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Evaluation of Total Concentrations and Bioaccessible Fractions of Essential and Toxic Elements in Amaranth, Quinoa and Chia by MIP OES

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The total concentrations and the bioaccessible fractions of Al, B, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, Ni, V and Zn were evaluated in 10 samples of pseudocereals, being 3 of amaranth, 3 of quinoa and 4 of chia. The samples were decomposed in a digester block with a reflux system under conditions optimized and the analyses were performed by microwave-induced plasma optical emission spectrometry. In all grains investigated, the highest total concentrations were found for the macroelements K, Mg and Ca, as well as the microelements Fe, Mn, and Zn. When the bioaccessible fraction was evaluated, negative correlations were observed between the content of total phenolic compounds and the bioaccessibility for most elements, in all samples. Among the elements considered essential in our diet, K (99%) and Cu (59%) presented the highest percentages of bioaccessible fraction. Among those elements considered potentially toxic, Ba did not exceed 15% bioaccessible fraction and Al presented concentrations lower than the limit of detection in all samples.

Keywords: pseudocereals, elemental composition, total concentration, bioaccessibility

Introduction

The population's concern about food in general has been changing over time. In this context, consumers have been showing a growing interest in functional food, seeking in these types of food, not only its basic nourishing function, but also their additional health benefits.[1]

In 2016, the Brazilian Consumer Protection Institute (IDEC) conducted an opinion survey on the use of the term "integral" in products based on whole grains that use wheat bran, other sources of fiber or other whole grains. The results showed the way consumers check if the food is whole, with 61.3% saying they check the list of ingredients, demonstrating that there is a real interest by most consumers in consuming healthier food.[2] Thus, it is necessary to look for alternative crop species with high productivity and a more complete nutritional profile than traditional crops. Pseudocereals such as amaranth (*Amaranthus*), quinoa (*Chenopodium quinoa*) and chia (*Salvia hispanica*) of Andean origin are among the candidate foods to replace traditional cereals such as wheat, rice, and corn. These pseudocereals are unconventional sources of protein with excellent nutritional value, with important levels of essential amino acids and high bioavailability, in addition to being considered good sources of vitamins, fiber, phenolic compounds and essential elements.[3-6]

Essential elements are nutrients of significant importance for the proper functioning of the human body and are needed to activate enzymes to produce hormones, in addition to playing a significant role in bone structure and biological processes. Deficiencies or excesses can disrupt the body's normal biochemical functions.[7] Amaranth and quinoa have significant levels of K, Fe, Zn, Mg and Ca[3,6] while for chia, the reports refer to K, Ca, Fe, Zn, Mg, Mn and Cu.[4,7]

The elemental composition of pseudocereals is variable because many factors can influence their characteristics, such as geographic location and climate, type of soil where they are grown, fertilizers employed, environmental pollution, among others, which can interfere with the absorption of elements by the plant.[8,9] Therefore, there is an interest in the determination of essential and non-essential elements from a nutritional point of view, focused on the adequacy of essential nutrients in the diet, as well as monitoring the content of potentially toxic elements.[4,10]

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However, it is also important to determine, in addition to the total concentration of the elements, their bioaccessible fraction in the food, as this is the fraction of the nutrient that can be released into the gastrointestinal tract during digestion and thus become available for absorption by the human body.[11] *In vitro* methods that simulate the gastrointestinal fluids of each stage of the digestive process have been successfully used to assess the bioaccessible fractions of essential and potentially toxic elements in various foods.[12,13]

The microwave-induced plasma optical emission spectrometry (MIP OES) technique has been successfully applied to determine the total concentrations and bioaccessible fractions of metals in several types of foods, with accurate results and good limits of detection.[13-16] This is a multi-element technique considered to have a low operational cost, due to the replacement of flammable or expensive gases by nitrogen that is easily obtained from the atmospheric air, for the maintenance of the plasma and besides, the linearity of MIP OES is larger when compared with the flame atomic absorption spectrometry (F AAS) technique.[17]

In the literature, few reports were found referring to the determination of the total concentration and the bioaccessible fraction of essential and toxic elements in chia, amaranth and quinoa samples. Santana *et al.*[18] determined the total content and estimated the bioaccessible fraction of Cu, Fe, Mn, and Zn in chia seeds (*Salvia hispanica* L.). Souza *et al.*[19] determined the total concentration of Ca, Cu, Fe, K, P, Na, and Zn and evaluated the bioaccessibility of Cu, Fe, and Zn total in nutritious flours (amaranth, plum meal, carrot, etc.). In all cases, the samples were analyzed by ICP OES.

Considering the importance of the consumption of these grains as a health benefit, as well as the lack of information on the bioaccessibility of inorganic constituents in pseudocereals, the aim of this study was to evaluate the total concentrations and bioaccessible fractions of essential and potentially toxic elements in amaranth, quinoa and chia. To determine the total concentrations, an analytical method was optimized, that involved some steps such as sample preparation, through an acid decomposition with a reflux system, method validation and data processing. The evaluation of the bioaccessible fractions was performed using an *in vitro* digestion method. Finally, it was investigated whether the total phenolic compounds present in the studied samples act as nutritional or antinutritional factors, in relation to the bioaccessibility of the elements.

Experimental

Instrumentation

An Agilent 4200 microwave induced plasma optical emission spectrometer (Agilent Technologies, Melbourne, Australia) equipped with an Inert One Neb nebulizer and a double-pass glass cyclonic spray chamber (Agilent Technologies, Melbourne, Australia) was used in this study. The nitrogen used to generate the plasma was supplied from an Agilent 4107 nitrogen generator (Agilent Technologies, Melbourne, Australia) with air supplied from an air compressor (model MSV12, Schulz, Joinville, SC, Brazil). For ignition of the plasma, a small flow of Ar gas with a purity of 99.996% was also used (Agilent Technologies). The instrumental operating conditions are presented on Supplementary Information (SI) section.

For sample decomposition, a digester block (model MA-4025, Marconi, Piracicaba, SP, Brazil) was used. In each digester tube, a cold finger with continuous water recirculation through a thermostatic bath (model Q-214M2 083, Quimis, Diadema, SP, Brazil) (ca. 15 ºC) was introduced to avoid losses by volatilization of analytes and reagents, as described in a previous work by Oreste *et al.*[20] For the determination of the dissolved solid content, a heating plate (Magnus, SP, Brazil) was employed, with the evaporation of the acids carried out at 150 °C in with subsequent drying at 180 °C, in an oven for sterilization and drying model 1.2 (Odontobrás, SP, Brazil). For determination of moisture content, the same oven was employed at 105 °C.

To determine the bioaccessible fractions, the samples were ground in a in a domestic knife mill (Philips Walita, 400 W). Also, a pH meter (pHS-3B model, PHtec, Curitiba, PR, Brazil), a Dubnoff bath with stirring and heating at 37 °C (model Q226M2, Quimis, Diadema, SP, Brazil) and a 11,000-rpm centrifuge (model 5804R, Eppendorf, Hamburg, Germany) were employed. For determination of phenolic compounds, a spectrophotometer (mono beam) was used for evaluations in 750 nm, brand Pró-Análise UV1100 (Cotia, SP, Brazil).

