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Aluminum content and effect of *in vitro* digestion on bioaccessible fraction in cereal-based baby foods

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ABSTRACT

The aim of this work was to determine the total concentration and the effect of *in vitro* digestion on the bioaccessible fraction of aluminum (Al) in 35 different cereal-based baby food samples and estimate the exposure to this element considering the consumption of this product. Total Al content was determined by inductively coupled plasma optical emission spectrometry after oxidative microwave digestion. An *in vitro* digestion method was applied and optimized to evaluate the bioaccessible fraction. The methods performance was efficient for both approached analysis and presented limits of detection and quantitation of 53 µg kg^{-1} and 89 µg kg^{-1}, respectively. Total concentration and bioaccessibility varied according to the product composition (rice, oat, wheat, barley, corn, multicereal and fruit). Multicereals and fruit-based (plum) cereals presented the highest total Al concentrations (8.82 mg kg^{-1} and 7.49 mg kg^{-1}, respectively), whilst lower values were observed for corn and rice flour cereals (0.92 mg kg^{-1} and 1.09 mg kg^{-1}, respectively). The bioaccessible fraction varied from 1.5% to 10.4% in the evaluated samples. Exposure to Al was estimated and compared with the Provisional Tolerable Weekly Intake (PTWI) of 2 mg kg^{-1} body weight. The results showed that the daily consumption of three portions of cereals contributes up to 10.48% of the PTWI, when considering the total Al concentration reported in this study.

1. Introduction

Infant cereals have become an excellent alternative as a complementary food for children, as they are produced from corn, rice, wheat and oat flour, and are usually enriched with specific nutrients. This requires consideration of the correct balance of nutrients (proteins, carbohydrates, fats, vitamins, and minerals), energy density, quality of essential fatty acids, chemical and biological hazards and the correct introduction of potential allergic ingredients (Ljung, Palm, Grandér, & Vahter, 2011; MacLean et al., 2010; Pandelova, Lopez, Michalke, & Schramm, 2012; Pehrsson, Patterson, & Khan, 2014). Based on these considerations, it is essential to monitor both essential and potentially toxic elements, since in excess the latter can cause several physiological disorders (Machado, Cesio, & Pistón, 2017; Peixoto, Devesa, Vélez, Cervera, & Cadore, 2016; Pereira et al., 2018).

Aluminum (Al) is a toxic element that may accumulate in lungs, liver, kidney, thyroid and brain. It has been reported that children are highly vulnerable to Al exposure because of their immature renal system, exhibiting a narrow tolerance to this non-essential element (Bouglé et al., 1999). Al can act as a potent neurotoxin causing impaired mental development in long-term exposure and also be incorporated into the bones of infants, resulting in a weakened bone structure. Furthermore, human exposure to Al has been identified as a possible contributor to neurodegenerative/neurodevelopmental diseases, such as multiple sclerosis and Alzheimer's disease (Ahmed, Mohammed, Amin, & Abdel-Raheem, 2016; Mold, Umar, King, & Exley, 2018).

Therefore, due to the relevant concern about Al toxicity, besides determining the total Al concentration in foods, it is also important to evaluate its interaction with physiological fluids during digestion, considering the action of enzymes and food matrix components. This consists of studies related to bioaccessibility (the amount of the element that is released into the gastrointestinal tract from its matrix) and bioavailability (the fraction of the element which is absorbed from the gastrointestinal tract by the human organism, reaches the bloodstream and is then used in biological functions) (Machado et al., 2017; Minekus et al., 2014; Peixoto et al., 2016).

The literature reports several studies on bioaccessibility and

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Table 1
Description of infant cereals samples.

