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Article

Cu, Fe, Mn and Zn Distribution in Protein Fractions of Brazil-Nut, Cupuassu Seed and Coconut Pulp by Solid-Liquid Extraction and Electrothermal Atomic Absorption Spectrometry

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Neste trabalho foram determinados Cu, Fe, Mn and Zn em extratos de castanha-do-Pará (*Bertholletia excelsa*), semente de cupuaçu (*Theobroma grandiflorum*) e polpa de coco (*Cocos nucifera*) usando extração sólido-líquido e espectrometria de absorção atômica com atomização eletrotérmica. Foram empregadas diferentes soluções extratoras (mistura de metanol e clorofórmio 1:2 v/v, água, 0,5 mol L^{-1} NaCl, 70% v/v etanol e 0,5 mol L^{-1} NaOH) para extração de lipídios, albuminas, globulinas, prolaminas e glutelinas, respectivamente. As concentrações de proteínas encontradas na castanha-do-pará foram maiores do que aquelas encontradas na semente de cupuaçu e polpa de coco. Nas frações lipídicas foram encontradas maiores concentrações de Fe em castanha-do-pará e semente de cupuaçu e Zn em polpa de coco. As determinações dos elementos nos extratos proteicos de castanha-do-pará indicaram que eles estão ligados, preferencialmente, às albuminas, globulinas e glutelinas. O mesmo comportamento não foi observado para semente de cupuaçu e polpa de coco. As concetrações dos elementos variaram para os diferentes extratos proteícos. Em geral, as maiores concentrações de proteínas foram determinadas nos extratos em NaOH. Por outro lado, as menores concentrações de Cu, Fe, Mn e Zn foram encontradas nessa fração.

In this work Cu, Fe, Mn and Zn were determined in extracts of Brazil-nut (*Bertholletia excelsa*), cupuassu (*Theobroma grandiflorum*) seeds and coconut (*Cocos nucifera*) pulp, using solid-liquid extraction and electrothermal atomic absorption spectrometry. Different extractant solutions (mixture of methanol and chloroform 1:2 v/v, water, 0.5 mol L^{-1} NaCl, 70% v/v ethanol, and 0.5 mol L^{-1} NaOH) were used for extracting lipids, albumins, globulins, prolamins and glutelins, respectively. The protein concentrations determined in Brazil-nut are higher than those observed for cupuassu and coconut pulp. The analysis of lipid fractions reveals high concentations of Fe in Brazil-nut and cupuassu seed and Zn in coconut pulp. For Brazil-nut, these elements are mainly associated to albumin, globulin and glutelin fractions. The same behaviour was not observed for cupuassu seed and coconut pulp. The element concentrations changed in each protein fraction. In general, high concentrations of proteins were determined in NaOH extractants. On the other hand, low concentration of Cu, Fe, Mn and Zn were found in this fraction.

Keywords: solid-liquid extraction, electrothermal atomic absorption spectrometry, Brazil-nut, cupuassu, coconut

Introduction

Foods are one of the main sources of metals for human body. Most of the metal ions present in foods are bound to specific proteins or enzymes and exert their effects as active or structural centers, command process such as catalysts, substrate binding and activation, transportation and storage.[1,2] In this way, the knowledge of the foods compositional, nutritional and functional properties is fundamental for defining their quality. In general, nutritional properties are characterized by both the abundance and bioavailability of essential nutrients.[3,4]

Efforts have been made to understand the interactions of metals with proteins and enzymes present in foods. Terms like metal-binding proteins and metalloproteins are more and more frequent in the food chemistry literature. A recent review describes the state-of-art, tendencies and analysis related to the metal-binding proteins and metalloproteins.[5] Metal-binding proteins and metalloproteins represent a large portion of the number of proteins. It has been estimated that around 40% of all proteins and enzymes contain metal ions in their structures.

