated by in vitro methods (solubility, dialyzability, and uptake and transport by Caco-2 cells). *Journal of Agricultural and Food Chemistry, 53*, 3721–3726.

Poitevin, E. (2016). Official methods for the determination of minerals and trace elements in infant formula and milk products: A review. *Journal of AOAC International, 99*, 42–52.

Sager, M., McCulloch, C. R., & Schoder, D. (2018). Heavy metal content and element analysis of infant formula and milk powder samples purchased on the Tanzanian market: International branded versus black market products. *Food Chemistry, 255*, 365–371.

Sanches, V. L., Peixoto, R. R. A., & Cadore, S. (2019). Phosphorus and zinc are less bioaccessible in soy-based beverages in comparison to bovine milk. *Journal of Functional Foods, 65*. Article 103728.

Shani-Levi, C., Alvito, P., Andrés, A., Assunção, R., Barberá, R., Blanquet-Diot, S., et al. (2017). Extending in vitro digestion models to specific human populations: Perspectives, practical tools and bio-relevant information. *Trends in Food Science & Technology, 60*, 52–63.

Theodoropoulos, V. C. T., Turatti, M. A., Greiner, R., Macedo, G. A., & Pallone, J. A. L. (2018). Effect of enzymatic treatment on phytate content and mineral bio-accessibility in soy drink. *Food Research International, 108*, 68–73.

Vieira da Silva, S., Mattanna, P., Bizzi, C. A., Richards, N. S. P. S., & Barin, J. S. (2013). Evaluation of the mineral content of infant formulas consumed in Brazil. *Journal of Dairy Science, 96*, 3498–3505.

Zou, L., Pande, G., & Akoh, C. C. (2016). Infant formula fat analogs and human milk fat: New focus on infant developmental needs. *Annual Review of Food Science and Technology, 7*, 139–165.