Reagents and standards

All reagents used were of analytical grade. Solutions were prepared with ultrapure water obtained from a glass distiller (model MA-075, Marconi, Piracicaba, SP, Brazil) followed by deionization through a column with cationic and anionic mixed resin (model CS1800, Permution, Brazil). For decomposition, nitric acid (Synth, Diadema, SP, Brazil) purified by doubly sub boiling distillation in

a quartz system (model MA-075, Marconi, Piracicaba, SP, Brazil) and 35% (v/v) hydrogen peroxide (Êxodo Científica, Sumaré, SP, Brazil) were employed. Calibration solutions were prepared from a multielement standard solution 6 for ICP TraceCERT® (Sigma Aldrich, Buchs, Switzerland) containing 100 mg L^{-1} of each analyte. Anhydrous D-glucose (dextrose) (Synth, Diadema, SP, Brazil) was used in the analysis of dissolved organic carbon. The resulting solutions from the decomposition were filtered with quantitative filter paper C42 (blue stripe), diameter 125 mm (Unifil, Germany). For bioaccessibility studies the following reagents were used: α-amylase from *Aspergillus oryzae* (PCode 101642338), pepsin from porcine gastric mucosa (PCode 101947953), bile extract porcine (PCode 1003443762) and pancreatine from porcine pancreas (PCode 1001987024) (Sigma-Aldrich, Saint Louis, Missouri, USA); $CaCl_2(H_2O)_2$, NaOH, KCl, NaCl, $MgCl_2(H_2O)_6$ and KH_2PO_4 (Synth, Diadema, SP, Brazil), $(NH_4)_2CO_3$ (Baker, San Bernardino County, USA), $NaHCO_3$ and HCl (Merck, Darmstadt, Germany). For polyphenol determination, methanol, Na_2CO_3 and Folin-Ciocalteu reagent (Sigma-Aldrich, Buchs, Switzerland) and HCl (Merck, Darmstadt, Germany) were used.

Samples

In total, four chia samples (Chia-A, Chia-B, Chia-C and Chia-D); three white amaranth samples (Amaranth-A, Amaranth-B, Amaranth-C) and three quinoa samples (two white quinoa-Quinoa-A and Quinoa-B and one black quinoa-Quinoa C) were analyzed. The amaranth, quinoa and chia grains were acquired in supermarkets of Pelotas (Rio Grande do Sul), in bulk and packaged form. The type of species of each pseudocereals obtained commercially was not informed and they were identified according to the locality of origin, provided on the package: São Paulo, Paraná and Rio Grande do Sul. Only those obtained from Brazilian Agricultural Research Company (EMBRAPA) from Pelotas provided information on the cultivars: BRS Alegria variety of amaranth (Amaranth-B) and BRS Piabiru variety of quinoa (Quinoa-B). In the laboratory, the samples were stored in decontaminated glass bottles and kept at room temperature and dry air.

Optimization of the acid decomposition procedure

For sample preparation using acid decomposition with reflux system, in the presence of 5.0 mL of HNO_3 65% (v/v), a study was carried out using a chia sample called Chia-A and the following variables were monitored: sample mass (250, 500 and 750 mg), heating temperature of the digester block (130, 150 and 170 ºC) and decomposition time (2, 3 and 4 h). First, the temperature was fixed at 150 °C and the time of 3 h, and the mass variation was performed, then, the temperature of the digester block and the decomposition time of the sample were varied. After setting these parameters, the volume of H_2O_2 35% (v/v) (2, 3, 4 and 5 mL) was evaluated to finalize the decomposition of each sample studied. In this last step, the temperature was reduced to 120 ºC, to avoid possible sample losses, since at higher temperatures, after the addition of H_2O_2, strong effervescence was observed, especially for larger volumes.

To evaluate the decomposition process, after changing each condition of the study, the dissolved solids content and the acidity content of the resulting solution were determined. The manufacturer of the MIP OES recommends that the solutions introduced in the equipment should have a maximum of 3.0% (m/v) of dissolved solids and 5.0% (v/v) acidity to preserve the optical parts of the equipment.[21] The dissolved solids content, acidity and moisture were determined based on the physical-chemical methods for food analysis described by the Adolfo Lutz Institute.[22]

For last, the efficiency of the decomposition method was also evaluated, through the determination of dissolved organic carbon in the obtained decomposed solutions. For this determination, a 5.0% (m/v) carbon solution was prepared by dissolving anhydrous dextrose in deionized water. The calibration curve was constructed with standard solutions with concentrations ranging from 0.05 to 1.0% (m/v), with a residual acidity of 5.0% (v/v). A 193.027 nm carbon wavelength was used for the measurements by MIP OES.

Acid decomposition method

After choosing the best conditions for acid decomposition method, the samples were prepared as follows 750 mg of grains were weighed directly into the digestion tubes and subsequently, 5.0 mL of HNO_3 65% (v/v) were added. Soon after, the reflux system was coupled at digestion tubes and the mixture was heated in a digester block to 150 °C for 2 h. After this period, the solutions were cooled to room temperature. To finish the decomposition of amaranth and quinoa samples, 3.0 mL of H_2O_2 were added and the solutions returned to heating in the digester block for another 1 h at 120 °C. For the chia samples, were added 4 mL of H_2O_2 divided in two steps. In the first, 2.0 mL of H_2O_2 were added and the solutions returned to heating in the digester block for another 1 h to 120 °C. After this time, more 2.0 mL of H_2O_2 was added and the mixture was heated again at 120 °C for more 1 h. At the end of this step, the samples were transferred to polypropylene flasks and the

final volume of 50.0 mL was filled with ultrapure water. Before being analyzed by MIP OES, the solutions were filtered on quantitative filter paper and diluted 2.5 times. Samples and analytical blanks were prepared in the same way and in triplicate.

The accuracy of the method for the determination of analytes total concentrations in amaranth, quinoa and chia samples was first evaluated through the decomposition of two Certified Reference Materials: rice flour (IRMM-804) and tomato leaf (CRM-Agro C1003a), using the same conditions earlier optimized for the chia samples. In addition, analyte addition tests were applied at the three concentration levels in the three samples (amaranth, quinoa, and chia), and the additions were performed before decomposition. The added concentrations were based on the results previously obtained in the samples and in linear ranges of the analytical curves obtained by MIP OES.

In vitro digestion method

The *in vitro* digestion method was applied to estimate the bioaccessible elemental fraction and was based on three sequential extraction steps (salivary, gastric and intestinal digestion). The method was adapted from the model proposed in the literature by Minekus *et al.*[11] and composition of the fluids are presented in Table S1 (SI section). For chia samples it was necessary to reduce the amount of mass due to the mucilage present in its composition that causes it to increase the viscosity of the solution, thus hindering the separation of the bioaccessible fraction (supernatant), from the non-bioaccessible fraction (solid part). For this reason, this sample was chosen to demonstrate each step of the method used. For amaranth and quinoa, the sample mass was 5 g.

The samples were ground for 10 s to simulate the ingestion of pseudocereals after chewing. Approximately 750 mg of each chia sample were weighed into polypropylene flasks. In the step that simulated the mouth, 4.0 mL of synthetic saliva and 1.0 mL of 7.5 mM $CaCl_2$ were added. The pH was adjusted to 7.0 after the addition of 1.0 mol L^{-1} NaOH, and the mixture was subsequently placed in a thermo-agitator water bath at 37 ºC for 10 min. In the stage where the stomach was simulated (second stage), 9.1 mL of gastric juice and 700 µL of 2.0 mM $CaCl_2$ were added, and the pH of the mixture was adjusted to 3.0 with the addition of 1.0 mol L^{-1} HCl. The flasks were left in the water bath at 37 °C for 2 h. Finally, in the step that simulated the intestine, 18.5 mL of intestinal juice and 1.35 mL of 9.0 mM $CaCl_2$ were added. The pH was adjusted to 7 with 1.0 mol L^{-1} NaOH, and the mixture was kept in the water bath for 2 h at 37 °C. Finally, the solutions were placed in an ice bath for 20 min and then centrifuged for 10 min at 11,000 rpm to separate the bioaccessible fraction (supernatant), which was used to determine the analytes by MIP OES, from the non bioaccessible fraction (solid part). Analytical blanks were run in parallel to check for the presence of analytes in the reagents. Before being analyzed by MIP OES, these solutions were diluted 3 times. The non-bioaccessible fraction, corresponding to the solid part obtained by centrifugation, was subjected to acid decomposition to assess the bioaccessibility accuracy through a mass balance.[14]

Determination of polyphenol contents

The Folin-Ciocalteu method, described by Louzada *et al.*[23] was used to determine the content of total phenolic compounds in the investigated samples. The only difference in the method was in relation to the sample mass used, which was 150 mg. The results were expressed in milligrams of gallic acid equivalents (EAG) *per* gram of sample.