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In these species, metals bind to proteins with high-affinity interactions. Transitions metal ions, such as Cu(II), Fe(II), Mn(II), Mo(II), and Zn(II), and non-metal as Se(IV), have the strongest coordinating interactions. As a consequence, they are found in the majority of metalloproteins.[5] Fruits are one source of metalloproteins, where the elements are mainly associated to albumins and globulins.[3]

The most common means to accomplish element speciation consist of combined and/or coupled systems, in which species are separated before the selective detection of the elements.[6] The coupling of chromatography by using different separation mechanisms with inductively coupled plasma mass spectrometry (ICP-MS) detection has been the most widely used analytical tool for element speciation.[5-8]

In the latest years, fruits and nuts from Amazon region have arisen interest among the scientific community due to the high concentrations of some essential elements in their compositions.[9-17] Special attention are devoted to Brazil-nut (*Bertholletia excelsa*), known as "castanha-do-Pará". This nut is a rich source of nutrients, such as proteins (10-20%, m/m), fat (50-70%, m/m), carbohydrates (10-20%, m/m), vitamins, minerals and dietary fiber.[13-17] The concentrations of Ag, Al, Ag, As, Ba, Ca, Co, Fe, Hg, Mg, Mn, Mo, Pb, Se, Sr, and Zn associated to different molecular weight fractions of Brazil-nut were determined.[14-22] Andrade *et al.*[16] studied the elemental distribution of soluble organic species in a methanol plus chloroform mixture and water, as well as insoluble metal species in these extractants. Chunhieng *et al.*[17] separated proteins of the Brazil-nut using sequential extraction with a mixture of methanol plus chloroform, water, sodium chlorine solution, ethanol, and sodium hydroxide solution to obtain lipids, albumins, globulins, prolamins, and glutelins fractions, respectively. Caruso and co-workers[18] studied the distribution of various elements of nutritional and toxicological interest in different molecular weight fractions, ranging from 0.18 to 14 kDa. Chemical speciation has been accomplished for As and Se in Brazil-nut[20-22] and Ni, Cu, Zn and Mn in different edible nuts.[19,23]

Cupuassu (*Theobroma grandiflorum*) has a white-yellow pulp with pleasant acidic taste and a strong fragrance, which is widely consumed as juice, ice cream and jam.[24] Seeds can be processed in a similar fashion to its close cousin and cocoa to produce cupuassu chocolate, named cupulate.[24,25] Cupulate has a good market potential as an exotic alternative to cocoa, and it could be an income source to the local Amazonian population.[24] However, the chemical composition of pulp and seed are scarcely studied. The majority studies of their chemical composition are related to volatile substances responsible by aroma, such as linalol, α-terpinol, 2-phenylethanol, myrcene and limonene, diols and methoxy-2,5-dimethyl-3(2H)-furanone.[24,25] In the pulp composition can be found proteins (9%, m/m), fats (13%, m/m), carbohydrates (49%, m/m), and also macro and micronutrients, such as Na (26 mg kg^{-1}), K (340 mg kg^{-1}), Mg (56 mg kg^{-1}), P (130 mg kg^{-1}), Fe (160 mg kg^{-1}), Zn (5.3 mg kg^{-1}), Cu (2.6 mg kg^{-1}) and Mn (2.1 mg kg^{-1}).[26]

The water and pulp of coconut (*Cocos nucifera*) are largely consumed by population. Green coconut water is a nutritious, refreshing, isotonic and low caloric drink. It presents some nutritional and therapeutic properties, being a natural, acid and sterile solution, which contain mineral salts, sugars, vitamins and proteins.[26] Additionally, it has been advised to gastric disturbs treatment, inhibition of vomit caused by cholera, treatment of dysentery and for infant feeding.[27-29] The concentrations of Cu, Fe, Mn and Zn found in coconut water ranged from 3-112, 49-323, 1-5 and 0.04-0.18 μg L^{-1}, respectively.[27,29]

The edible pulp of the mature coconut is usually employed in the production of coconut flakes, coconut milk and oil.[30] The elemental composition of coconut pulp is scarcely known, however the fractionation and characterization of protein was studied.[30-32] Kwon *et al.*[30] carried out solid-liquid extraction by using deionized water, NaCl solution, 2-propanol, glacial acetic acid and NaOH solution. The determination of proteins in each fractions resulted in albumins (21%, m/m), globulins (40%, m/m), prolamins (3.3%, m/m), glutelins-1 (14%, m/m), glutelins-2 (4.8%, m/m) and unextractable protein (8.1%, m/m).

