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Avaliação da Retenção Mineral em Leguminosas Verdes Submetidas ao Tratamento Térmico

Resumo: A produção de leguminosas tem grande impacto na agricultura familiar, além de grande importância nutricional. O consumo de feijão verde é generalizado no Brasil. Técnicas culinárias, além do processamento industrial, podem levar à perda de micronutrientes e espécies tóxicas por mecanismos de lixiviação. Este estudo avaliou a influência do tratamento térmico na retenção de Ca, Fe, Zn, Mn, Mg, Cu, Cr, Co, Ba, Cd, Pb nas seguintes leguminosas. Os elementos químicos característicos de cada uma dessas espécies de leguminosas podem ser usados em estudos dos padrões de identidade das leguminosas avaliadas. Em geral, o mangalô (*Lablab purpureus* L. Sweet) apresentou um menor número de elementos cujas concentrações se alteram significativamente quando um tratamento térmico é realizado. O feijão guandu mostrou uma maior diversidade de elementos químicos que diferem significativamente após o cozimento, enquanto o feijão caupi está em uma posição intermediária.

Palavras-chave: Composição mineral; elementos traços; leguminosas verdes; PCA; ICP OES.

Abstract

Legume production has a great impact on family farming, as well as great nutritional importance. The consumption of green beans is widespread in Brazil. Culinary techniques, besides industrial processing, can lead to the loss of micronutrients and toxic species by leaching mechanisms. This study assessed the influence of heat treatment on the retention of Ca, Fe, Zn, Mn, Mg, Cu, Cr, Co, Ba, Cd, Pb in the following green legumes. The characteristic chemical elements of each of these legume species can be used in studies of the identity patterns of the evaluated legumes. In general, *mangalô* (*Lablab purpureus* L. Sweet) had a smaller number of elements whose concentrations change significantly when a thermal treatment is performed. Pigeon pea showed a greater diversity of chemical elements that differ significantly after cooking, while cowpea beans are at an intermediate position.

Keywords: Mineral composition; trace elements; green legumes; PCA; ICP OES.

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Evaluation of Mineral Retention in Green Legumes Submitted to Thermal Treatment

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1. Introduction

The consumption of green beans is widespread in Brazil, especially in the northeastern region, creating jobs and income for the rural and urban populations of the North and Northeast regions. Legume production has a major impact on family farming. Depending on the region, there may be up to three harvests of beans during the year are still produced by family agriculture and represent about 70 % of the total bean production in Brazil.[1] Brazilian bean production in the 2013/2014 harvest was 3.4 million tons, and Brazil was the 3rd largest producer in the world, accounting for 12 %

production, behind Myanmar, Southeast Asia (16.4 %) and India (15.7 %).[2]

The frequent consumption of legumes by the population is considered an effective alternative to reduce the risk of several chronic degenerative diseases.[3,4] In addition, legumes provide various minerals to the diet, notably iron, zinc, calcium, phosphorus and magnesium. Although they have this nutrient range in their composition, there are processes and interactions between compounds in the beans that can lead to losses, especially of mineral elements.[5,6] The protein phaseolin, present in beans, has the majority of the essential amino acids, except methionine, which is found at low concentrations.[7] In addition, they have oligosaccharides and phenolic compounds, which provide beans with antioxidant properties.[8] Therefore, the frequent consumption of legumes by the population is considered an effective alternative to reduce the risk of several chronic degenerative diseases.[3,9]

Pigeon pea (*Cajanus cajan* (L.) is popularly known as pigeon pea or *Andu* in Brazil. It is believed that pigeon pea is native to India, where it spread to Africa 4,000 years ago.[10] The protein is rich in lysine, leucine and histidine, but poor in methionine, threonine and tryptophan. Cowpea, string bean or macassar bean (*Vigna unguiculata* (L.) Walp.) stands out for its socioeconomic importance for the families in the North and Northeast regions of Brazil. Cowpea beans is the main raw material used in the preparation of *acarajé* (Intangible Cultural Heritage), and the large-scale production of cowpea flour is necessary to meet the demand for this food.[11] The legume *Lablab purpureus* is known by several names in different parts of the world, such as lab-lab bean (Australia) and bean *frijol jacinto* (Colombia). In Brazil, the legume has several names, such as *mangalô*, hyacinth bean, ear-of-priest, and bean-of-stone. It is believed that the wild forms of Lablab originated in India, spreading to many tropical and subtropical countries.[12,13]