Statistics

The results of the total concentration were submitted to analysis of variance (ANOVA) with comparison of means by the Tukey's test at the significance level of 5% comparing the different samples of each pseudocereal. Pearson's correlation was used to evaluate the correlation between polyphenol content and the bioaccessible fraction of the analytes, as well as the correlation between total concentration and bioaccessibility. The concentrations of analytes evaluated in certified reference materials (IRMM-804) and (CRM-Agro C1003a) were submitted to Student's *t*-test at a significance level of 5%. The results were statistically evaluated using Statistica Software 7.0.[24]

Results and Discussion

Effect of sample mass

The sample mass is an important parameter for the evaluation of homogeneity and for the complete decomposition of organic matter during the sample preparation process. For this purpose, different masses of Chia-A (250, 500 and 750 mg) were evaluated to assess the decomposition of organic matter and the adequacy of the sample to the working conditions established for the MIP OES. This evaluation occurred in the presence of 5.0 mL of HNO_3 65% (v/v) at 150 °C during 3 h. Figure 1a shows the results of the effect of mass variation under acid

decomposition conditions at 150 °C. As it can observed, the increase in mass caused an increase in the dissolved solids content, but without exceeding the maximum limit (3.0% m/v).

For acidity, an inverse effect was observed, there was a decrease in acidity with increasing mass. This behavior was also observed in the works by Sampaio *et al.*[17] and Pereira *et al.*[16] For this reason, the quantity of 750 mg mass was chosen, because a greater amount of mass allows a higher consumption of acid and favors obtaining better limits of detection of the method.

In relation to the moisture content, their determination allows the removal of water and other residues that can be volatilized, establishing the amount of dry sample that will be used in the methodology. The values found in this study for amaranth, quinoa and chia were 12.9; 13.1 and 8.3%, respectively. This way, for amaranth and quinoa samples that presented a moisture content of approximately 13%, the results were calculated considering the dry mass of 653 mg.

Effect of heating temperature and decomposition time

Figures 1b and 1c show the results concerning the effect of heating temperature of the digester block and decomposition time, respectively. Temperature is a critical factor for the elimination of all organic matter, so it should be sufficient for the activation energies of chemical processes to be reached and chemical bonds to be broken.

As can be seen in Figure 1b, for 750 mg, the results showed that the lowest values of dissolved solids were obtained when the digester block was heated at 150 °C, while the lowest residual acidity found was at 130 °C, which was close to that found at 150 °C. Previous studies[17,25] using acid decomposition with a reflux system demonstrated that a temperature of the digester block of 150 °C or close was efficient for the decomposition of complex matrices, such as fish samples and sugarcane-derived products. Therefore, the temperature of 150 °C was chosen.

Time is a less critical factor, but it should be enough for the entire sample mass to be efficiently decomposed. To evaluate the influence of decomposition time, 750 mg of chia sample were submitted to acid decomposition with 5.0 mL of HNO_3 65% (v/v) during 2, 3 and 4 h at 150 °C. The results obtained are shown in Figure 1c. As the decomposition time varied, the acidity and dissolved solids contents remained practically constant. For the three times evaluated, the residual acidity was above the recommended

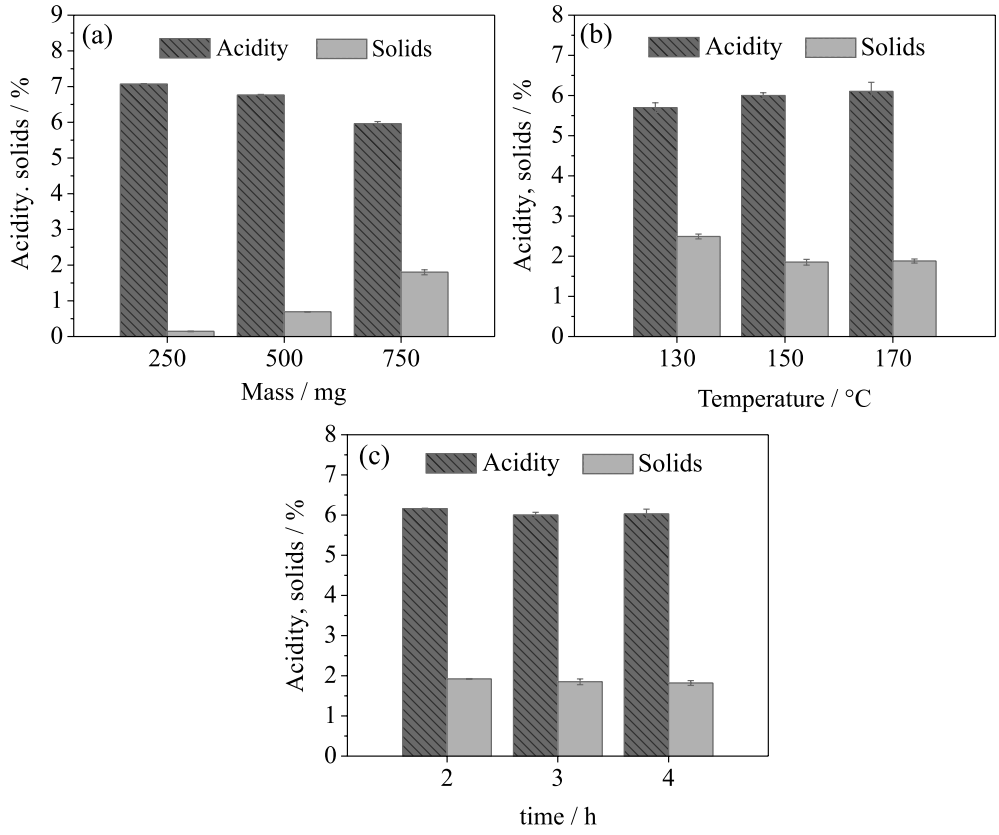

Figure 1. (a) Effect of mass variation on the acid decomposition (fixed conditions: 5.0 mL of HNO_3 65% (v/v); 3 h of decomposition at 150 °C); (b) effect of heating temperature of digester block (fixed conditions: 750 mg of sample; 5.0 mL of HNO_3 65% (v/v); 3 h of decomposition); (c) effect of decomposition time (fixed conditions: 750 mg of sample; 5.0 mL of HNO_3 65% (v/v) at 150 °C). Final volume: 50 mL.

of 5.0% (v/v). Thus, 2 h was chosen because it was the shortest decomposition time and the one that presented results like the longer times.

Effect of H_2O_2 volume and dissolved organic carbon

The efficiency of acid decomposition using H_2O_2 as auxiliary oxidant was evaluated by comparing the obtained results of acidity and dissolved solids in the three samples (amaranth, quinoa, and chia) decomposed only with HNO_3 65% (v/v) and samples decomposed with HNO_3 65% plus additions of different volumes of H_2O_2 35% (v/v), to obtain a clear digest and within the recommended limits. The results obtained are shown in Figure 2.