Based on the importance of these food as mineral source and the necessity to characterize their mineral composition, the goal of this study was to access the distribution of Cu, Fe, Mn and Zn in different proteins fractions of the Brazil-nut, cupuassu seed and coconut pulp after sequential solid-liquid extraction with a mixture of methanol plus chloroform, water, NaCl solution, ethanol, and NaOH solution. The determinations of these elements were carried out by electrothermal atomic absorption spectrometry (ET AAS).

Experimental

Instrumentation

Analysis of protein extracts was carried out using a ZEEnit® 60 model atomic absorption spectrometer (AnalytikjenaAG, Jena, Germany) equipped with a transversely heated graphite atomizer, pyrolytically coated graphite tube, transversal Zeeman-effect

background corrector. Copper, Fe, Mn and Zn hollow cathode lamps were used. All measurements were based on integrated absorbance values. The instrumental conditions for the spectrometer and the heating programs for the graphite tube atomizer are shown in Table 1. Argon 99.998%, v/v (Air Liquide Brasil, São Paulo, Brazil) was used as purge gas.

A Ultrospec 2100 pro spectrophotometer (Biochrom LTD, Cambridge, UK) equipped with xenon lamp and wavelength range from 190 to 900 nm was used for protein determination at 590 nm.

The digestions of samples and solid residues obtained after extractions were performed in a closed vessel microwave oven, model Microwave 3000 (Anton Paar, Graz, Austria).

An orbital shaker (Quimis, São Paulo, Brazil) was used to mix the samples and each extractant for 30 min at 250 rpm.

A vacuum filtration system of borosilicate glass, model XX15 047 00 (Millipore, USA) and a 0.45 μm Nylon® membrane filter (Millipore, USA) was used to separate the supernatant from solid material after extraction.

Reagents and samples

All solutions were prepared from analytical-grade chemicals and using high purity deionized water obtained from a Milli-Q water purification system (Millipore, Belford, MA, USA). Tritisol standard analytical solutions of 1000 mg L^{-1} of Cu(II) [$Cu(NO_3)_2$], Fe(III) [$FeCl_3$], Mn(II) [$Mn(NO_3)_2$] and Zn(II) [ZnO] (Merck, Darmstadt, Germany) were used to prepare the reference analytical solutions by sucessive dilutions. Chloroform, ethanol, methanol, NaCl and NaOH (Merck) were used for the solid-liquid extractions. Nitric acid (Merck), distilled in a quartz sub-boiling still (Marconi, Piracicaba, SP, Brazil) and 30%, v/v H_2O_2 (Merck) were used to prepare the oxidant mixture for the *in situ* thermal sample pretreatment during the heating program, and for digesting the samples in a microwave oven. Triton X-100 from (Merck) was used to dilute fractions.

Coomassie Blue G-250 (CBG) and ovoalbumin from BioAgency (São Paulo, Brazil) and GE Healthcare (Amersham Bioscience, São Paulo, Brazil) were used to determine the total proteins. The methanol and 85%, m/m of phosphoric acid (Sigma) were used to prepare the CBG.

Brazil-nuts, cupuassu seeds (from the Amazonian region) and coconut pulps (from the northwest of Brazil) were purchased at local markets.

Procedure

All glassware and polypropylene flasks were cleaned with detergent solution, soaked in 10%, v/v HNO_3 for 24 h, rinsed with Milli-Q water and stored into a closed polypropylene container. All solutions preparation and sequential solid-liquid extractions were conducted in a class-100 laminar flow bench (Veco, Campinas, SP, Brazil) to avoid airborne contamination.

