Chapter 7

Sample Preparation for Determination of Bioaccessibility of Essential and Toxic Elements in Legumes

Wagna Piler Carvalho dos Santos and
Rita Maria Weste Nano

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.69850

Abstract

The methods used to estimate the bioavailability of elements have different approaches. These tests are based on selective extraction or simulation of the physiology of the gastrointestinal tract. The sample preparation methods require studies about extraction procedures, thermal treatment, and decomposition of organic matter. The method of decomposing organic matter assisted by microwaves introduced adequate results for most chemical elements in pulses. The content of the elements present in the extracts obtained by employing the method physiologically based extraction test (PBET) is lower than those obtained by simple bioaccessibility extraction test (SBET) due to complexing effects of metal ions. The mineral content in the gastric and intestinal stages can vary significantly with the investigated leguminous species and the elements. The thermal processing can affect the concentrations of the elements analyzed in samples from leguminous species. This results from the heat capacity to change the speciation of chemical elements. The change speciation may modify the solubility and mobility of chemical species under the conditions of the gastrointestinal tract, which alters the bioavailability. In this sense, it can be concluded that the domestic cooking process can influence the nutritional and toxicological potential of pigeon pea, cowpea, and mangalo.

Keywords: legumes, bioaccessibility, sample preparation, ICP OES, minerals

1. Introduction

Grain legumes represent an important food group due to the related nutritional and socioeconomic aspects, especially for Brazil, which occupies the position of major producer and

consumer of common bean (*Phaseolus vulgaris* L.). Its nutritional importance is due to the presence of important nutrients, such as proteins, vitamins, and minerals. Among these, we highlight the essential minerals present in larger quantities known as macroelements (Ca, K, Mg, P) and trace elements (Co, Cr, Cu, Fe, Mn, and Mo). Knowledge of the total concentration of these elements does not provide sufficient nutritional information for the elucidation of absorption mechanisms as essential nutrients. On the other hand, toxic elements, such as Pb and Cd, may also be present, being considered contaminants. Thus, studies on the bioavailability of nutrients and contaminants are needed to ensure food safety.

Bioavailability and chemical speciation are multidisciplinary areas, which have gained space in the scientific community in recent years. Research into the interactions of various chemical forms, the presence of antinutritional agents, and their absorption into living organisms involves many scientific fields [1–3]. The bioaccessibility and bioavailability of different species of essential or toxic elements are also important because the essentiality depends on the chemical form of the element that is absorbed as well as its toxic potential [1].

The methods employed to determine the bioavailability of a chemical species in the human body are generally very laborious and provide results that are discordant to each other or are not comparable. Therefore, the development and standardization of analytical processes are activities of interest, since they can contribute with advances in the understanding of natural processes related to the environment and nutrition.

The legumes belong to the species *P. vulgaris* L., the botanical family Leguminosae. Intake of legumes (e.g., common beans, lentils, peas, cowpeas) in the Brazilian diet should be encouraged. However, the total amount of a nutrient does not reflect the amount available to the body through absorption. The accessibility of a chemical species to normal metabolic and physiological processes is known as bioavailability. For the assessment of bioaccessibility, the composition of the food should be considered. The legumes have several antinutritional factors that negatively interfere in the bioavailability of elements [2]. The phytic acid (and phytates) is known as a food inhibitor which chelates micronutrient and prevents it to be bioavailable for monogastric animals, including humans, because they lack enzyme phytase in their digestive tract [3].

The bioavailability can be divided into three phases: availability in the intestinal lumen by absorption, adsorption and/or retention in the body, and use by the body. Several factors may influence the bioavailability of minerals, which may be of dietary or physiological origin [4, 5]. The bioavailability of a chemical species can be estimated by means of the percentage of bioaccessibility of this species. Bioaccessibility assays performed using in vitro methods are the focus of this work.

The oral bioaccessibility of a substance can be defined as the fraction soluble in the conditions of the gastrointestinal tract and that is available for absorption [6, 7]. However, some nutrients do not need to be digested to be absorbed and others, even hydrolysates, cannot be absorbed. Iron may be strongly bound in the absorbed chelate structure, with no release of the metal ion to the cells and incorporation by the proteins [8].

http://dx.doi.org/10.5772/intechopen.69850

Many factors and promoters act on the bioavailability of trace elements, such as chemical form of the mineral in the food, food binders, redox activity in food components, interactions between the minerals, and the individual's physiological state [2, 6]. Therefore, the concept of bioavailability of micronutrients should recognize all important factors, as well as the rates of use of the absorbed nutrient and the rates of exchange and excretion, which can vary considerably, due to (i) intrinsic factors, that is, mechanisms of absorption and metabolic processes and mutual interactions, and (ii) extrinsic, such as solubility, size of molecules, and synergistic or antagonistic chemical effects [6].