Sample composition	n	Ingredients
Muticereals	9	Wheat flour, sugar, rice flour, corn flour, oatmeal, barley flour, vitamins and minerals
Fruit based (banana and apple)	6	Rice flour, corn starch, dehydrated banana, dehydrated apple, vitamins and minerals
Rice flour and oat	6	Rice flour, oatmeal, malt extract, vitamins and minerals
Fruit based (plum)	3	Rice flour, sugar, oatmeal, malt extract, dehydrated plum, vitamins and minerals
Corn flour	6	Corn flour, sugar, vitamins and minerals
Rice flour	5	Rice flour, sugar, vitamins and minerals

bioavailability of chemical elements in different foods and cereal products (Versantvoort, Oomen, Van de Kamp, Rompelberg, & Sips, 2005; Fu & Cui, 2013; Khouzam, Pohl, & Lobinski, 2011; Laparra, Velez, Barbera, Montoro, & Farre, 2007; Do Nascimento da Silva, Leme, Cidade, & Cadore, 2013; Peixoto et al., 2016; Bhatia et al., 2013; Domínguez-González et al., 2010; Vitali, Dragojevic, & Sebecic, 2008; Yang et al., 2014). Hemaltha, Platel, and Srinivasan (2007) evaluated the bioaccessibility of Fe and Zn in different cereal-based flours. The bioaccessible concentration of Cu, Fe, Mg, Mn and Zn in instant cereal and infant formula samples was determined by Do Nascimento da Silva, Farias, and Cadore (2018). Leśniewicz, Kretowicz, Wierzbicka, and Żyrnicki (2012) used breakfast cereal samples to determine the bioavailable concentration of Al, Ca, Cu, Fe, Mg, Mn, P, Sr and Zn by *in vitro* simulation of the digestive system. Vitali et al. (2008) assessed the bioaccessible concentration of Ca, Mg, Mn and Cu in biscuit samples. Erdemir and Gucer (2016) evaluated the bioaccessibility of Mn in wheat flour samples. However, bioaccessibility studies of inorganic contaminants, particularly Al, are still limited with regard to food for infants and children, and only little information has been found in the literature (Ayivor et al., 2011; Souza et al., 2019).

Due to the importance of monitoring toxic elements in infant cereals intended for nutrition, the present study aims to: (i) evaluate the total Al concentration and its bioaccessible fraction in infant cereals using an *in vitro* digestion method; (ii) quantify the Al concentrations using optical emission spectrometry with an inductively coupled plasma source (ICP OES); and (iii) estimate the weekly intake contribution related to the Provisional Tolerable Weekly Intake (PTWI) through the consumption of infant cereals commercialized in Brazil.

2. Material and methods

2.1. Materials, reagents and equipment

All the reagents used in the study were of analytical grade or above. Water (18.2 MΩ cm) was purified using reverse osmosis (Gehaka, São Paulo, Brazil) while a sub-boiling distiller was employed for the purification of nitric acid (Distillacid, Berghof, Eningen, Germany). To determine Al, the sample was first submitted to acid digestion using distilled HNO_3 and 30% H_2O_2 (m/v) (Merck, Darmstadt, Germany). The analytical curves were prepared from a certified 100 mg L^{-1} standard solution (Specsol, Quimlab, Jacareí, Brazil) in a 0.5% HCl solution (v/v) (Merck, Darmstadt, Germany). The accuracy and precision of the method were evaluated by the analysis of certified reference materials: egg powder (EGGS-1, National Research Council, Canada) and a diet (Typical Diet, NIST SRM 1548a).

The samples were digested in a closed microwave-assisted digestion system (Start E, Milestone, Sorisole, Italy) equipped with 24 teflon flasks with internal volumes of 50 mL. The total Al content was determined using an ICP OES (model 5100 VDV, Agilent Technology, Tokyo, Japan) equipped with a double-step nebulization camera, a sea-spray nebulizer (U-series with Ezylok) and 99.996% pure liquid argon (Air Liquide, São Paulo, Brazil). The optimized operational conditions of the equipment were: radio frequency generator power (1400 W); nebulizer argon flow rate (0.5 L min^{-1}); principal argon flow rate (12 L min^{-1} Ar); auxiliary argon flow rate (1 L min^{-1} Ar); sample flow rate (0.5 L min^{-1}); axial vision mode; number of replicates ($n = 3$) and wavelength for Al (396.152 nm). The external calibration method was used to determine the Al content by ICP OES through a standard curve in the range from 2 to 200 μg L^{-1}.