Table 1. Instrumental parameters and heating program for the determination of Cu, Fe, Mn and Zn

	Cu[a]	Fe[b]	Mn[c]	Zn[d]
Spectrometer setup				
Wavelength / nm	224.8	248.3	279.5	213.9
Spectral resolution / nm	0.8	0.8	0.2	0.2
Lamp current / mA	4.0	4.0	4.0	4.0
Heating program for the element determinations in lipidic fractions				
Step	Temperature / ºC	Heating rate / (ºC s^{-1})	Hold / s	Ar flow rate / (mL min^{-1})
Drying	130	10	20	1000
Pyrolysis I	400	10	20	1000
Pyrolysis II	1000[a-c]/700[d]	100	20	1000
AZ*	1000[a-c]/700[d]	0	6	1000
Atomization	2200[a-d]/2300[c,d]	2200[a,b]/2300[c,d]	5	0, 100[d]
Cleaning	2500	1200	2	1000
Heating program for the element determinations in other fractions and digested samples				
Step	Temperature / ºC	Heating rate / (ºC s^{-1})	Hold / s	Ar flow rate / (mL min^{-1})
Drying	110	10	15	1000
Drying	130	10	20	1000
Pyrolysis	1000[a-c]/700[d]	100	20	1000
AZ*	1000[a-c]/700[d]	0	6	1000
Atomization	2200[a,b]/2300[c,d]	2200[a,b]/2300[c,d]	5	0, 100[d]
Cleaning	2500	1200	2	1000

[a]Cu; [b]Fe; [c]Mn; [d]Zn; *AZ = auto-zero.

About 200 g of samples were ground in a household food grinder. Masses around 5.0 g of the ground samples were used to solid-liquid sequential extraction with 10 mL of methanol and chloroform (1:2 v/v); deionized water; 0.5 mol L^{-1} NaCl; 70%, v/v ethanol; and 0.5 mol L^{-1} NaOH.

The first extraction, using a mixture of methanol and chloroform (1:2 v/v), was performed to separate lipids. This procedure was carried out twice using an orbital shaker. At the end, the defatted nut was submitted to sequential extractions with deionized water, NaCl, ethanol, and NaOH. Separation of all supernatants was performed using a filtration system with 0.45 μm membrane filter.

The final solid was digested in a closed vessel microwave oven, using a diluted oxidant mixture (2.0 mL HNO_3, 1.0 mL H_2O_2, and 3.0 mL water). The microwave heating program presents four steps (Temperature / ºC; ramp / min; hold / min): 1 (140; 5; 1), 2 (180; 4; 5), 3 (200; 4; 10), 4 (0, 0, 20). The same procedure was used to digest the ground samples. In both cases, the digestates were diluted to 10 mL with deionized water.

Copper, Fe, Mn and Zn were determined in all fractions and in the digested solutions by ET AAS, after appropriate dilution with deionized water. For all elements, aliquots of 10 μL of the samples were injected into the graphite furnace without chemical modifier.

For lipidic fractions analysis, 900 μL of each sample were mixed with 100 μL of 10 % m/v Triton X-100 directly in the autosampler cups. The preparation of aqueous calibration solutions was made using 900 μL of deionized water and 100 μL of analytical reference solutions (200 to 800 μg L^{-1} of Cu and Fe, 100 to 250 μg L^{-1} of Mn, and 10 to 40 μg L^{-1} of Zn in 10%, m/v Triton X-100). Due to the high organic content in the methanol and chloroform extractant, an aliquot of 10 mL of an oxidant mixture (15%, v/v H_2O_2 and 1%, v/v HNO_3) was co-injected with 10 mL of the samples into the graphite furnace. In this case, the oxidant mixture avoided the formation of carbonaceous residue onto the integrated platform.[27,33]

Addition of 40 μg L^{-1} Cu and Fe, 15 μg L^{-1} Mn and 1 μg L^{-1} Zn and recovery tests were performed for the digested samples and for all fractions, except for the lipidic fractions.