Thus, in 1997, at the International Bioavailability Conference, the term bioavailability began to refer to the fraction of any ingested nutrient with the potential to meet physiological demands on target tissues. In 2001, the concept incorporated three aspects: bioconversion, bioefficacy, and bioefficiency [2].

The methods employed to estimate the bioavailability of elements rely on different approaches [9]. In vivo tests make use of guinea pigs (rabbits, rats, pigs, and monkeys). The bioaccessible fraction of the nutrients is determined by the analysis of the animal's nails, hair, and blood after administration of the diet of interest. These tests require specialized professionals and specific infrastructure for their realization, besides having execution times and high costs [10, 11]. In vitro tests may be attractive due to higher analytical speed and lower cost. These tests are based on selective extraction or simulate the physiology of the gastrointestinal tract and can be classified into two categories, static and dynamic, and do not include the microorganisms present in the digestive tract, nor do they consider the adsorption mechanisms that preferentially occur in the duodenal epithelium [10].

The in vitro bioaccessibility of minerals varies significantly, depending on the mineral and the type of the food matrix [3]. On the other hand, it may also change with variations in the sample preparation step. The effects of heat treatment on the bioavailability of some minerals in food matrices were investigated. Different species of legumes consumed in India were studied (*Cicer arietinum*, *Phaseolus aureus*, *Phaseolus mungo*, *Cajanus cajan*, *Vigna catjang*, *P. vulgaris*). Most of the works use gastric simulation with simple extraction tests. In addition, few studies have done any kind of comparison of the results using the in vivo method as a reference [11–15].

The possible explanations for this fact are high cost of the in vivo method, high time of analysis, complexity of the tests, and ethical implications [14]. These aspects make it difficult to perform the experiments, making in vitro tests more attractive, which are based on the selective extraction or simulate the physiology of the gastrointestinal tract.

Due to the limitations of the in vivo assays to estimate the bioavailability of metals, since the 1990s it is recommended to replace them by in vitro methods, requiring, for such development and validation. The in vitro approach enables the faster and more accessible generation of information that allows human health risk assessments related to exposure to a specific toxic agent [7].

Many in vitro methods are employed to estimate the bioaccessibility of certain chemical species. A brief comparison of examples of these methods was presented, in which four are framed as static methods and only one as dynamic [10, 11]. The simplest of these is the simple bioaccessibility extraction test (SBET) method proposed by the Consortium for Research on Solubility and Bioavailability (SBRC). Recognized research groups updating in the area are evaluating the measurement of methods for assessing bioaccessibility, such as the Scientific Group on Methods for a Chemical Safety Assessment (SGOMSEC) was established in 1979 [13]. Also more recently the in vitro method recommended by the European Unified Research Group (BARGE) is used to estimate the bioaccessibility of trace elements [14].

The SBET method was developed based on the work initially described by Ruby et al. and simulates the mobilization of the substances in the gastric conditions of the stomach, disregarding the intestinal compartment [6]. Since it only simulates the gastric phase, it generally provides overestimated bioaccessibility results, due to the low pH of the medium and the absence of an intestinal phase. The development, validation, and standardization of these methods are areas that still demand studies [15].

In vitro laboratory tests to predict the bioavailability of metals from a solid matrix that simulate the physicochemical conditions of solutions found in the stomach and in the human duodenum are called bioaccessibility tests and can be known as physiologically based extraction test (PBET) [16]. These tests do not include the microorganisms present in the digestive tract, nor do they consider the adsorption mechanisms that occur preferentially in the duodenal epithelium [10]. The oral bioaccessibility of a substance was then defined as the fraction that is soluble in gastrointestinal tract conditions and is available for absorption [6, 7]. Bioaccessibility values become very useful in the nutritional analysis of foods when it is considered that any soluble nutrient is susceptible to absorption in the human intestine. Leguminosae (chickpeas, lentils, cowpeas, and green peas) are sources of essential elements, particularly K, P, Ca, Cu, Fe, and Zn [17]. Generally, they provide sufficient amounts of Fe, Ca, and P required in a human diet. However, it is necessary to mention the possible presence of inorganic contaminants in plants, which, although generally inferior to foods of animal origin, should also be investigated, since it poses an imminent risk to health maintenance [18]. In recent work, concentrations of Pb in samples of carioca beans varied between 4.6 and 6.2 $\mu g\ g^{-1}$ [19]. These concentrations are above the maximum tolerance limit (LMT) recommended by the Brazilian legislation for legumes in natura or industrialized (0.50 $\mu g\ g^{-1}$) [20]. According to the Codex Alimentarius, in the 2006 review, these levels were set at 0.2 $\mu g\ g^{-1}$ for Pb in legumes in natura and 1.0 $\mu g\ g^{-1}$ for legumes (processed green peas and beans and peas) [21].