The average chemical composition of cowpea (*Vigna unguiculata* L. Walp) and Andu beans (*Cajanus cajan* L.), in dry basis (g %) is respectively: protein (20.2 and 19); lipids (2.4 and 2.1); carbohydrates (61.2 and 64); dietary fiber (23.6 and 21.3); minerals in mg 100 g^{-1}: Ca (78 and 129); Mg (178 and 166); Mn (1,43 and 1,02); P (355 and 269); Fe (5,1 and 1,9); Na (10 and 2,0); K (1083 and 1215); Cu (0,70 and 0,57); Zn (3,9 and 2,0); vitamins in mg 100 g^{-1}: thiamine (0,14 and 1,06); riboflavin (0,03 and ND); pyridoxine (0,26 and 0,07).[14] Souza *et al*. reported concentrations (mg. 100 g^{-1}) of Fe (13.6); Zn (4.8); Mn (2.1); Cu (1.9); P (384); K (1273); Ca (148) e Mg (153) in the mangalo bean (*Lablab purpureus* L. Sweet).[15]

The presence of antinutritional factors may affect mineral bioavailability.[16,17] Therefore, the availability and use of nutrients by the body are influenced by different thermal processes.[18,19]

Many authors have studied the influence of different thermal processes on the nutritional value of foods.[20-22] Sucupira and Xereza and Souza affirm that thermal processes can influence the nutritional value of foods by changing their nutritional properties and depends on different factors, such as: chemical species, processing method, time and temperature of heating.[21] Wang *et al* evaluated the effect of domestic conductive cooking on bean (*Phaseolus vulgaris* L.) and chickpea (*Cicer arietinum* L.) composition, observing that after the thermal processing of the legumes there was no significant effect on the concentrations of Ca, Cu, Fe and Zn, but there was a significant loss of K and Mg and an increase of Mn and P.[24] Compared different heat treatments to evaluate the potassium content in green beans. The thermal processing reduced significantly the potassium content, especially the ones preceded by immersion.[20] Statistical tools have been used to better present and explain data obtained in scientific researches such as surface responses, analysis of variance (ANOVA), principal component analysis (PCA), among others. The PCA is a multivariate analysis chemometric tool that allows investigations with a large number of data available and is certainly among the most important, also because it is the basis for most of the other multivariate data analysis methods.[24] As an exploratory analysis tool, the PCA allows to reveal the existence or not of anomalous samples, relationships between measured variables and relations or groupings between samples, making it possible to eliminate some original variables that have little information. The main characteristic of the PC is that they are obtained in descending order of maximum variance, for example, the main component 1 (PC1) holds more statistical information than the main component 2 (PC2), which in turn has more statistical information than the component the main 3 (PC3) and so on.[26-27]

This study aimed to evaluate the influence of thermal treatments on the retention of major and trace elements (Ca, Fe, Zn, Mn, Mg, Cu, Cr, P, Se, Mo, Co, Ni) and those potentially toxic (As, Ba, Cd, Pb), in the following green legumes *Vigna unguiculata* L. Walp (caupi bean), *Cajanus cajan* L. (andu bean) and *Lablab purpureus* L. Sweet, (mangalô bean) using Principal component analysis (PCA).

2. Materials and Methods

2.1. Samples, reagents and standard solutions

Samples of the species *Vigna unguiculata* L. Walp, *Cajanus cajan* L. and *Lablab purpureus* L. Sweet (green maturation stage) were obtained at popular fairs and cooperative associations of family agriculture producer the State of Bahia, Brazil. Mangalô and pigeon pea samples were produced in the North Center Bahia Mesoregion, Microregion of Feira de Santana. Cowpea samples were produced in the Bahia Mesoregion of the São Francisco Valley, Microregion of Juazeiro. This work is an exploratory analysis aiming to find some sample patterns of elemental composition after heat treatments. Thus, this work has no intention to carry out a wide evaluating of this legumes that would requires large representative sample to cover aspects such as legume growing stages, geographic location, weather climate and others.

The accuracy of analytical procedure was verified by the analysis of the INCT-SBF-4 certified reference material (Institute of Nuclear Chemistry and Technology, Department of Analytical Chemistry, Warsaw, Poland).