After each addition of H_2O_2, it was possible to observe visual changes in the sample solutions, such as reduction in fat material on the walls of the digestion tube. For the amaranth and quinoa samples with the addition of up to 3 mL of H_2O_2 there was a decrease in acidity and dissolved solid contents when compared to the sample without the addition of auxiliary oxidant. Already with 4 mL, the residual acidity began to increase (Figures 2a and 2b). Thus, it was decided to add 3 mL of H_2O_2 35% (v/v), followed by heating at 120 °C for 1 h, because it was possible to obtain a clear digested solution that was consistent with the pre-established limits for the MIP OES technique, with acidity of 4.91 ± 0.07 and 4.89 ± 0.04% (v/v), and dissolved solids 0.35 ± 0.003 and 0.34 ± 0.01% (m/v), for amaranth and quinoa samples, respectively.

For the chia samples, after one hour of decomposition in the presence of H_2O_2, there was still a residual fatty material. Thus, for this sample, larger volumes of H_2O_2 (2, 3, 4 and 5 mL) were evaluated. With a volume of 4 mL of H_2O_2, it was possible to obtain a clear digest, with acidity of 5.09 ± 0.10% (v/v) and dissolved solids content of 1.05 ± 0.04% (m/v). Thus, two additions of 2 mL of H_2O_2 35% (v/v) were fixed, totaling a volume of 4 mL, which proved to be satisfactory for the objectives of the study. After each addition, the solutions were heated for a further 1 h at 120 °C.

The decomposed samples showed low levels of dissolved organic carbon and the values obtained were 0.04, 0.05, and 0.15%, for amaranth, quinoa, and chia, respectively, which demonstrates the efficiency of the process. When comparing the data obtained, it was observed that the carbon contents obtained in some studies[18,26] are lower than those reported.

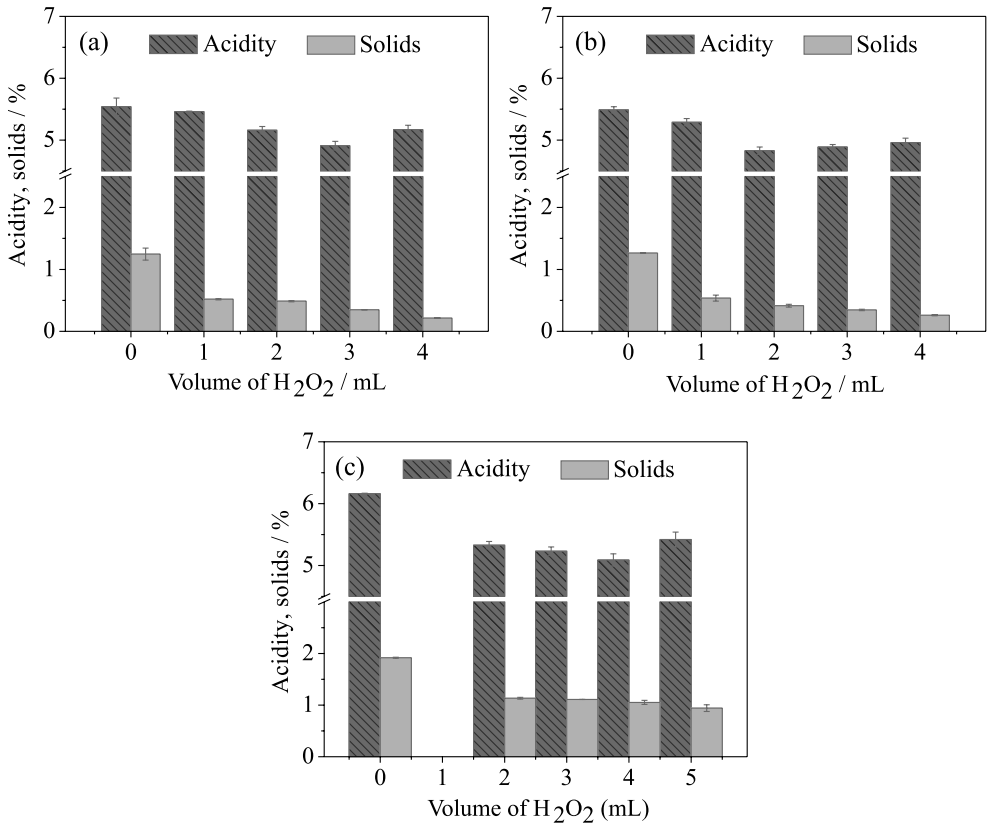

Figure 2. Effect of H_2O_2 addition on the decomposition of 750 mg of sample. Conditions for (a) amaranth and (b) quinoa: (0) decomposition using 5.0 mL of HNO_3 at 150 °C during 2 h. Additions of H_2O_2: 1.0; 2.0; 3.0 and 4.0 mL, followed by heating at 120 °C for 1 h. (c) chia: (0) decomposition using 5.0 mL of HNO_3 at 150 °C during 2 h. Additions of H_2O_2: 2.0; 3.0; 4.0 and 5.0 mL, followed by heating at 120 °C for 2 h.

Total concentrations of analytes in pseudocereals

Figures of merit

The figures of merit for determination of total concentrations are presented in Table S2, SI section. Analytical curves were prepared using aqueous standard solutions in 2.0% (v/v) HNO_3 with concentrations ranging from 0.1 to 5.0 mg L^{-1}. Adequate linear correlation coefficients in all curves were obtained ($R > 0.998$). The limits of detection of the method ($LOD_{(m)}$) for amaranth and quinoa samples ranged from 0.019 to 0.574 mg kg^{-1} and for chia samples, from 0.017 to 0.500 mg kg^{-1} and were suitable for the determinations of the investigated analytes in pseudocereals samples.

The accuracy of the method was evaluated using the certified reference materials rice flour (IRMM-804) and tomato leaf (CRM-Agro C1003a) and the obtained results are shown in Table S3, SI section. The recoveries ranged from 90 to 113% and the application of the Student's *t*-test at a confidence level of 95% showed no significant differences between the values found and the certified values. The relative standard deviation (RSD) was lower than 5.9% for all analytes.

Also, the analyte addition test was applied, to verify the accuracy of the results for all elements. Additions were based on the linear range of the calibration curve (mg L^{-1}) and on the concentrations determined in the samples. Tables S4, S5 and S6 in SI section present the obtained results for additions in amaranth, quinoa, and chia samples, respectively. The average recoveries ranged from 81 to 118% for amaranth; 80 to 118% for quinoa and 82 to 116% for chia. The RSD was lower than 9.5% for all analytes, proving the method's accuracy.

Analytical results

The results obtained for the total concentrations of the analytes Al, B, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, Ni, V and Zn, in the amaranth and quinoa samples are presented in Table 1 and in the chia, samples are shown in Table 2. Variations in the analyte concentrations were observed when varied brands of amaranth, quinoa and chia grains were evaluated. The results were submitted to ANOVA with comparison of averages by Tukey's test at a significance level of 5%, to show where there was a significant difference ($p < 0.05$) between the analytes investigated in the varied brands of each pseudocereal. These variations can be attributed to the form of plant cultivation or soil characteristics depending on the region where they were cultivated and, associated with the sensitivity and tolerance of crops to the addition of nutrients such as chemical elements, as they may vary between species and cultivars of the same species. In some cases, the amount of nutrient adequate for one cultivar can be toxic for another and the effects can be observed in relation to the growth and yield of the plant.