2. Methods and materials

2.1. Collection of samples

The choice of samples (grain legumes) was based on being foods consumed in the Northeast, especially species such as cowpea (*Vigna unguiculata* L. Walp), pigeon pea (*C. cajan* L.), and mangalo (*Lablab purpureus* L. Sweet). Samples of cowpea and pigeon pea were obtained in

http://dx.doi.org/10.5772/intechopen.69850

the municipality of Ipirá, a micro-region of Feira de Santana, Bahia, Brazil. Mangalo samples were obtained in the city of Santo Amaro, metropolitan region of Salvador, Bahia, Brazil.

2.2. Reagents and standard solutions

The concentrations of the working solutions of the trace elements Ba, Ca, Cd, Cr, Cu, Fe, K, Mn, Mo, Mg, P, Pb, and Zn (Merck, Germany) were prepared from stock solutions containing 1000 mg L^{-1}. All solutions were prepared with analytical grade reagents and ultrapure water, specific resistivity of 18.2 MΩ cm^{-1} in purification system Milli-Q® (Millipore, Bedford, MA, USA).

As certified reference materials of grain legumes as well as compatible materials for analysis of bioaccessibility of minerals in food matrices are not available on the market, certified reference materials from National Institute of Standards and Technology (NIST, Gaithersburg, Maryland, USA): 1515 apple leaf and 1570a spinach. These materials were used to evaluate the accuracy of the analytical procedure employed for the determination of the total content of the analytes.

The following reagents were used: hydrochloric acid (Carlo Erba, Italy), nitric acid (Merck, Germany), hydrogen peroxide 30% m/v (Synth, São Paulo, Brazil), and glycine (Vetec, Rio de Janeiro, Brazil). Standard buffer solutions pH 7.00 ± 0.05 and pH 4.00 ± 0.02 (Haloquímica, São Paulo) were used for the calibration of the pH meter. All materials used in the collection, storage, and preparation of the samples were previously washed with detergent and decontaminated with nitric acid (10% v/v) for a minimum period of 24 h and rinsed with ultrapure water, with specific resistivity of 18.2 MΩ cm^{-1}.

2.3. Instrumentation

To perform the thermal treatment of the samples, the following equipment was used: drying oven with Fanem forced circulation, model 520, and oven, Panasonic brand, with an output/consumption power of 900 W/1450 W, a frequency of 2450 MHz, and power output/consumption (resistance) of 950 W/1010 W. For cooking in a pressurized system, Teflon™ coated aluminum pan, whose heat source for cooking the food was a Bunsen nozzle, was used.

For the drying of the samples, a lyophilizer Terroni Fauvel LT 1000/8 (São Carlos, São Paulo) was used. The dried samples were ground in an 8000 M ball mill (Spex Sample Prep, USA). The Tecnal (São Paulo) digester block and ETHOS One microwave (Milestone, Italy) were used for the acid decomposition procedure of the samples.

In the bioaccessibility assays, the vials were incubated in a Tecnal incubator, model TE-420, at 37°C and shaking at 100 rpm.

The optical emission spectrometer with inductively coupled argon plasma (ICP OES) with VISTA PRO axial vision (Varian, Mulgrave, Australia) was used to determine the analytes. This instrument is equipped with a solid-state detector with CCD array (charge-coupled device) and operates at wavelengths in the range of 167–785 nm. It has an end-on gas interface, which with the front flow countercurrent gas protects the pre-optical region from overheating and

removes the colder zone from the plasma. The spectral lines were selected considering the intensities of the emission signals of the analytes and the background signal, the standard deviation of the measurements, the adequate sensitivity for the determination of the elements present in high and low concentrations in the matrices, as well as the profile of the spectra and the possibility of interference.

2.4. Preparation of the samples

Pretreatment of samples consisted of selection, washing, freeze-drying for 48 h, milling for 2 min, sieving (nylon mesh, <300 μm), packaging, and storage. The green, fresh, and moist grains of the legumes were washed, selected, drained, packed in polypropylene bottles, and preserved under freezing at −30°C.

The bioaccessibility of the minerals in the legumes was evaluated in the cooking process, for which the samples were heat treated in three types of heating and two time levels: (a) under pressure at 15 psi (3 and 6 min), (b) in the oven (20 and 40 min), and (c) in a microwave oven (6 and 12 min). During cooking, the conditions were determined: sample mass/volume ratio (100 g sample/300 mL water), oven temperature 2000°C, and 100% microwave power. For oven and microwave oven processes, the cooking of the samples was performed in beakers of 1000 mL. After the cooking time set for each experiment, the grains were drained in a plastic sieve. This stage simulates the domestic procedure that is usually performed in the preparation of these foods. For comparison of the results, samples that were not heat treated were also analyzed. For each species three sub-samples were produced. After cooking the samples were dried in an oven with air circulation at 60°C and ground in a ball mill. The dried and ground samples were stored in decontaminated plastic bottles and kept at refrigeration temperature (<10°C).