The following reagents were used for the bioaccessibility assay: $NaHCO_3$ [(> 99.7%) Merck KGaA, Darmstadt, Germany], alpha-Amylase from *Aspergillus oryzae* (30 U mg^{-1}) (Sigma-Aldrich, Saint Louis USA), lactase (85,300 USP) (Sigma-Aldrich, Saint Louis USA), pepsin porcine gastric mucosa (> 250 U mg^{-1}) (Sigma-Aldrich, Saint Louis USA), bile from bovine and ovine [(bile acid mixture) Sigma-Aldrich, Saint Louis USA] and pancreatin from porcine pancreas (8 × USP) (Sigma-Aldrich, Saint Louis USA). A dubnoff shaking water bath (NT 230, Nova Técnica, Piracicaba, Brazil) and pHmeter (Starter 3100, Ohaus, Parsippany, EUA) were also employed.

2.2. Samples

A total of 35 samples were acquired in the city of Campinas, SP, Brazil from distinct batches and different brands (designated as A, B and C), according to the following compositions: multicereals (A, B and C); banana and apple (A and B); rice flour and oat (A and B); corn flour (A and B); rice flour (A, B and C) and plum (B). All the collected samples were evaluated in relation to the total Al content while only the most contaminated sample of each type of product, considering all brands, had its bioaccessibility assessed. The samples were stored in a dry place at room temperature (25 °C) and the determinations of Al carried out in triplicate. The sample preparation was conducted according to the intended analysis. For total Al determination, the sample was weight directly into the microwave vessels, while for bioacessible fraction the cereal was prepared with milk, according to the label recommendation. The main ingredients contained in the products are shown in Table 1.

2.3. Determination of total Al

A sample of 0.5 g of infant cereal was weighed into a digestion flask, and 8 mL of purified HNO_3 plus 2 mL of H_2O_2 were added and maintained in contact overnight. The flasks were then sealed, transferred to the microwave digestor and digested using 4 heating ramps applying 1000 W of power: (a) room temperature to 70 °C in 5 min; (b) from 70 °C to 120 °C in 5 min; (c) from 120 °C to 170 °C in 5 min; and (d) maintained at 170 °C for 25 min. After cooling, the flasks were opened and the resulting solution transferred to a 25 mL Falcon tube.

2.4. In vitro digestion

2.4.1. Dialysis method

The *in vitro* digestion model was performed according to Perales, Barberá, Largada, and Farré (2006) with some adaptations considering the infant's gastrointestinal system. The enzymes solution quantities and concentrations were optimized considering the infant gastrointestinal capacity. Approximately 2.5 g of dry infant cereal were transferred to clean erlenmeyer flasks and mixed with whole milk to a final volume of 15 mL. The pH of samples was adjusted to 7.0 and 4 mL of saliva were added for salivary digestion. The mixture was incubated at 37 °C in a shaking water bath for 5 min. Thereafter, the samples were acidified to pH 2.0 with 6 mol L^{-1} hydrochloric acid (HCl) with addition of 0.3 mL of a porcine pepsin preparation (1.6 g of porcine pepsin in 10 mL 0.1 mol L^{-1} HCl).The mixture was incubated at 37 °C in a shaking water bath for 2 h. To stop the gastric digestion phase, the samples were maintained for 10 min in an ice bath. The gastric digest solution added by pancreatin-bile was titrated with 0.5 mol L^{-1} $NaHCO_3$ (Merck KGaA, Darmstadt, Germany) solution to determine the volume of base needed to increase pH digests to about 7.5. The optimum $NaHCO_3$ concentration was calculated from titratable acidity (Shiowatana, Kitthikhun, Sottimai, Promchan, & Kunajiraporn, 2006), using standard 1 mol L^{-1} $NaHCO_3$ as titrant. This concentration of $NaHCO_3$ changed the pH of the dialysate to 5.0–6.0 after 30 min of dialysis and gradually increased the pH to 7.0–7.5 upon the addition of pancreatin bile extract. Dialysis membrane (Sigma-Aldrich, Saint Louis, EUA) with cut-off from 12.000 to 16.000 Da and porosity of 25 Å) containing 20 mL of freshly prepared 0.5 mol L^{-1} $NaHCO_3$ solution and water, were immersed in pepsin digests and incubated in a shaking water bath at 37 °C. After 30 min, 0.75 mL of pancreatin-bile salt mixture (0.4 g porcine pancreatin and 2.52 mg bile bovine per 100 mL of 0.1 mol L^{-1} $NaHCO_3$) were added and incubated in a shaking water bath at 37 °C for more 2 h. To stop intestinal digestion, the samples were maintained for 10 min in ice bath. Dialysates were transferred to a Falcon tube and used for Al determination. The results were expressed in percentages and the analysis of bioaccessibility was carried out according to each composition and brand, taking into account the samples with the highest values of total Al. The Al bioacessible fraction content was determined by ICP OES using the previously described conditions (model 5100 VDV, Agilent Technology, Tokyo, Japan).