When comparing the three analyzed pseudocereals, chia presented the highest total concentrations for most

Table 1. Determined total concentrations of Al, B, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, Ni, V, Zn and total phenolic contents (TPs) in amaranth and quinoa samples (n = 3)

Analyte	Concentration ± standard deviation / (mg kg^{-1})					
	Amaranth-A	Amaranth-B	Amaranth-C	Quinoa-A	Quinoa-B	Quinoa-C
Al	5.4 ± 0.1^{c}	5.7 ± 0.1^{b}	7.7 ± 0.1^{a}	10.8 ± 0.6^{b}	13.9 ± 1.1^{a}	8.1 ± 0.5^{c}
B	5.0 ± 0.1^{b}	8.5 ± 0.8^{a}	8.0 ± 0.1^{a}	6.5 ± 0.1^{b}	8.5 ± 0.6^{a}	8.5 ± 0.5^{a}
Ba	5.73 ± 0.01^{a}	5.74 ± 0.01^{a}	1.91 ± 0.01^{b}	1.91 ± 0.01^{b}	< 0.019	5.5 ± 0.4^{a}
Ca	1364 ± 27^{b}	1587 ± 38^{a}	1308 ± 36^{b}	523 ± 51^{b}	658 ± 14^{a}	626 ± 31^{a}
Cu	3.4 ± 0.1^{b}	13.5 ± 1.1^{a}	4.4 ± 0.3^{b}	5.3 ± 0.1^{b}	13.1 ± 1.1^{a}	6.2 ± 0.5^{b}
Fe	64 ± 3^{b}	80.8 ± 3.3^{a}	75.7 ± 2.2^{a}	49.4 ± 2.8^{a}	48.2 ± 1.2^{a}	47.6 ± 1.1^{a}
K	4724 ± 73^{b}	4449 ± 106^{c}	5314 ± 108^{a}	8135 ± 82^{a}	6200 ± 158^{b}	6459 ± 137^{b}
Mg	1746 ± 27^{c}	3119 ± 36^{a}	2455 ± 15^{b}	1830 ± 93^{b}	2458 ± 77^{a}	1872 ± 20^{b}
Mn	21.0 ± 0.1^{c}	51.1 ± 2.9^{a}	39.6 ± 1.1^{b}	39.4 ± 1.2^{b}	60.6 ± 1.1^{a}	58.7 ± 1.1^{a}
Na	30.5 ± 1.8^{a}	11.4 ± 1.1^{c}	22.2 ± 1.9^{b}	493 ± 9^{a}	23.1 ± 2.2^{b}	6.2 ± 0.6^{c}
Ni	< 0.096	< 0.096	< 0.096	1.91 ± 0.01^{b}	0.38 ± 0.001^{c}	2.4 ± 0.1^{a}
V	3.8 ± 0.1^{c}	19.1 ± 0.1^{a}	12.8 ± 1.1^{b}	11.5 ± 0.1^{b}	16.7 ± 1.1^{a}	10.9 ± 1.0^{b}
Zn	16.5 ± 1.1^{c}	49.8 ± 1.9^{a}	31.3 ± 2.2^{b}	36.2 ± 2.0^{b}	42.7 ± 1.1^{ab}	47.2 ± 4.0^{a}
	(mg EAG g^{-1})					
TPs	3.34 ± 0.09^{a}	0.55 ± 0.03^{c}	1.61 ± 0.10^{b}	0.60 ± 0.04^{b}	0.65 ± 0.01^{b}	1.07 ± 0.06^{a}

^{a,b,c} Results followed by different letters on the same line have a significant difference, comparing the sample of different brands (Tukey test, $p \leq 0.05$). Amaranth: (A) São Paulo; (B and C) Rio Grande do Sul. Quinoa: (A) São Paulo; (B and C) Rio Grande do Sul. EAG: gallic acid equivalents.

Table 2. Determined total concentrations of Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, Ni, V, Zn and total phenolic contents (TPs) in chia samples (n = 3)

Analyte	Concentration ± standard deviation / (mg kg^{-1})			
	Chia-A	Chia-B	Chia-C	Chia-D
Al	64.1 ±1.1^{b}	234 ± 1.1^{a}	37.2 ± 2.5^{d}	50.0 ± 2.9^{c}
Ba	31.1 ± 1.9^{c}	36.1 ± 2.5^{b}	25.02 ± 0.07^{d}	42.2 ± 1.9^{a}
Ca	6527 ± 154^{c}	8385 ± 471^{b}	6378 ± 152^{c}	9540 ± 353^{a}
Cu	24.1 ± 1.0^{a}	20.5 ± 1.0^{b}	15.03 ± 0.06^{c}	23.9 ± 1.0^{a}
Fe	80.8 ± 0.8^{b}	99.4 ± 0.9^{a}	71.97 ± 0.05^{c}	78.3 ± 1.6^{b}
K	7143 ± 85^{b}	8069 ± 177^{a}	6944 ± 102^{b}	7059 ± 115^{b}
Mg	3759 ± 33^{b}	4841 ± 309^{a}	3848 ± 101^{b}	3430 ± 104^{b}
Mn	67.2 ± 1.9^{b}	79.9 ± 4.4^{a}	40.0 ± 1.6^{c}	64.98 ± 0.04^{b}
Na	16.66 ± 0.01^{d}	53.3 ± 3.3^{b}	44.2 ± 2.5^{c}	72.1 ± 4.8^{a}
Ni	1.67 ± 0.01^{a}	< 0.083	< 0.083	1.66 ± 0.01^{a}
V	14.4 ± 1.0^{a}	7.7 ± 0.3^{d}	11.66 ± 0.01^{b}	9.7 ± 0.3^{c}
Zn	77.8 ± 3.5^{a}	53.3 ± 1.7^{c}	60.5 ± 1.0^{b}	63.3 ± 1.7^{b}
	(mg EAG g^{-1})			
TPs	2.38 ± 0.08^{b}	3.41 ± 0.09^{a}	3.27 ± 0.08^{a}	2.46 ± 0.10^{b}

^{a,b,c,d} Results followed by different letters on the same line have a significant difference, comparing the sample of different brands (Tukey test, $p \leq 0.05$). Chia: (A) São Paulo; (C) Paraná; (B and D) Rio Grande do Sul. EAG: gallic acid equivalents.

elements (Al, B, Ba, Ca, Cu, Fe, K, Mg, Mn, and Zn); for Na and Ni the highest concentrations found were in quinoa samples and for V, the highest concentration was observed in amaranth. When evaluating the varied brands of each sample, Amaranth-B, Quinoa-B and Chia-D showed the highest concentrations for most of the elements evaluated and these three samples come from Rio Grande do Sul.

In the three grains evaluated, the macrominerals K, Ca and Mg were the elements that presented higher concentrations. K concentrations ranged between 4449 and 8135 mg kg^{-1}, with higher values in the Quinoa-A sample. For K, the high concentrations found in all grains can be explained by its high mobility in plants due to its low affinity with organic forms and is the main element responsible for nutrient and metabolite transport of human body.[26] For Ca, the concentrations ranged from 523 to 9540 mg kg^{-1} and for Mg, ranged from 1746 to 4841 mg kg^{-1}, wherein the Chia-D and Chia-B samples exhibited the highest concentrations, respectively. Sodium was the macromineral with the lowest concentration in the investigated samples, ranging from 6.2 to 493 mg kg^{-1}.

Compared to the database of Brazilian Food Composition Table (TBCA),[27] the results for total concentrations agree for almost all macrominerals, in at least one sample of each pseudocereal, and some differences can be explained because different cultivars were evaluated, and they were produced in different climatic conditions. For Na, the concentrations obtained in this study were lower than the concentrations reported by the TBCA database, except for the Quinoa-A which showed a high concentration (493 ± 9 mg kg^{-1}), which may be due to a natural accumulation of Na in the soil from the use of fertilizers and pesticides.

Among the microminerals, Fe, Mn and Zn were the ones that presented greater quantity in the investigated samples. These elements act on the immune system and perform numerous physiological functions that contribute to the proper functioning of the human organism.[28] The concentrations for Fe ranged between 47.6 and 99.4 mg kg^{-1}, with higher values in the Chia-B sample. For Mn, the concentrations ranged from 21 to 79.9 mg kg^{-1} and for Zn, from 16.5 to 77.8 mg kg^{-1}, with the highest concentrations found in Chia-B and Chia-A samples, respectively.