The total protein concentration in water, NaCl, ethanol, and NaOH fractions was determined the by the Bradford method.[34] The Bradford reagent was prepared using 10 mg of CBG, 5.0 mL of methanol and 10 mL of 85%, v/v phosphoric acid. The final volume of 100 mL was completed with deionized water. The protein standard was prepared dissolving 4.0 mg of ovoalbumin in 2.0 mL of deionized water, submitted to Vortex stirring for 2 min and diluted 10 times with deionized water. The spectrophotometer calibration was performed using analytical reference solutions of 4, 6, 8, 10, 12, 16 and 20 μg of ovoalbumin in 1.0 mL of Bradford reagent.

Water, NaCl, and NaOH extracts of the Brazil-nut samples were diluted 100 times and ethanol extract was diluted 10 times. A volume of 50 μL of these solutions was used for protein determination. For the cupuassu samples, H_2O and NaCl extracts were diluted twofold and NaOH extract was diluted 20 times. A volume of 100 μL of these solutions was used for protein determination. Finally, H_2O, NaCl and NaOH coconut sample extracts were diluted 20 times and 100 μL of these solutions were used for protein determination. Aliquots of 100 μL of cupuassu and coconut ethanol extracts were analyzed without dilution. In all cases it was used a volume of 1.0 mL of Bradford reagent.

Results and Discussion

Protein distribution in each fraction

Previous investigations studied the total protein determination in Brazil-nut and coconut pulp after extraction of the defatted cake with NaOH solution (pH = 10) and protein precipitation with HCl. In the sequence, fractionation of proteins was carried out from the dried pellet using water, NaCl, ethanol and NaOH solutions. Using this procedure, the total amount of protein extracted from Brazil-nut and coconut were 84%, m/m and 67.9%, m/m, respectively.[17,29] Therefore, the sequential extraction with these solvents did not extract all protein content of these samples. In our study, proteins were directly extracted from the defatted cake using water, NaCl, ethanol and NaOH solutions for albumins, globulins, prolamins and glutelins extraction, respectively.

The protein concentration in each extractant of Brazil-nut, cupuassu seed and coconut pulp samples are shown in Tables 2-4. It can be seen that, protein concentrations found in Brazil-nut are higher than those observed for cupuassu seed and coconut pulp. Albumins, globulins and glutelins were the predominant proteins in Brazil-nut samples (n=3), as was observed by Chunhieng *et al*.[17] On the other hand, glutelin (NaOH solution) was the predominant protein in cupuassu seed and coconut pulp samples (n=3).

Cu, Fe, Mn and Zn determination in each fraction

At the begining of this work, Brazil-nut, cupuassu seed and coconut pulp samples were ground and digested for the total determination of Cu, Fe, Mn and Zn.

Table 2. Total protein contents and Cu, Fe, Mn and Zn concentrations in each extracted fraction of Brazil-nut samples

Extractants(fraction)	Protein / (mg g^{-1})	Concentration ± SD* Cu / (μg g^{-1})	Fe / (μg g^{-1})	Mn / (μg g^{-1})	Zn / (μg g^{-1})
Water(albumin)	29 ± 6	1.1 ± 0.1	0.03 ± 0.02	0.16 ± 0.01	2.1 ± 0.2
NaCl(Globulin)	33 ± 4	1.1 ±0.1	0.005 ± 0.001	0.11 ± 0.01	0.85 ± 0.01
Ethanol(prolamin)	4.2 ± 0.3	0.05 ± 0.01	0.03 ± 0.01	0.011 ± 0.002	0.027 ± 0.001
NaOH(glutelin)	37 ± 7	1.2 ± 0.1	0.08 ± 0.01	0.053 ± 0.005	1.1 ± 0.1
CH_3Cl/CH_3OH[a] (lipid)	-	0.06 ± 0.01	0.03 ± 0.01	0.028 ± 0.001	<LD[b]
Final solid(non-extracted)	-	1.1 ± 0.1	8.5 ± 0.6	1.7 ± 0.1	2.2 ± 0.4
Total 1[c]	-	4.7 ± 0.2	8.7 ± 0.6	2.1 ± 0.1	6.3 ± 0.4
Total 2[d]	-	4.4 ± 0.3	8.7 ± 0.5	2.4 ± 0.1	6.9 ± 0.4

[a]Chloroform:methanol (2:1 v/v); [b]detection limit (LD) = 3.8 ng g^{-1}; [c]sum of the masses found in extracted and non-extracted fractions; [d]analytes concentrations in digested sample; *SD = standard deviation (n=3).