2.5. *Preliminary exposure assessment and risk characterization*

The Al exposure along the week was calculated considering 3 servings per day (21 g cereal/portion) and the mean value of total Al concentration found in each type of cereal. The calculations were performed considering 15 kg as the body weight (bw) of a child (2–6 years old), as recommended by the Food and Agriculture Organization/World Health Organization (FAO/WHO) (2011).

For risk characterization, the most frequently used approach is the quantitative comparison with the Provisional Tolerable Weekly Intake (PTWI), which represents the amount of the substance that can be weekly ingested in a lifetime without significant health risks. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established a PTWI for Al of 2 mg kg^{-1} bw applied to all the Al compounds in foods, including food additives (Food and Agriculture Organization/World Health Organization (FAO/WHO), 2011; Codex Alimentarius Commission (CAC), 2018).

2.6. *Statistical analysis*

The means obtained for each batch of the same brand, and between different brands, were compared by variance analysis (ANOVA) and Tukey's test ($p < 0.05$). All statistical analyses were made using the Statistica 7.0 software package (Statsoft, USA).

3. Results and discussion

3.1. *Total aluminum content in infant cereals*

The results obtained for total Al concentration in the infant cereal samples are presented in Table 2.

It was observed that the highest levels of Al are present in multicereal (8.82 mg kg^{-1}) and cereal with plum (7.49 mg kg^{-1}) from brand B, followed by multicereal brand C (7.36 mg kg^{-1}) and rice flour and oat from brand B (5.64 mg kg^{-1}). Within each brand, multicereals always presented the highest average Al concentration, suggesting an influence of the product composition on the levels of the contaminant. The lowest amounts were found in samples from brand A, including cereals made with corn flour (0.92 mg kg^{-1}), rice flour (1.09 mg kg^{-1}) and rice flour and oat (1.16 mg kg^{-1}).

Table 2
Mean values, standard deviation (SD) and concentration range for Al in infant cereal samples.

Brand	Composition	n	Aluminum (mg kg^{-1})	
			Mean ± SD	Range
A*	Multicereals	3	3.46 ± 0.19	3.13–3.64
	Fruit (banana and apple)	3	2.45 ± 0.19	2.20–2.76
	Rice flour and oat	3	1.99 ± 0.48	1.16–2.60
	Corn flour	3	1.17 ± 0.16	0.92–1.34
	Rice flour	2	1.30 ± 0.21	1.09–1.54
B	Multicereals	3	7.13 ± 1.74	4.65–8.82
	Fruit (banana and apple)	3	3.18 ± 0.23	2.93–3.55
	Rice flour and oat	3	4.95 ± 0.43	4.08–5.64
	Fruit (plum)	3	5.30 ± 1.67	3.40–7.49
	Corn flour	3	4.65 ± 0.57	3.81–5.40
	Rice flour	2	3.80 ± 0.13	3.65–3.88
C	Multicereals	2	6.59 ± 0.66	5.51–7.36
	Rice flour	2	3.22 ± 0.14	3.05–3.32

* A: Statistical analysis indicated that the mean Al level considering products from brand A is different from B and C ($p < 0.05$).