Other microminerals present in the samples studied, but in a smaller proportion, are the Cu and V. They act in tissue formation, bone mineralization and as a component of several enzymes.[29,30] Cupper presented concentrations ranged from 3.4 to 24.1 mg kg^{-1} while for V, the concentrations ranged between 3.8 at 19.1 mg kg^{-1}, wherein the highest values were found in Chia-A and Amaranto-B samples, respectively.

The total concentrations obtained for the microminerals Fe, Mn, Zn and Cu, for the most part, agree with those available in the TBCA database for amaranth and quinoa samples, while for chia samples, only the Fe results showed good agreement. However, the results of this study for Mn, Zn and Cu in chia samples agree with those obtained by Llorent-Martínez *et al.*,[31] da Silva *et al.*[4] and Rubio *et al.*[32] For V, the obtained values of concentration, for the three

samples, are higher than those reported in the literature.[33,34]

Ni and B are essential elements for plants, but in relation to the human diet, studies that clarify their biological functions and the necessary concentrations of these elements have not yet been presented.[35,36] For Ni, the concentration values obtained in chia and quinoa samples are close to those reported in another studies[31-33,37] and ranged between 0.38 to 2.4 mg kg^{-1}. Regarding the element B, the concentrations ranged from 5 to 8.5 mg kg^{-1} and no data were found on its concentration in amaranth and quinoa grains for comparison.

Al and Ba are considered non-essential and have toxicological potential. Al concentrations ranged from 5.4 to 234 mg kg^{-1}, with the highest concentration in Chia-B sample which can be explained by the soil conditions in Brazil, which has unusually acidic soil with high Al content.[38] The concentration values for Al showed a variation and agreed with the results found in the literature for chia and quinoa samples.[34,37] For amaranth, the values obtained were slightly lower than those reported in a study conducted with amaranth flours.[19]

Ba, also considered a non-essential element, has toxicological potential. Chronic human exposure to Ba in excess is associated with adverse outcomes, including heart and/or renal failure, pulmonary edema, respiratory paralysis, and gastric and respiratory hemorrhages.[39,40] Ba concentrations ranged between 1.91 and 42.2 mg kg^{-1}. The chia samples presented the highest levels and the results obtained in this study agree with those reported by Llorent-Martínez *et al*.[31] Finally, among the Ba values obtained in this study in quinoa samples, there is agreement with the result found by Bolaños *et al*.,[34] but the value obtained for amaranth by these authors is lower than all the values found in this work.

Total polyphenol content (TPs)

The obtained results for total polyphenol content (TPs) in the amaranth and quinoa samples are presented Table 1 and for chia samples in Table 2, in the last lines. The chia samples showed the highest concentrations of TPs, ranging from 2.38 to 3.41 mg EAG *per* g of sample. The obtained results are within the range found in other studies for chia 1.77 to 7.89 mg EAG *per* g of sample.[41] For amaranth samples, the values ranged from 0.55 to 3.34 mg EAG *per* g of sample and in other studies, the values reached up to 5.24 mg EAG *per* g of sample.[42]

The amaranth varieties BRS Alegria and quinoa BRS Piabiru were evaluated by Palombini *et al.*,[43] who found 0.22 and 0.63 mg EAG *per* g of sample, for amaranth and quinoa, respectively. In the present study, for these varieties, the values found were 0.55 and 0.60 mg EAG *per* g of sample, for Amaranth-B and Quinoa-B, respectively. The value obtained for the Amaranth-B variety was higher than that found by Palombini *et al*.[43] This may be because plant secondary metabolites, such as phenolic compounds, vary from generation to generation depending on environmental factors. Our samples were obtained from distinct locations in a different year, thus the effect of these factors on phenolic compounds should be considered, as well as the differences in the methods used for the determination of TPs. Considering all the quinoa samples evaluated, the obtained values ranged from 0.60 to 1.07 mg EAG *per* g of sample. The black quinoa variety (Quinoa-C) presented the highest value of TPs, and this result is similar to those obtained by Diaz-Valencia *et al.*[44] for the same variety (0.96 mg EAG *per* g of sample).

Bioacessible fraction

Figures of merit

In the present study, the bioaccessible fractions of Al, B, Ba, Cu, Fe, K, Mg, Mn, Ni, V and Zn were evaluated in amaranth, quinoa, and chia samples from different brands. However, it was not possible to evaluate the bioaccessibility of Ca and Na because these elements are present in substantial amounts in the reagents used to simulate the *in vitro* gastrointestinal digestion. Table S7, SI section, shows the figures of merit for the determination of the analytes bioaccessible fractions in amaranth, quinoa, and chia samples.

The samples Amaranth-A, Quinoa-A and Chia-A were used to evaluate the accuracy. After simulating the three main stages of the digestive system, the bioaccessible fraction (supernatant) was collected for direct analysis and the non-bioaccessible fraction (solid phase) was decomposed as described earlier. The results presented in Table 3 for amaranth and quinoa and in Table 4 for chia showed that the sum of both concentrations was close to the total concentration (TC) initially obtained for each analyte, proving the accuracy of the method.[14]

The recoveries obtained with the mass balance ranged from 80 to 115% for amaranth and quinoa, respectively, and from 80 to 111% for chia sample, proving the accuracy of the method. Thus, the method was applied to determine the bioaccessible fractions of the analytes in amaranth and quinoa (brands B and C) and chia samples (brands B, C and D). The results are presented in Tables 5 and 6, respectively.

K and Mg, which are present in high concentrations in the investigated samples, also showed high percentages of bioaccessibility. For K it reached 99% in amaranth, 97% in quinoa and 62% in chia sample. Mg presented

Table 3. Results of total concentrations (TC), bioaccessible fractions (BF) and non-bioaccessible fractions (NBF) in Amaranth-A and Quinoa-A samples (n = 3)

Analyte	Amaranth-A				
	TC / (mg kg^{-1})	NBF / (mg kg^{-1})	NBF / %	BF / (mg kg^{-1})	BF / %
Al	5.4 ± 0.1	5.3 ± 0.1	98	< 0.003	–
B	5.0 ± 0.1	4.1 ± 0.1	82	0.98 ± 0.001	20
Ba	5.7 ± 0.1	4.3 ± 0.2	75	0.30 ± 0.001	5
Cu	3.4 ± 0.1	1.31± 0.01	38	2.0 ± 0.01	59
Fe	64 ± 3	33.7 ± 2.3	53	19.1 ± 1.0	30
K	4724 ± 73	305 ± 2	6	4582 ± 142	97
Mg	1746 ± 27	347 ± 19	20	1066 ± 12	61
Mn	21.0 ± 0.1	13.5 ± 0.1	64	3.4 ± 0.1	16
V	3.8 ± 0.1	1.90 ± 0.01	50	2.45 ± 0.01	64
Zn	16.5 ± 1.1	14.2 ± 0.7	86	1.96 ± 0.01	12
Analyte	Quinoa-A				
	TC / (mg kg^{-1})	NBF / (mg kg^{-1})	NBF / %	BF / (mg kg^{-1})	BF / %
Al	10.8 ± 0.6	9.7 ± 0.1	90	< 0.003	–
B	6.5 ± 0.1	3.8 ± 0.1	58	2.0 ± 0.1	31
Ba	1.91 ± 0.01	1.8 ± 0.1	94	0.28 ± 0.001	15
Cu	5.3 ± 0.1	5.0 ± 0.1	94	1.13 ± 0.01	21
Fe	49.4 ± 2.8	24.1 ± 1.3	49	20.5 ± 0.2	41
K	8135 ± 82	407 ± 27	5	8019 ± 189	99
Mg	1830 ± 93	501 ± 13	27	1266 ± 4	69
Mn	39.4 ± 1.2	24.0 ± 0.1	61	8.0 ± 0.3	20
Ni	1.91 ± 0.01	0.95 ± 0.04	50	0.60 ± 0.01	31
V	11.5 ± 0.1	4.5 ± 0.1	39	5.1 ± 0.1	44
Zn	36.2 ± 2.0	26.2 ± 1.4	72	3.1 ± 0.1	9

NBF: percentage of non bioaccessible fraction; BF: percentage of bioaccessible fraction.