Table 3. Total protein contents and Cu, Fe, Mn and Zn concentrations in each extracted fraction of cupuassu seed samples

Extractants(fraction)	Protein / (mg g^{-1})	Concentration ± SD* Cu / (μg g^{-1})	Fe / (μg g^{-1})	Mn / (μg g^{-1})	Zn / (μg g^{-1})
Water(albumin)	1.9 ± 0.3	0.30 ± 0.01	0.10 ± 0.01	0.43 ± 0.05	1.4 ± 0.1
NaCl(Globulin)	1.5 ± 0.1	0.12 ± 0.01	0.09 ± 0.01	0.22 ± 0.01	0.99 ± 0.01
Ethanol(prolamin)	1.2 ± 0.1	0.030 ± 0.001	0.07 ± 0.01	0.10 ± 0.02	0.70 ± 0.02
NaOH(glutelin)	16 ± 2	0.08 ± 0.01	0.07 ± 0.02	0.027 ± 0.001	0.30 ± 0.02
CH_3Cl/CH_3OH[a] (lipid)	-	0.03 ± 0.01	0.60 ± 0.04	0.21 ± 0.02	0.024 ± 0.003
Final solid(non-extracted)	-	0.27 ± 0.01	2.1 ± 0.5	0.26 ± 0.02	8.1 ± 0.7
Total 1[b]	-	0.51 ± 0.01	2.8 ± 0.5	0.67 ± 0.03	12 ± 1
Total 2[c]	-	0.84 ± 0.02	3.0 ± 0.5	1.06 ± 0.21	14 ± 1

[a]Chloroform:methanol (2:1 v/v); [b]sum of the masses found in extracted and non-extracted fractions; [c]analytes oncentrations in digested sample; *SD = standard deviation (n=3).

Table 4. Total protein contents and Cu, Fe, Mn and Zn concentrations in each extracted fractions of coconut pulp samples

Extractants(fraction)	Protein / (mg g^{-1})	Concentration ± SD* Cu / (μg g^{-1})	Fe / (μg g^{-1})	Mn / (μg g^{-1})	Zn / (μg g^{-1})
Water(albumin)	4 ± 1	0.21 ± 0.01	0.05 ± 0.01	0.10 ± 0.02	0.07 ± 0.02
NaCl(Globulin)	2 ± 1	0.039 ±0.003	0.041 ± 0.001	0.33 ± 0.06	0.11 ± 0.01
Ethanol(prolamin)	0.5 ± 0.1	0.012 ± 0.001	1.2 ± 0.1	0.018 ± 0.003	0.033 ± 0.001
NaOH(glutelin)	9 ± 1	1.2 ± 0.1	0.34 ± 0.01	0.010 ± 0.005	0.25 ± 0.02
CH_3Cl/CH_3OH[a] (lipid)	-	0.07 ± 0.01	0.35 ± 0.01	0.003 ± 0.001	0.92 ± 0.09
Final solid(non-extracted)	-	0.040 ± 0.005	4.5 ± 0.2	0.49 ± 0.02	2.3 ± 0.6
Total 1[b]	-	0.9 ± 0.1	6.9 ± 0.1	0.94 ± 0.06	3.7 ± 0.6
Total 2[c]	-	1.1 ± 0.1	6.4 ± 0.2	1.02 ± 0.03	3.4 ± 0.2

[a]Chloroform:methanol (2:1 v/v); [b]sum of the masses found in extracted and non-extracted fractions; [c]analytes concentrations in digested sample; *SD = standard deviation (n=3).