In the work performed by Ayivor et al. (2011), 16 samples of infant cereals marketed in Ghana containing rice, oats, fruits, maize, corn, wheat and multicereals presented levels of Al varying from 2.89 to 11.07 mg kg^{-1}. The highest concentrations were observed in apple muesli (11.07 mg kg^{-1}), wheat cereal (8.13 mg kg^{-1}), and fruit-based cereals (7.5 mg kg^{-1}). The authors reported that the presence of Al may be related to the manufacturing processes of these foods or to the packaging and emphasized concern about Al levels found in the samples.

In a recent study developed by Souza et al. (2019), levels of Al were reported in nine samples of infant cereals marketed in Brazil and the obtained mean concentrations were: 13.8 mg kg^{-1}, 16 mg kg^{-1}, 5.75 mg kg^{-1} and 17.1 mg kg^{-1}, for rice, rice and oat, corn flour and multicereals, respectively. These values are higher than those found in the current study, which may be related to the applied digestion method (open block) facilitating sample contamination with Al present in the air.

3.2. *Bioaccessibility of Al by in vitro gastrointestinal digestion*

Table 3 shows the bioaccessible fractions of Al present in cereal-based infant foods. As previously mentioned, the bioaccessibility experiments were performed using 2.5 g of infant cereals prepared with 15 mL of whole milk to simulate the ready-to-eat consumption as recommended by manufacturers.

It is possible to observe that Al bioaccessibility varied from 1.5% to 10.4% and may be influenced by the flour composition and ingredient list. Higher values were verified in products containing only one type of flour (rice flour or corn flour). Among multicereals, the obtained values (%) were: 2.2, 3.6 and 5.0 for brands A, B and C, respectively. The brand C showed high bioaccessible fraction compared to brands A and B and this may be due to the flour types and quantities used in its composition. According to the labels, three types of flour (wheat, corn and rice) are declared in brand C whilst in brands A and B five types (wheat, rice, corn, oats and barley) are present, which may contain more fiber contributing to the reduction of the bioaccessible fraction.

Further, some studies have associated low mineral absorption with the presence of antinutritional factors, such as phytates, which are present in plant seeds, grains and wheat bran (Schons, Ries, Battestin, & Macedo, 2011). Their ability to chelate poorly soluble multivalent cations (De Carli, Rosso, Schnitzler, & Carneiro, 2006), such as $Fe^{2+/3+}$,

Table 3
Mean values *(n = 3)* for total Al in ready-to-eat cereal (cereal prepared with milk), bioaccessible fraction and percentage of bioaccessibility (%).

Brands	Composition	Al concentration in ready-to-eat cereal (μg kg^{-1})		% Bioaccessibility
		Total	Bioaccessible fraction	
A	Multicereal	938 ± 18	21.2 ± 1.6	2.2
	Fruit (banana and apple)	326 ± 41	11.4 ± 0.5	3.4
	Rice flour	197 ± 17	16 ± 1.6	8.1
	Rice flour and oat	953 ± 11	14.0 ± 1.0	1.5
	Corn flour	346 ± 19	36.1 ± 11	10.4
B	Muticereals	1034 ± 69	37.0 ± 4.7	3.6
	Fruit (plum)	1852 ± 681	48.1 ± 6.2	2.6
	Fruit (banana and apple)	774 ± 90	26.7 ± 2.0	3.4
	Rice flour	1185 ± 50	79.6 ± 1.5	6.7
	Corn flour	842 ± 23	64.2 ± 2.5	7.6
C	Muticereals	1028 ± 21	52.3 ± 3.8	5.0
	Rice flour	757 ± 40	47.2 ± 1.7	6.2

* The bioaccessibility analysis was performed for samples with the highest values of total Al, according to each composition and brand.

Ca^{2+}, Cu^{2+}, and Zn^{2+}, may reduce the bioaccessibility of bound cations (Ries, 2010).