Table 4. Results of total concentrations (TC), bioaccessible fractions (BF) and non-bioaccessible fractions (NBF) in Chia-A sample (n = 3)

Analyte	TC / (mg kg^{-1})	NBF / (mg kg^{-1})	NBF / %	BF / (mg kg^{-1})	BF / %
Al	64.1 ± 1.1	63.8 ± 1.1	99	< 0.017	–
Ba	31.1 ± 1.9	23.9 ± 1.0	77	1.42 ± 0.001	5
Cu	24.1 ± 1.0	20.8 ± 1.2	86	5.20 ± 0.01	22
Fe	80.8 ± 0.8	68.0 ± 3.8	84	14.20 ± 0.02	18
K	7143 ± 85	2257 ± 216	32	4408 ± 218	62
Mg	3759 ± 33	1350 ± 13	36	2116 ± 90	56
Mn	67.2 ± 1.9	45.8 ± 0.8	68	8.3 ± 0.3	12
Ni	1.67 ± 0.01	< 0.042	–	1.41 ± 0.003	84
V	14.4 ± 1.0	13.2 ± 1	92	2.8 ± 0.1	19
Zn	77.8 ± 3.5	63.3 ± 4.7	81	< 0.084	–

NBF: percentage of non bioaccessible fraction. BF: percentage of bioaccessible fraction.

bioacessible fractions of up to 61% for amaranth, 72% for quinoa and 58% for chia, indicating that these elements are more soluble in the conditions presented by the gastrointestinal system. These results agree with those presented by Souza *et al.*[45] who report higher bioaccessible concentrations of K and Mg in cereal samples.

Among the microelements, the highest bioaccessible fractions were observed for Cu and V, mainly in the amaranth sample (59% for Cu and 64% for V). The bioaccessibility of Fe, Mn and Zn was less than 50% for all samples. The highest fractions of Fe (41%) and Mn (20%) were found in quinoa. In amaranth, the highest fraction

Table 5. Results of bioaccessible fraction (BF) and percentage of bioaccessible fraction (BF) in samples amaranth and quinoa (B and C) (n = 3)

Analyte	Amaranth-B		Amaranth-C	
	BF / (mg kg^{-1})	BF / %	BF / (mg kg^{-1})	BF / %
Al	< 0.003	–	< 0.003	–
B	1.9 ± 0.1	22	1.5 ± 0.1	19
Ba	0.28 ± 0.001	5	< 0.001	–
Cu	5.0 ± 0.3	37	0.84 ± 0.001	19
Fe	14.9 ± 0.5	18	19.5 ± 0.4	26
K	4132 ± 82	93	4905 ± 247	92
Mg	1597 ± 124	51	1233 ± 19	50
Mn	6.7 ± 0.1	13	5.9 ± 0.2	15
V	6.9 ± 0.2	36	3.6 ± 0.3	28
Zn	2.2 ± 0.2	4	3.1 ± 0.1	10
Analyte	Quinoa-B		Quinoa-C	
	BF / (mg kg^{-1})	BF / %	BF / (mg kg^{-1})	BF / %
Al	< 0.003	–	< 0.003	–
B	1.7 ± 0.1	20	1.0 ± 0.01	12
Ba	< 0.001	–	< 0.001	–
Cu	3.3 ± 0.2	25	0.3 ± 0.001	5
Fe	13.5 ± 1.1	28	11.1 ± 0.3	23
K	5360 ± 327	86	5946 ± 341	92
Mg	1418 ± 2	58	1347 ± 88	72
Mn	6.2 ± 0.1	10	7.4 ± 0.1	13
Ni	0.28 ± 0.001	74	0.6 ± 0.001	25
V	5.6 ± 0.2	33	3.2 ± 0.2	29
Zn	1.13 ± 0.01	3	1.15 ± 0.01	2

Table 6. Results of bioaccessible fraction (BF) and percentage of bioaccessible fraction (BF) in chia samples (B, C and D) (n = 3)

Analyte	Chia-B		Chia-C		Chia-D	
	BF / (mg kg^{-1})	BF / %	BF / (mg kg^{-1})	BF / %	BF / (mg kg^{-1})	BF / %
Al	< 0.017	–	< 0.017	–	< 0.017	–
Ba	1.42 ± 0.002	4	1.41 ± 0.002	6	2.70 ± 0.25	6
Cu	1.41 ± 0.01	7	1.40 ± 0.01	9	5.66 ± 0.01	24
Fe	4.48 ± 0.33	4	9.91 ± 0.02	14	1.49 ± 0.13	2
K	5038 ± 109	62	3684 ± 95	53	3841 ± 218	54
Mg	2501 ± 3	52	2248 ± 59	58	1988 ± 100	58
Mn	9.45 ± 0.01	12	4.28 ± 0.04	11	8.51 ± 0.01	13
Ni	< 0.042	–	< 0.042	–	1.42 ± 0.002	85
V	1.5 ± 0.1	20	2.1 ± 0.1	18	< 0.169	–
Zn	< 0.084	–	< 0.084	–	< 0.084	–

was found for Zn (12%). In chia, Zn had bioaccessible concentration results below the limit of detection for all samples. This fact was also observed in the studies reported by Santana *et al.*[18] who explain that one of the factors that inhibit the bioaccessibility of Zn in chia may be the presence of fibers in greater amounts, as it tends to form insoluble compounds with this component.

Ni showed high bioaccessibility in chia and quinoa samples, which had presented values above the limit of detection when determining the total concentration, being 85 and 74% respectively. B presented total concentration above the limit of detection only for amaranth and quinoa, reaching a maximum of 31% of bioaccessibility in quinoa.

Al and Ba, considered potentially toxic elements,

showed low percentages of bioaccessible fractions, and Ba did not exceed 15% of bioaccessible fraction in the quinoa sample. For Al, the bioaccessible concentrations determined were below the limit of detection. The low bioaccessibility of Al may be associated with the presence of phytates, influence of intestinal pH or precipitation in the form of phosphates.[46] The presence of compounds such as phytic acid and phenolics, which are formed in greater amounts due to the processes of storage, fermentation, germination, processing, and digestion of grains, can have a negative effect on bioaccessibility, when they act as anti-nutritional.

In general, chia presented the lowest bioaccessible fractions, because the presence of tannins and phytic acid in their composition, even if in small amounts and associated with fibers, which are normally available in high concentrations in this food, may act creating difficulties in the release of some elements.[47] The nature of the proteins is also a factor that influences the bioaccessible fraction.[48] Phytic acid has chelating agents that bind to minerals and negatively affect bioaccessibility. The tannin-type polyphenols, on the other hand, bind to components that are present in the food matrix and form complexes through hydrogen bonds that reduce the bioaccessibility of these elements.[47]

When samples of varied brands were evaluated, variations in the percentage of the bioaccessible fraction of the elements were observed for the three grains (amaranth, quinoa, and chia). Therefore, an explanation to be considered for these variations in the bioaccessible fractions is the possible interaction with inhibitor compounds (phenolic compounds, phytates, fibers and proteins) as well as interactions between minerals, because according to the literature,[3,6,49] there are competing minerals, for example, where there is a higher concentration of Ca, as observed in this study, it can result in a decrease in the absorption of minerals such as Fe and Zn.