2.5. Sample acid decomposition procedure

For the digestion block acid decomposition procedure, 500 mg of the previously dried and ground samples was weighed directly into digestion tubes, and 5.0 mL of 65% w/w nitric acid was added. The digestion was started with a gradual increase in temperature, starting at 50°C, rising to 100°C, and ending at 150°C, maintaining this temperature for 30 min. At the time the temperature reached 150°C, hydrogen peroxide (H_2O_2) was slowly added in 1 mL portions. At the end of the 15 min, 10 mL peroxide was added to each tube. To promote the condensation and reflux of gases and vapors generated in the digestion, minimizing contamination and loss of the volatile chemical elements, cold fingers were adapted to the cold digestion flasks [22]. At the end of the digestion, due to the presence of particulate, the solution obtained was filtered on medium filtration filter paper into 25 mL volumetric flasks. The volume of the flask was filled with ultrapure water, with resistivity of 18.2 MΩ cm^{-1}.

A microwave-assisted decomposition procedure was also employed. In the procedure, 9.0 mL of nitric acid, 4.0 mol L^{-1}, 1.0 mL of hydrogen peroxide 30% v/v, and 500 mg of the sample were used. The heating program consisted of four steps: (1) ramp of 6 min and temperature of 90°C, (2) 5 min at 90°C, (3) ramp of 10 min and temperature of 190°C, and (4) 10 min at 190°C. The volume of the digested mixture was adjusted to 15.0 mL with ultrapure water. The solutions were stored in polypropylene bottles at refrigeration temperature.

http://dx.doi.org/10.5772/intechopen.69850

2.6. Procedure for bioaccessibility testing

2.6.1. Simple bioaccessibility extraction test (SBET)

The bioaccessibility assays were conducted by the SBET method with mangalo pulse samples. For this assay, a 0.4 mol L-1 glycine solution in acid medium (HCl, pH = 1.5) was used to simulate gastric digestion. To this, 0.25 g of sample was incubated with the prepared solution; the temperature was adjusted to 37°C and a rotation speed to 100 rpm for 1 h. Subsequently, the mixture was vacuum filtered on filter membranes of cellulose acetate having porosity of 0.45 μm (Millipore). The extracts were stored in plastic vials under refrigeration temperature for a maximum time of 24 h.

The use of these membranes increases the time of analysis and may increase the risk of contamination. In this way, the influence of the separation of the solid phase (legume sample) from the solution (bioaccessible fraction), obtained after sample incubation stage, was evaluated. To this end, the membrane filtration step was replaced by centrifugation at 3000 rpm for 15 min. Of the sample-solution mixture, the bioaccessibility of Cu, Mn, and Zn was determined. Samples from the three species were used for this evaluation.

2.6.2. Physiologically based extraction test (PBET)

To estimate the analytes' bioavailability, the PBET method was used, which involves the simulation of gastric digestion conditions, followed by the simulation of intestinal digestion conditions [23, 24]. For this, the sample was incubated at 37°C for 1 h with pepsin at pH 2.5 (simulated gastric digestion) and then at pH 7.0 with pancreatin and bile extract (simulating intestinal digestion). Each batch consisted on average of four samples selected randomly, in triplicate of gastric and intestinal digestion, plus three gastric digestion blank samples and three intestinal digestion blank samples, totaling 30 beakers. Each beaker (except the blank samples) contained 0.300 g of the sample and 30.0 mL of gastric solution prepared on the day. Gastric solution was prepared with 1.25 g pepsin, 500 mg malate, 500 mg of citrate, 500 uL of acetic acid, and 420 uL of lactic acid diluted in 1.0 L of ultrapure water, and the pH was adjusted to 2.5 with hydrochloric acid solution. The mixture was incubated at 37°C under orbital shaking at 100 rpm for 1 h. The pH of the mixture was adjusted to 7.0 with $NaHCO_3$ solution. To the intestinal phase, 15 mg of pancreatin and 52.5 mg of bile salts were added, and this mixture was incubated for 4 h under the same conditions of the gastric phase.

2.7. Determination of analytes

The concentration of each analyte in the digests after acid decomposition of the samples and in the extracts of the bioaccessibility assays was obtained using inductively coupled plasma optical emission spectrometry (ICP OES). The optical system of the ICP OES was calibrated with a multielement reference solution, and torch alignment was performed with a 5.0 mg Mn/L solution. The spectral lines were selected considering the intensities of the emission signals of the analytes and the background signal, the standard deviation of the measurements, the adequate sensitivity for the determination of the elements present in high and low concentrations in the matrices, as well as the profile of the spectra and the possibility of interference.

3. Results and discussions

3.1. Determination of mineral contents

Initially, the accuracy of the procedure used to determine the total analyte content was checked using CRM 1515 and 1570a. The average extraction efficiency and RSD of K, P, Ba, Cu, Mn, and Zn were, respectively, 93% and less than 5%. The lowest extraction efficiency was due to Fe in CRM 1515 (83%).