The heating program of the atomizer were optimized for each analyte, using digestate and extractant solutions. Pyrolysis and atomization curves were obtained and they confirmed the possibility to carry out the determinations without chemical modifier, except for the lipidic fraction that required the use an oxidant mixture containing 15%, v/v H_2O_2 + 1%, v/v HNO_3 to carry out *in situ* organic decomposition inside the graphite tube.[33]

The recoveries for 40 μg L^{-1} Cu and Fe, 15 μg L^{-1} Mn and 1 μg L^{-1} Zn added in digested and extracted fractions ranged from 85 to 104% for Cu, 80 to 114% for Fe, 80 to 111% for Mn, and 70 to 101% for Zn. Except for Zn in water extractant of the Brazil-nut (70% of recovery), aceptable recoveries were obtained for all elements. The relative standard deviation for three replicates of samples were always lower than 6%.

The total concentration of the analyts are shown in Tables 2-4. It is important to highlight that elemental concentration is dependent of soil characteristics, physiology of plant, water source composition, fertilizers, insecticides, pesticides, and fungicides used in the plantations.[35]

Cu, Fe, Mn and Zn distribution in each fraction

Copper, Fe, Mn and Zn concentration in albumins, globulins, prolamins and globulins fractions of the Brazil-nut, cupuassu seed and coconut pulp samples are shown in Tables 2-4, respectively. They also indicate the total concentration of the analytes obtained from the digested samples and from the sum of non-extracted fraction and all extracted and non-extracted fractions. The sum of the element concentrations in each fraction agrees at a confidence level of 95% (*t*-test) with the total concentration found in the digested samples, except for the Cu and Mn in cupuassu seed.

The analysis of lipidic fraction for each sample indicated higher concentrations of Fe in Brazil-nut and cupuassu seed than coconut pulp. On the other hand, higher concentration of Zn was found in lipidic fraction of coconut pulp. This fact may be explained because plant membranes contain sulfolipids, in which a sulfonated glucose residue is joined to a diacylglycerol in glycosidic linkage.[6,36] In sulfolipids, the sulfonate group bears a fixed negative charge that can interact with cationic species as metal ions.

The concentration of the analytes found in non-extracted fractions indicated that the majority of them (Fe and Mn in Brazil-nut, Fe and Zn in cupuassu, and Cu, Fe, Mn and Zn in coconut pulp) stayed bound to some matrix (organic substance or biomolecule). These elements could be bound to other protein groups not extracted with the extractants selected for this work.

The main amino acids constituents of albumins, globulins, prolamins and glutelins are rich in sulfur and charged groups such as methionine, cysteine, glutamic acid, arginine, aspartic acid and lysine.[32,37,38] The metal ions present high-affinity for these kinds of amino acids. For Brazil-nut, all analytes are mainly in albumin, globulin and glutelin fractions. It can be seen a close relation between the protein and analyte concentrations for Brazil-nut samples (Table 2). This pattern was not observed for cupuassu seed and coconut pulp samples (Tables 3 and 4). For these samples, high concentration of proteins were found in NaOH extractant (glutelins group). On the other hand, low concentration of Cu, Fe, Mn and Zn were found in this fraction. In general, the concentration of all analytes was not proportional to the glutelins concentration. It is know that NaOH is not a selective protein extractant.[17,30] For this reason, we can suppose that other proteins and non-extracted albumins, globulins and prolamins may be present in this fraction.

Conclusions

Solid-liquid extraction combined with electrothermal atomic absorption spectrometry can be considered as a powerful technique for fractionation and determination of elements associated to proteins. A preliminary relationship amoung Cu, Fe, Mn and Zn to different protein groups was observed, indicating the possible linkage of these elements to albumins, globulins, prolamins and glutelins. The identification and quantification of these elements for different protein fractions is only an initial step for characterizing metaloproteins in Brazil-nut, cupuassu seed and coconut pulp. The application of other separation techniques such as size exclusion chromatography (SEC), gel electrophoresis (GE), and mass spectrometry (MS) are required to identify these unknow species.

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