Regarding fruit-based cereals (banana and apple), brand A declares five types of flour (rice, corn, barley, oats and quinoa) plus fruit extract which increases the total fiber content. This component may contribute to a lower (3.4%) bioaccessibility of Al. The sample containing plum (brand B) presented lower bioaccessibility (2.6%) compared to banana and apple composition of the same brand once the former contains rice and oat flour and dehydrated plum extract, whilst only rice flour and dehydrated banana and apple are constituents of the latter.

3.2.1. Comparison of the present study with reported studies

In the current literature, few studies are related to the bioaccessibility of contaminants, especially Al in infant foods. In a recent study conducted by Souza et al. (2019), the Al bioaccessible fraction of corn flour, rice and oat, and rice cereals was below the method limit of detection (LOD) of 0.158 mg kg^{-1}. Only a multicereal sample presented Al concentration of 1.04 mg kg^{-1} in the dialysable fraction which corresponds to 6% of bioaccessibility, similar to the obtained value for brand C (5.0%) in the present study. However, it is important to note that Souza et al. (2019) assessed the bioaccessibility using a standardized static *in vitro* method simulating human gastrointestinal digestion (Minekus et al., 2014), without optimization and necessary adaptations for the infant gastrointestinal system, characterizing an exploratory study. Cabrera-Vique and Marta Mesías (2013) found that Al bioaccessible fraction ranged from 0.30% to 17.26% which is consistent with some results of the current study.

Willett (1990) observed that Al bioaccessible fraction could present values ranging from 0.06% to 27% in experimental animals, and from 0.001% to 24% in humans. Greger (1985) reported Al absorption lower than 1%, which is also consistent with some of the results of the present study. Another study (Shuping, 1996) indicated that Al ions in the diet cannot be penetrated by the hydrated charged ions at the intestinal level due to the duodenal mucosa membranes.

Moreover, it should be emphasized that the wide difference between total Al content in diets and its bioaccessibility is also in agreement with Mehra and Baker (2007), which found that although 1 L of tea can provide > 100% of the safe daily dietary intake of Al, the 'available' absorption percentage in the intestine is only 4.96% for loose tea and 9.13% for tea bag samples. Many factors can influence bioaccessibility of Al and its impact in the real exposure to this element. It has been reported that the chemical forms present in the intestinal tract may cause variations of 10-fold in the oral absorption of Al from food (EFSA, 2008).

Table 4
Al exposure expressed as % of the Provisional Tolerable Weekly Intake (PTWI), considering total Al concentration (mean).

Brands	Composition	Total Al (mg kg^{-1})	% PTWI
A	Multicereal	3.46	5.09
	Rice flour and oat	2.45	2.93
	Rice flour	1.99	1.91
	Fruit (banana and apple)	1.17	3.60
	Corn flour	1.30	1.72
B	Muticereals	7.13	10.48
	Rice flour	3.18	5.59
	Fruit (banana and apple)	4.95	4.67
	Fruit (plum)	5.30	7.79
	Corn flour	4.65	6.84
C	Muticereals	3.80	9.69
	Rice flour	6.59	4.73

3.3. Evaluation of the potential risk related to the intake of Al from infant cereals

Table 4 presents the results of Al exposure with regard to the contribution of the PTWI of 2 mg kg^{-1} bw (Food and Agriculture Organization/World Health Organization (FAO/WHO), 2011), considering total Al concentration.

It is possible to observe that despite the high total Al content infant cereal, the contribution to the PTWI is low as follows (%): 5.09–10.48 for multicereal, 2.93 for rice flour and oat, 1.91–5.59 for rice flour, 3.60–4.67 for fruit-based products (banana and apple) and 1.72–6.84 for corn flour, taking into account brands A, B and C.