The traces of Ba, Cu, Fe, Mn, and Zn were determined in the cowpea, pigeon pea, and mangalo samples after acidic decomposition. The samples did not undergo any heat treatment except drying the grains in a greenhouse with 60°C air circulation and milling. The concentrations in mg kg^{-1} and interval at the 95% confidence level of the metals were Ba 8.9 ± 0.1, Cu 8.90 ± 0.1, Fe 37.1 ± 0.4, Mn 16.7 ± 0.2, and Zn 30.6 ± 0.4 for cowpea; Ba 0.07 ± 0.01, Cu 3.94 ± 0.01, Fe 50.2 ± 0.06, Mn 23.6 ± 0.04, and Zn 47.3 ± 0.7 for Pigeon pea; and Ba 1.50 ± 0.01, Cu 11.4 ± 0.01, Fe 82.9 ± 0.1, Mn 28.9 ± 0.01, and Zn 43.5 ± 0.2 for mangalo.

The contents of the major elements Ca, K, Mg, and P were determined in the samples. The concentrations in mg kg^{-1} and interval at the 95% confidence level of the metals were Ca 817 ± 8, K 137,914 ± 792, Mg 1155 ± 9, and P 2086 ± 27 for cowpea; Ca 829 ± 3, K 137,914 ± 68, Mg 1785 ± 2, and P 5149 ± 4 for pigeon pea; and Ca 356 ± 1, K 183,923 ± 150, Mg 1975 ± 1e, and P 5939 ± 7 for mangalo.

3.2. Step of separation of the in vitro method

The ratio between the content of the analyte present in the sample and the filtrate corresponds to the bioaccessibility of the minerals. This ratio is then multiplied by a factor of 100, and the result is expressed as a percentage of bioaccessibility, as shown in Eq. (1):

$$\%B = \frac{Y}{Z} \times 100 \quad (1)$$

where Y is the content of the element in the bioaccessible fraction and Z is the total content of the element [12].

The bioaccessibility of a chemical species can be defined as the fraction soluble in the conditions of the gastrointestinal tract and that is available for absorption. In in vitro methods, as in the SBET method, 0.45 μm membrane filters are used. The filtration stage aims to separate the fraction dissolved in the gastric simulation of the solid phase.

The two separation procedures were compared: (a) vacuum filtration in cellulose acetate membrane of 0.45 μm porosity and (b) centrifugation at 3000 rpm for 15 min. Analytes were determined using ICP OES.

The bioaccessibility results of the solid phase separation tests using membrane filtration and centrifugation of three bean species showed the following intervals in membrane filtration: Cu 93–111%, Ba 0–41%, Fe 40–58%, Mn 97–116%, Zn 105–106%, Ca 97–121%, K 95–97%, Mg

http://dx.doi.org/10.5772/intechopen.69850

101–103%, and P 62–75%. The results for solid phase separation using centrifugation were Cu 94–113%, Ba 0–41%, Fe 39–52%, Mn 107–111%, Zn 105–109%, Ca 98–107%, K 98–100%, Mg 102–108%, and P 64–76%.

The results were analyzed by applying a paired *t*-test at a 95% confidence level. The comparison between the variances of the methods was analyzed using an *F* test. No significant difference ($p > 0.05$) was observed between the means ($n = 3$) of the percentages of bioaccessibility of Cu and Zn, obtained with the procedure employing centrifugation, when compared to the filtration procedure.

3.3. Bioaccessibility: gastric and intestinal phases

In vitro methods that simulate only the gastric phase give limited information about the potential bioavailability of the nutrient or toxic species. The PBET in vitro method simulates gastric conditions including the use of enzymes from this compartment as well as simulates the intestinal compartment promoting alkaline pH change and biliary enzyme addition. For the determination of the bioaccessibility of the minerals, the PBET method was used for the cowpea, pigeon pea, and mangalo samples. The results presented in **Table 1** refer to the percentages of bioaccessibility of Ca, Cu, Fe, K, Mg, and Zn in samples previously dried in an oven with 60°C air circulation and ground.

It is observed that potassium and iron presented the lowest percentage of bioaccessibility considering the three legumes species investigated. It is observed that iron presented the lowest bioaccessibility in the pigeon pea sample. Also, this legume presented the lowest bioaccessibility of potassium when compared to mangalo and cowpea. Mangalo had the lowest bioaccessibility for calcium (39.0%) and the opposite behavior for magnesium (92.3%). Cowpea also presented high bioaccessibility of Mg (97.0). The percentages of bioaccessibility for the Cu and Zn elements varied in a relatively narrow range, comparing with the other elements and the three legumes.

Concentrations of Cu, Fe and Zn, Ca, K, and Mg were also determined in the extracts of the gastric phase and intestinal phase for comparison purposes. The percentage of bioacessible Fe in the intestinal phase varied, respectively: cowpea, 0% at 27 ± 4%; mangalo 0% at 1.8 ± 0.7%;

Elements	% of bioaccessibility		
	Cowpea	Pigeon pea	Mangalo
Ca	79.4	70.3	39.7
K	17.0	7.1	13.0
Mg	97.0	66.1	92.3
Cu	57.8	49.9	62.3
Fe	24.8	3.1	39.9
Zn	43.7	39.3	52.4

Table 1. Mean bioaccessibility of trace elements and higher in legume samples using the PBET method.

and pigeon pea, 14 ± 2% at 83 ± 3%. And the percentages for Cu, 0% at 119 ± 14% (cowpea), 0% at 35 ± 9% (mangalo), and 0% at 76 ± 3% (pigeon pea).