The reagents and standard solutions used were: 65 % (w w^{-1}) HNO_3 (Merck, Germany), 30% v v^{-1} H_2O_2 (Vetec, Brazil) and 1000 mg L^{-1} standard solutions (Specsol, Brazil) of As, Ba, Ca, Cd, Cr, Co, Cu, Fe, Mn, Mg, Mo, Ni, P, Pb, Se and Zn. In the working solution, the concentration of the trace elements (As, Ba, Cd, Cr, Co, Cu, Fe, Mn, Mo, Ni, Pb, Se and Zn) was 40.0 mg L^{-1}. For macro elements Ca, Mg, K and P, a stock solution at 1,000 mg $L^{-1}$was used.

All solutions were prepared with ultrapure water with specific resistivity of 18.2 MΩ cm provided by the Milli-Q® Purification System (Millipore, Bedford, MA, USA). All glassware and materials in general were decontaminated in a 10 % (v v^{-1}) HNO_3 bath for at least 24 hours and washed extensively with ultrapure water.

2.2. Instrumentation

For the implementation of the thermal treatment, the following equipments were used: domestic cooker and domestic microwave oven (Eletrolux), with an output power/consumption of 900 W/1450 W; frequency 2450 MHz, power output/consumption (resistance) of 950 W/1010 W.

For drying of the samples lyophilizer LIOTOP L101 (Liobras, São Carlos, Brazil) was used. The dried samples were ground in an analytical mill IKA A11. A microwave assisted digestion system (ETHOS One – Milestone, Italy) was used for acid decomposition.

An inductively coupled plasma optical emission spectrometer, ICP OES (OPTIMA

7300 DV – *PerkinElmer*, USA), with axial viewing and a Charge Coupled Device (CCD) detector, was used.

2.3. Sample preparation

The samples were submitted to the initial pretreatment stage. This step consisted of selection, washing, lyophilization, grinding, packaging and storage. The seeds were selected and washed with ultrapure water and three sub-samples were produced of each legume species. The sample was separated into three portions: two of them were submitted to heat treatments and one was stored fresh. The thermal treatments were carried out using conductive heating in domestic stove and microwave oven, under the following conditions: sample mass (60 g), ultrapure water volume (150 mL) and cooking time (6 minutes). To conduct the heat treatment, aluminum pots with internal ceramic coating and glass vials were used for the microwave oven to simulate domestic cooking conditions. After cooking, the beans were separated from the coating liquid (supernatant). The samples of boiled seeds were then conditioned and kept under a freezing temperature of -31 °C for 72 hours for further lyophilization, for 48 hours. The supernatants were kept under refrigeration (<10 °C) in Falcon® tubes. The dry beans were then milled in an analytical mill at 28000 rpm for 40 seconds, to obtain a fine flour, which was packed in polyethylene bottles and kept in a desiccator until microwave-assisted acid digestion.

2.4. Sample decomposition

The fresh samples and boiled seeds were decomposed by microwave-assisted diluted acid digestion (Gonzalez et al., 2009). In this procedure, 9.00 mL of 65 % (w w^{-1}) HNO_3, 4.00 mol L^{-1}, 1.00 mL of 30 % (v v^{-1}) H_2O_2 and 500 mg of the sample were used.[28] For the supernatant, that is, the coating liquid obtained by sieving the beans after cooking, 10.00 mL of supernatant, 4.00 mL of 65 % w w^{-1} HNO_3 and 1.00 mL of 30 % v v^{-1} H_2O_2 were used in decomposition. The heating program had four stages: step 1 (6 minutes ramp to 90 °C); step 2 (4 minutes at 90 °C); step 3 (18 minutes ramp to 190 °C); step 4 (7 minutes at 190 °C).[28] The fresh sample decomposition of the three pretreated portions was performed in eight replicate and the samples of boiled seeds were performed in triplicate. The volume of the digested mixture was adjusted to 15.0 mL with ultrapure water and the solutions were stored in previously decontaminated 50.0 mL Falcon® tubes.[29]

2.5. Determination of analytes

The analytical curve was prepared in 2.0 mol L^{-1} HNO_3. The ICP OES operating conditions were: measured power, 1300W; signal integration time, 1 s; plasma gas flow, 15 L min^{-1}; auxiliary gas flow, 1.5 L min^{-1}; 0.70 L min^{-1} nebulization gas flow; 0.70 mL min^{-1} sample pump flow. For sample introduction, a cyclonic chamber and a GemCone™ nebulizer - Low Flow were used. The chosen atomic (I) and ionic (II) lines (nm) were: As I 188.979; Ba II 233.527; Ca II 317.933; Cd I 228.802; Cu I 324.752; Co II 228.616; Cr II 267.716; Fe II 238.204; Pb II 220.353; P I 213.517; Mo II 202.031; Mn II 257.610; Mg II 279.077; Na I 589.592; Ni II 231.604; Se I 196.026; Zn II 213.857.