Polyphenols are a class of compounds that can act as a nutritional or anti-nutritional factor regarding bioaccessibility, depending on whether they carry the element for soluble fraction, which corresponds to the part available for absorption. Thus, the total polyphenol contents found were correlated with the bioaccessible fraction of the elements, as well as the correlation between total concentration and bioaccessibility was also evaluated. Table 7 shows the results of correlations found. The bioaccessible fractions values for Al in all samples and for Zn in the chia sample were below the limit of detection of the method.

The chia samples showed the highest polyphenol contents, followed by amaranth and quinoa samples. Considering the results obtained and using Pearson's correlation coefficient, it is possible to conclude that for chia, a higher content of polyphenols is associated with a lower bioaccessible fraction. Phenolic compounds showed a strong negative correlation (0.6 to 1.0) with Cu and Ni, followed by moderate negative correlation (0.3 to 0.6) with Ba and a weak negative correlation (0.1 to 0.3) with Fe and Mn. Amaranth samples showed similar behavior, with predominance of negative correlations, with B, Cu, Mg, Mn, and V strong negative correlations and with Zn, a moderate negative correlation between polyphenol content in the samples and the bioaccessible fractions of the elements. For quinoa, a higher amount of polyphenols indicated a low bioaccessibility for B, Cu, Fe and V, which presented a strong negative correlation, followed by Ba, K and Zn, which showed a moderate negative correlation.

Table 7. Pearson's correlation coefficient[a] (r) for the percentage of bioaccessible fractions of B, Ba, Cu, Fe, K, Mg, Mn, Ni, V and Zn *versus* total concentration (TC) and total polyphenols (TPs) in amaranth, quinoa, and chia samples

Analyte	Amaranth		Quinoa		Chia	
	TC	TPs	TC	TPs	TC	TPs
B	+0.95	–0.99	–0.73	–0.98	ND	ND
Ba	–0.99	+0.20	–0.17	–0.58	+0.79	–0.53
Cu	+0.93	–0.60	+0.93	–0.64	+0.85	–0.98
Fe	–0.68	+0.74	+0.99	–0.76	–0.31	–0.16
K	+0.95	+0.46	+0.99	–0.40	+0.93	+0.29
Mg	+0.97	–0.94	+0.88	+0.13	+0.97	+0.88
Mn	+0.99	–0.99	–0.81	+0.09	+0.98	–0.28
Ni	ND	ND	+0.97	+0.41	+0.99	–0.99
V	+0.93	–0.92	+0.73	–0.96	+0.67	+0.11
Zn	+0.14	–0.33	–0.91	–0.57	ND	ND

[a]Significance level > 5% in the established correlations; ND: bioaccessible fraction not detected.

Pearson's correlation also showed that a high total concentration of Fe results in a low bioaccessibility of this element in the amaranth and chia samples, which showed a strong and moderate negative correlation, respectively. Amaranth and quinoa showed a strong and weak negative correlation for Ba. Only in quinoa there was a strong negative correlation for B, Mn, and Zn. This negative correlation between the total concentration and the bioaccessible fraction observed for some elements may be a question of ionic balance that occurs during gastrointestinal digestion, since the greater the amount of ions in the sample, the less soluble they may become.

Contribution of bioaccessible elements to the human diet

One way to assess the contribution of elements to our diet is to compare the discovered bioaccessible concentrations with the recommended daily intake (RDI) values of minerals, which correspond to the amounts that need to be consumed daily to maintain the proper functioning of the human body.[50]

Table 8 shows an estimated daily intake for Cu, Fe, K, Mg, Mn, and Zn, considering the RDI for adults, which was calculated considering the intake of 20 g of cereals *per* day, an amount recommended by the World Health Organization (WHO).[51] The contribution levels were obtained based on the bioaccessible fraction of each element in each sample. However, it is necessary to consume other foods combined with amaranth, quinoa, and chia throughout the day to aid in supplementation, since the bioaccessible fraction in the food is lower than the total concentration, which did not correspond to 100% contribution to the RDI of these minerals, showing the relevance of evaluating the bioaccessible fraction of foods. For B, Ni and V, there is no RDI, but the estimate of tolerable intake for adults is 20, 1 and 1.8 mg *per* day, respectively.[52] The highest values found for these elements considering the three samples investigated were: B (0.04 mg *per* 20 g quinoa); Ni (0.03 mg *per* 20 g chia) and V (0.14 mg *per* 20 g amaranth).

The element Al presented a quantity released during gastrointestinal digestion below the limit of detection, so it should not represent any risk associated with its consumption in these grains. To assess the risks related to Ba consumption, a long-term mean dietary barium intake for adults has been found to be 0.75 mg *per* day (range 0.44-1.8 mg Ba *per* day) was considered.[53] The maximum value obtained in this study was 0.054 mg *per* 20 g found in chia, and, even with a daily intake, the concentration does not exceed the limit.

Conclusions

Identifying the elemental composition of the foods we are consuming is extremely important and the method used in this study proved to be adequate for quantifying the total concentrations of analytes such as Al, B, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, Ni, V and Zn in grains such as amaranth, quinoa and chia, using the MIP OES technique. The efficiency of the decompositions was ensured by the low levels of dissolved carbon obtained in the samples and by presenting good accuracy and precision, which were proven by the analysis of certified materials, analyte addition tests and by the RSD values obtained.

Bioaccessibility varied according to the type of pseudocereal and the way in which each element can be bonded to the organic compounds of the grain, influences its release in the gastrointestinal tract. Polyphenols had a negative influence on the bioaccessibility of most elements in all samples investigated.

The results obtained provide additional data on the content of essential elements for a healthy diet, such as the actual amounts of some elements that are bioaccessible to humans, indicating their nutritional value. Among these elements, K and Cu presented the highest bioaccessible fractions, while Zn presented the lowest bioaccessible fractions and was below the limit of detection in chia samples. Finally, considering the bioaccessible values found for the essential elements and the RDI, Mg was the

Table 8. Contribution to the RDI of Cu, Fe, K, Mg, Mn, and Zn, considering the bioaccessible fraction in amaranth, chia, and quinoa samples. The results are expressed in a range of percentage of contribution

Analyte	RDI[a] / (mg *per* day)	Amaranth / %	Quinoa / %	Chia / %
Cu	0.9	4.4-11.1	0.7-7.3	3.1-12.6
Fe	14	2.1-2.8	1.6-2.9	0.2-2.0
K	4700	1.8-2.1	2.3-3.4	1.6-2.1
Mg	260	8.2-12.3	9.7-10.9	15.3-19.2
Mn	2.3	3.0-5.8	5.4-7.0	3.7-8.2
Zn	7	0.6-0.9	0.3-0.9	ND

[a]RDI: Recommended Daily Intake.[50] ND: not detected.

element that presented the greatest contribution, reaching a maximum value of 19.2%. This fact makes evident the need for additional consumption of other sources of nutrients to meet the daily requirements of minerals. The potentially toxic elements had low bioaccessible concentrations, showing no risk to the consumer's health when ingesting the grains analyzed in this study.

Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br as PDF file.

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Author Contributions

Caroline D. Clasen was responsible for conceptualization, investigation, methodology, validation, formal analysis, investigation, writing original draft; Sandy A. Silva for investigation, formal analysis, writing original draft; Kaiane Q. Ribeiro for investigation, formal analysis, writing original draft; Anderson S. Ribeiro for validation, resources, writing-review and editing, visualization; Mariana A. Vieira for conceptualization, validation, resources, writing - review and editing, visualization, supervision, funding acquisition. All authors reviewed the manuscript.

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