However, the bioavailability of Zn was 52 ± 7% and 36 ± 1% (mangalo) and 74 ± 3% and 70 ± 1% (pigeon pea) for the gastric and intestinal phases, respectively. The bioaccessibility of Mg, for all species, was higher in the intestinal phase.

Among the trace elements, it was observed that copper presented greater bioavailability (100%) in the intestinal phase, accompanied by iron and zinc, which also possessed higher bioavailability in the intestinal phase. This increased bioavailability may be explained according to the level of protein and carbohydrate aggregation in the in the gastric and intestinal phases. Protein degradation begins in the stomach, but it is only complete with the enzymes present in the intestine, whereas carbohydrate degradation begins in the mouth with the salivary amylase, is interrupted in the stomach, and continues again in the intestine, in the form of amino acids and glucose. If there was any fraction of "bound" metal in a protein or carbohydrate, it would now be "released" due to the breakdown of protein or carbohydrate.

For Ca, the gastric and intestinal phases were 58 ± 4% and 98 ± 4% (cowpea) and 89 ± 4% and 56 ± 3% (pigeon pea). For the mangalo sample, the bioaccessibility of Ca did not vary between phases (42 ± 2). The percentages of K in the gaseous phase were higher for pigeon pea (11.9 ± 0.4) and mangrove (8.2 ± 0.2).

Considering the extractions of the major elements, it was observed that it is in the intestinal phase that most of the absorption takes place. These elements, for the most part, are associated with carbohydrates and proteins, so the higher the level of breakage of these molecules, the more macroelements will be bioavailable.

3.4. Comparison of SBET and PBET methods

We compared the results between the SBET method and the PBET method, and it was observed that, among the trace elements, the higher extraction occurs in the SBET because the gastric medium is more acidic and it is easier to have these ions in solution. In this method, the gastric compartment was simulated with an extractor liquid containing only 0.4 mol L^{-1} glycine solution at pH 1.5 adjusted with concentrated hydrochloric acid. In the PBET method, pepsin, malate and citrate are used.

The chemical changes necessary for the digestive process are achieved with the aid of digestive tract enzymes. These enzymes catalyze the hydrolysis of native proteins into amino acids, from starches to monosaccharides and from fats to glycerol and fatty acids. During these digestive reactions, minerals and fat-soluble vitamins in food may become more available for metabolic functions [7].

Therefore, it is expected that the trace element contents present in the extracts obtained using the PBET method will be lower than those obtained by the SBET due to the complexing effects of the metal ions. Among copper, iron, and zinc, it was observed that, in iron, the highest percentage of extraction was mostly (100%) in the SBET, in the copper 50% of the samples

http://dx.doi.org/10.5772/intechopen.69850

extracted more in the SBET, and 50% in the PBET and the zinc was the only one that most of the samples had higher extraction percentages in PBET.

As expected, there was a higher percentage of extraction in the PBET when compared to the trace elements; however, the potassium had the highest extraction percentages (100%) in the SBET. Calcium showed high percentages also in the SBET, the opposite behavior observed for the manganese that presented higher percentages of extraction by the PBET method.

3.5. Cooking effect

Initially, comparing the analyte contents of the cooked samples with respect to the uncooked samples, significant differences were observed at the 95% confidence level in the concentrations of nickel, molybdenum, and barium. The levels of Ni, Se, Mo, Sn, and Ba differed significantly ($p < 0.05$) for analyte concentrations for the three legume species investigated.

With the analysis of the results, it is possible to observe that there is variation of the concentrations according to the fact that the samples are not processed thermally and according to the duration of the cooking. This fact was observed because the heat treatment can influence in the form, that is, in the speciation with which the chemical species presents itself in the food. From this influence the thermal processing can alter the mobility and the solubility of the elements in the conditions of the gastrointestinal tract, thus interfering in the bioaccessibility.

The alteration of speciation in the cooking process occurs because this process is capable of causing separation effects, fractionation of minerals, destruction of inhibitors, formation of complexes with metal ions, denaturation of enzymes that degrade inhibitors, or generation of compounds insoluble by oxidation and precipitation [25]. This ability to change the speciation of the elements also warrants observations on changes in analyte concentrations as the duration of the heating has been greater or less. With the rise of the heating period, the greater the power supply, therefore the more prone is the speciation change. In the majority of sample, it was verified that the longer the thermal processing time, the higher the analyte concentrations in the legume samples.