2.6. Chemometric data analysis

A data matrix containing the concentrations of 13 chemical elements determined in triplicate in 9 different bean samples (*Vigna unguiculata* L. Walp, *Cajanus cajan* L. and *Lablab purpureus* L. Sweet) was submitted to principal component analysis (PCA) using autoscale pre-processing[30,31] resulting in a 27 *X* 12 data matrix. This pre-treatment gives similar weights to the evaluated micro and macro constituents. The chemistry package Unscrambler 8.0 (CAMO, Norway) was used to perform the calculations.

3. Results and Discussion

3.1. Mineral composition

The accuracy of the procedure used to determine the total analyte content (Ca, Cu, Fe, Mg, Mn, Mo, P and Zn) was verified using soya bean flour (INCT-SBF-4) certified reference material. Recoveries of 92 to 117 % were obtained. The results are shown in Table 1.

The estimated limit of quantification for the method (LOQ) was calculated as 3.3-fold of the limit of detection (LOD).[32] The LOD and LOQ were calculated based on the measurement of the analytical signal of the digest blank assays (n = 15). The values of LOD and LOQ were (µg g^{-1}), respectively: As (0.7; 2), Ba (0.02; 0.06), Cu (0.03; 0.1), Cr (0.03; 0.1), Cd (0.02; 0.08), Co (0.02; 0.06), Fe (0.3; 1), Pb (0.1; 0.5), Mn (0.01, 0.04), Mo (0.07; 0.2), Ni (0.06; 0.2), Se (0.6; 2), Zn (0.3; 1). For the elements present at higher concentrations (major elements), the LOQ (mg g^{-1}) was, respectively: P (0.2; 0.6), Ca (0.003; 0.01), Na (0.02; 0.07), Mg (0.03; 0.1). The concentrations of the elements Cd, Co, Cr, Se and Pb were below the LOQ.

Table 1. Average values (n = 3) and standard deviations for certificates macro and trace elements (INCT-SBF-4) obtained by ICP OES after microwave assisted digestion

Elements	**Certified**	**Obtained**	**Recovery, %**
	(µg g^{-1})		
Cu	14.30±0.46	14.77±0.09	103
Fe	90.8±4.0	89.8±0.9	99
Mn	32.3±1.1	31.0±0.4	96
Mo	5.99±0.35	5.62±0.17	94
Zn	52.3±1.3	51.99±0.80	99
	(g $100g^{-1}$)		
K	2.423±0.083	2.223±0.072	92
Mg	0.3005±0.0082	0.2764±0.0059	92
P	0.6555±0.0335	0.7674±0.0143	117
Ca	0.2467±0.0170	0.2442±0.0002	99

Precision was evaluated based on the concentrations of the analytes present in higher amounts and traces and for eight real replicates of fresh legume samples. Coefficients of variation obtained as percentage ranges were 1.29-8.91 for pigeon pea; 0.75-15.6 for cowpea and 1.24-18.9 for *mangalô*.

Tables 2 and 3 show the mean concentrations and standard deviations for analytes in fresh samples and for samples thermally treated by conductive heating and by microwaves, as well as in the supernatants after cooking for the three legume species.

Table 2. Average concentration (mg $100g^{-1}$; n = 8) and standard deviation for trace elements in fresh legume beans (IN), (n = 3) after conductive heating (CH), beans cooked by microwaves (MW), supernatant liquid after conductive heating (SCH) and supernatant liquid after cooking by microwaves (SMW)