In a duplicate diet sampling study considering the main meals along the day (breakfast, lunch, afternoon meal, and dinner), a total dietary intake of Al was estimated in two population groups from southern Spain (family and university students) (Cabrera-Vique & Marta Mesías, 2013). Mean values for Al intakes were 2.93 and 1.01 mg/day in families and students, respectively, ranging from 0.12 to 10.00 mg/day. Assuming an average adult body weight of 60 kg, the mean dietary exposures to Al were 0.34 and 0.12 mg/kg bw/week in these groups, which amounted to 17% and 6% of the 2 mg/kg bw established as the PTWI, with very similar values (%PTWI) found in the present study. If the bioaccessibility results of the reported work could be expressed in terms of exposure assessment, the estimated contribution to the PTWI would be even lower, with values ranging from 0.02% to 0.15%. This demonstrates there is a stamped distinction between the 'intake' and 'uptake' of Al in the human body. Duffield and Williams (1988), defends that Al represents a classical example to emphasize this difference as Al ions in the diet are completely non-bioaccessible to the small intestine and unable to pass into the bloodstream. In the case that little quantities of Al do enter the blood plasma, they will in general be quickly discharged. Pennington and Jones (1989) have also reported that only a small percentage of ingested Al is absorbed by the intestines of healthy people and this is readily eliminated from the body by the kidneys.

Moreover, dietary Al intake is likely to form complexes with natural components of foods such as citric and lactic acids. It has also been suggested that in the human body, Al ions may compete with other elements such as calcium, magnesium or iron in different metabolic processes (Macdonald & Martin, 1988), which may explain the variability of the bioavailability of aluminum in relation to the diet composition.

3.4. Method validation

Accuracy, precision (repeatability), linearity, detection limit (LOD) and quantification limit (LOQ) were evaluated according to the National Institute of Metrology, Standardization and Industrial Quality (INMETRO, 2017). For the concentration range from 2 to 200 µg L^{-1} of Al, a satisfactory correlation coefficient value was found ($r^2 > 0.999$), showing the linearity of the analytical curve. The LOD and LOQ were determined using 10 analytical replicates of a reagent blank, multiplied by the sample dilution factor (40×), and the values found were: LOD (3 s) = 53 µg kg^{-1} and LOQ (5 s) = 89 µg kg^{-1}, with "s" being the standard deviation value of the 10 blank replicates. In order to determine the precision of the method, the coefficients of variation (CV) of analytical triplicates of a cereal sample were evaluated and the mean value found was 2.6%, satisfying the conditions recommended by the AOAC International (AOAC, 2013) with a maximum CV of 25% for the range concentration. The accuracy of the method was evaluated in two certified reference materials (CRM): Typical Diet (SRM 1548a - Typical Diet) and Egg Powder (NRC EGGS - Egg Power), containing a mean concentration of 500 and 55 µg kg^{-1} of Al, respectively. The obtained results varied from 87% to 97%, in accordance with the AOAC (2013) guidelines, which establish a range of 75–120%, for the studied concentrations. In addition, an infant cereal sample containing low Al levels was used to perform spike experiments in analytical triplicate (n = 3) at four different concentrations (25, 50, 100 and 200 µg kg^{-1}). Further, the obtained values already take into account the total Al concentration present in the referred sample. Recovery values ranged from 87 to 101%.

4. Conclusions

In the present study, it was possible to determine the total Al content in samples of cereal-based baby foods as well as its bioaccessible fraction through an adapted *in vitro* approach considering the gastrointestinal system of children. The employed analytical method showed suitable values for precision and accuracy for infant cereal samples. The differences observed for total Al concentrations may be related to the brand, type of cereal used in the formulation (oat, corn, rice, wheat, barley, among others) and flour composition, reflecting in the Al bioaccessibility. The intake of Al through cereals contributed up to 10.48% of the PTWI, when considering total concentration results, indicating no potential risk to health.

CRediT authorship contribution statement

Esther Lima de Paiva: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing - original draft. **Camila Medeiros:** Data curation, Formal analysis. **Raquel Fernanada Milani:** Formal analysis, Software. **Marcelo Antonio Morgano:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Visualization, Writing - review & editing. **Juliana Azevedo Lima Pallone**: Methodology, Writing - review & editing. **Adriana Pavesi Arisseto-Bragotto:** Conceptualization, Funding acquisition, Project administration, Supervision, Visualization, Writing - review & editing.

Declaration of Competing Interest

The authors declare no conflict of interest.

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