To evaluate the effect of cooking on the bioaccessibility of minerals, tests were performed using the SBET method. The statistical evaluation of the effect of the thermal treatment on the bioaccessibility of the minerals was performed by paired *t*-test, in which the following results were obtained: at the 95% confidence level.

Using oven cooking, the cooking time significantly interferes, at 95% confidence, in the bioaccessible percentage of Cu. However, the bioavailability of Zn and Fe is not significantly affected by changing the cooking time in the oven. When using the microwave oven for cooking, the bioaccessible percentage of Fe, Cu, and Zn does not differ significantly, at 95% confidence, with different cooking times being achieved. On the other hand, in the cooking under pressure, the bioavailability of Cu differs significantly, at 95% confidence, using different cooking times, since the bioavailability of Zn and Fe does not differ significantly for different cooking times.

Comparing the bioaccessibility percentages obtained in cooked and raw samples, there was a significant difference for Ba, Cu, Fe, K, P, and Zn. Higher percentages of bioaccessibility were obtained for Fe, K, and P in the extract of the unprocessed samples. However, opposite behavior was observed for Cu, i.e., thermal processing favored the availability of this element. However, the behavior of Ba and Zn differed from the others, since for the samples processed in microwave oven and under pressure, the bioaccessibility was lower for Ba and higher for Zn, when compared to the unprocessed samples. The species variable was also significant for these elements.

4. Conclusions

The study carried out between the SBET extraction procedure showed that centrifugation presented comparable results to the conventional method, which employs filtration. Thus, the proposed method for centrifugation is a safe, faster, and lower cost alternative for a phase separation of the SBET method.

Comparison of the traceability and major percentage bioaccessibility percentages using the SBET and PBET methods in the samples of the three legume species confirmed that smaller amounts of minerals are extracted when using the method that simulates gastrointestinal conditions when compared to the method that simulates only the condition of the stomach compartment. This leads in fact, in some cases, to overestimations of bioaccessibility. However, this behavior cannot be generalized, since it can vary from element to element and between food matrices.

The results suggest that the thermal processes investigated can influence the bioaccessibility of the macroelements (Ca, Mg, K, and P) and trace elements (Ba, Cu, Fe, and Zn) in the bioaccessibility of the minerals, i.e., thermal processing may result in increase or decrease in the bioaccessibility of the element.

Acknowledgements

The authors are grateful to the Fundação de Amparo a Pesquisa do Estado da Bahia (FAPESB), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and the Instituto Federal da bahia, for the grants and for their financial support.

Author details

Wagna Piler Carvalho dos Santos* and Rita Maria Weste Nano

*Address all correspondence to: wagna.ifba@gmail.com

Instituto Federal da Bahia (IFBA), Salvador, Brazil

References

[1] Moreda-Piñeiroa J, Moreda-Piñeirob A, Romarís-Hortasb V, Moscoso-Péreza C, López-Mahíaa P, Muniategui-Lorenzoa S, Bermejo-Barrerab P, Prada-Rodrígueza D. In-vivo and in-vitro testing to assess the bioaccessibility and the bioavailability of arsenic, selenium and mercury species in food samples. Trends in Analytical Chemistry. 2011;**30**(2):324-345. DOI: 10.1016/j.trac.2010.09.008

[2] Cozzolino SMF. Biodisponibilidade de nutrientes. 3rd ed. São Paulo: Manole; 2009. DOI: 10.1590/S1516-93322005000100017

[3] Gupta RK, Gangoliya SS, Singh NK. Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains. Journal of Food Science and Technology. 2015;**52**(2):676-684. DOI: 10.1007/s13197-013-0978-y

[4] House WA. Trace element bioavailability as exemplified by iron and zinc. Field Crops Research. 1999;**60**:115-141. DOI: 10.1016/S0378-4290(98)00136-1

[5] Peakall D, Burges J. Methodologies for assessing to metals: Speciation, bioavailability of metals, and ecological host factors. Ecotoxicology and Environmental Safety. 2003;**56**:110-121. DOI: 10.1016/S0147-6513(03)00055-1

[6] Ruby MV, Davis A, Kempton JH, Drexler JW, Bergstrom PD. Lead bioavailability: Dissolution kinetics under simulated gastric conditions. Environmental Science & Technology. 1992;**26**:1242-1248. DOI: 10.1021/es50002a614

[7] Ruby MV, Schoof R, Brattin W, Goldade M, Post G, Harnois M, Mosby DE, Casteel SW, Berti W, Carpenter M, Edwards D, Cragin D, Chappell DW. Advances in evaluating the oral bioavailability of inorganics in soil for use in human health risk assessment. Environmental Science & Technology. 1999;**33**:3697-3705. DOI: 10.1021/es990479z

[8] Fennema OR, Parkin K, Damodaran S. Food Chemistry. 4th ed. Boca Raton: CRC Press; 2008. p. 1160. ISBN: 9780849392726

[9] Nayak B, Madhavan Nair K. In vitro bioavailability of iron from wheat flour fortified with ascorbic acid, EDTA and sodium hexametaphosphate, with or without iron. Food Chemistry. 2003;**80**:545-550. DOI: 10.1016/S0308-8146(02)00341-2

[10] Bosso ST, Enzweiler J. Essays for determining lead (Bio)availability in contaminated soils: Review. Química Nova. 2008;**31**:394-400. DOI: 10.1590/S0100-40422008000200036

[11] Oomen AG, Hack A, Minekus M, Zeijdner E, Cornelis SC, Schoeters G, Verstraete W, Van De Wiele T, Wragg J, Rompelberg CJM, Sips A, Van Wijnen JH. Comparison of five in-vitro digestion model to study the bioaccessibility of soil contaminants. Environmental Science & Technology. 2002;**36**:3326. DOI: 10.1021/es010204v

[12] Hemalatha S, Platel K, Srinivasan K. Influence of heat processing on the bioaccessibility of zinc and iron from cereals and pulses consumed in India. Journal of Trace Elements in Medicine and Biology. 2007;**21**:1-7. DOI: 10.1016/j.jtemb.2006.10.002

[13] Goldstein BD. Methodologies for assessing exposures to metals: Speciation, bioaccessibility, and bioavailability in the environment, food, and feed. Ecotoxicology and Environmental Safety. 2003;**56**(1):1-2. DOI: 10.1016/S0147-6513(03)00045-9

[14] Tokalioğlu S, Clough R, Foulkes M, Worsfold P. Bioaccessibility of Cr, Cu, Fe, Mg, Mn, Mo, Se and Zn from nutritional supplements by the unified BARGE method. Food Chemistry. 2014;**150**:321-327. DOI: 10.1016/j.foodchem.2013.10.151

[15] Intawongse M, Dean JR. In-vitro testing for assessing oral bioaccessibility of trace metals in soil and food samples. Trends in Analytical Chemistry. 2006;**25**(9):876-886. DOI: 10.1016/j.trac.2006.03.010

[16] Ruby MV, Davis A, Schoof R, Eberle S, Sellstone CM. Estimation of lead and arsenic bioavailability using a physiologically based extraction test. Environmental Science & Technology. 1996;**30**:422-430. DOI: 10.1021/es950057z

[17] Iqbal A, Khalil IA, Ateeq N, Khan MS. Nutritional quality of important food legumes. Food Chemistry. 2006;**97**:331-335. DOI: 10.1016/j.foodchem.2005.05.011

[18] Kahlon TS, Smith GE, Shao Q. In vitro binding of acids by kidney bean (*Phaseolus vulgaris*), black gram (*Vigna mungo*), Bengal gram (*Cicer arietinum*) and moth bean (*Phaseolus aconitifolins*). Food Chemistry. 2005;**90**:241-246. DOI: 10.1016/j.foodchem.2004.03.046

[19] Santos WPC, Gramacho DR, Teixeira AP, Costa ACS, Korn MGA. Use of Doehlert design for optimizing the digestion of beans for multi-element determination by inductively coupled plasma optical emission spectrometry. Journal of the Brazilian Chemical Society (Impresso). 2008;**19**:1-10. DOI: 10.1590/S0103-50532008000100002

[20] Anvisa. Agência Nacional de Vigilância Sanitária. Sistema de Legislação em Vigilância Sanitária (VISALEGIS). Resolução RDC No. 42/2013. Ministério da Saúde. Agência. Available from: http://www.agricultura.gov.br/arq_editor/file/CRC/Resolu%C3%A7%C3%A3o%2042%20-%20ANVISA.pdf [Accessed: January 4, 2015]

[21] Codex Alimentarius. Codex General Standard for Contaminants and Toxins in Foods; CODEX STAN 193-1995, Rev.2-2006. Available from: http://www.codexalimentarius.net/download/standards/17/CXS_193e.pdf [Accessed: October 3, 2014]

[22] Nano RMW, Bruns RE, Ferreira SLC, Baccan N, Sousa RA, Cadore S. Statistical mixture design development of digestion methods for Oyster tissue using inductively coupled plasma optical emission spectrometry for the determination of metallic ions. Talanta. 2009;**80**:559-564. DOI: 10.1016/j.talanta.2009.07.025

[23] Navarro P, Arana G, Etxebarria N, Dean JR. Evaluation of the physiologically based extraction test as an indicator of metal toxicity in mussel tissue. Analytica Chimica Acta. 2008;**622**:126-132. DOI: 10.1016/j.aca.2008.05.052

[24] Turner A, Radford A. Bioaccessibility of trace metals in boat paint particles. Ecotoxicology and Environmental Safety. 2010;**73**:817-824. http://dx.doi.org/10.1016/j.ecoenv.2010.02.022

[25] Watzke HJ. Impact of processing on bfioavailability examples of minerals in food. Review. Trends in Food Science & Technology. 1998;**9**:320-327. DOI: 10.1016/S0924-2244(98)00060-0