Samples	As	Ba	Cu	Fe	Mo	Mn	Ni	Zn
Pigeon pea IN	3.67±0.28	10.05±0.32	14.21±0.28[a,c]	46.80±0.61[c]	0.943±0.048	9.37±0.20	3.01±0.27[a]	30.56±0.80[a,c]
Pigeon pea CH	3.94±0.16[b]	9.51±0.47	11.05±0.30[a,b]	46.8±1.2[b]	0.81±0.13	8.96±0.31	1.918±0.041[a]	26.56±0.33[a]
Pigeon pea MW	3.30±0.16[b]	10.03±0.30	12.46±0.54[b,c]	50.5±1.9[b,c]	1.04±0.25	9.56±0.26	2.38±0.40	28.0±1.3[c]
SCH	<LOQ	<LOQ	0.221±0.037[b]	<LOQ	<LOQ	<LOQ	0.03±0.01	0.27±0.04
SMW	<LOQ	<LOQ	0.092±0.006[b]	<LOQ	<LOQ	<LOQ	<LOQ	0.024±0.001
Cowpea IN	3.02±0.39	<LOQ	4.69±0.16[a,c]	71.8±2.1	0.258±0.040	20.1±1.0[a]	1.698±0.013[a,c]	32.8±1.4
Cowpea CH	2.97±0.42	<LOQ	3.85±0.14[a]	71.36±0.82	<LOQ	18.09±0.71[a]	0.94±0.40[a,b]	32.23±0.48
Cowpea MW	3.15±0.84	<LOQ	3.90±0.33[c]	73.3±3.0	0.373±0.064	20.3±1.7	0.22±0.16[b,c]	33.2±1.5
SCH	<LOQ	<LOQ	0.018±0.0006[b]	<LOQ	<LOQ	0.054±0.004[b]	0.083±0.026	0.03±0.01
SMW	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.0095±0.0046[b]	<LOQ	<LOQ
Mangalô IN	<LOQ	1.74±0.32	6.38±0.18[a,c]	71.46±0.74	3.62±0.14[a]	23.34±0.28	<LOQ	30.45±0.42[a,c]
Mangalô CH	<LOQ	1.64±0.34	5.51±0.12[a,b]	68.1±2.7	2.38±0.22[a]	24.0±2.0	<LOQ	27.2±1.4[a]
Mangalô MW	<LOQ	1.85±0.10	5.84±0.08[b]	70.6±3.0	2.86±0.73	23.35±0.26	<LOQ	26.68±0.53[c]
SCH	<LOQ	<LOQ	0.0677±0.0095[b]	0.63±0.15	0.13±0.02	0.07±0.04	<LOQ	0.34±0.05[b]
SMW	<LOQ	<LOQ	0.0188±0.0028[b]	0.39±0.12	0.05± 0.02	0.047±0.014	<LOQ	0.15±0.04[b]

Note: "a" means that there is a significant difference between IN and CH; "b" means that there is a significant difference between CH and MW; “c” means that there is a significant difference between IN and MW

The presence of As, Cu, Fe, Mo, Zn, Mn, P, Ca, Na and Mg in the three species was verified. The element Ba was determined in pigeon pea and *mangalô* for the three conditions and, for cowpea, only fresh. The element Ni was not quantified in m*angalô* under the three conditions; however, it was detected in pigeon pea and cowpea. Nevertheless, selenium was detected in *mangalô* under the three conditions but at concentrations below LOQ.

Table 3. Average concentration (g 100g^{-1}; n = 8) and standard deviation for major elements in fresh legume beans (IN), and (g 100g^{-1}; n = 3) after conductive heating (CH), in beans cooked in microwave (MW), in liquid cover after conductive heating (SCH) and in liquid cover after cooking in microwave (SMW)

Samples	P	Ca	Na	Mg
Pigeon pea IN	0.3659±0.0075^{a}	0.1291±0.0021	0.01603±0.00051	0.1742±0.0036^{a}
Pigeon pea CH	0.333±0.011^{a,b}	0.1223±0.0038^{b}	0.0179±0.0014	0.1543±0.0030^{a,b}
Pigeon pea MW	0.370±0.014^{b}	0.1319±0.0044^{b}	0.01717±0.00094	0.1672±0.0029^{b}
SCH	(3.2±0.3)x10^{-3b}	(0.679±0.069)x10^{-3b}	(0.436±0.038)x10^{-3b}	(2.09±0.26)x10^{-3b}
SMW	(1.932±0.063)x10^{-3b}	(0.312±0.028)x10^{-3b}	(0.249±0.006)x10^{-3b}	(1.05±0.05)x10^{-3b}
Cowpea IN	0.589±0.019^{a}	0.1206±0.0053^{a}	0.0127±0.0002^{a}	0.1007±0.0026^{a,c}
Cowpea CH	0.5091±0.0079^{a}	0.1066±0.0019^{a}	0.0160±0.0010^{a}	0.1703±0.0016^{a}
Cowpea MW	0.538±0.0048	0.1142±0.0084	0.0136±0.0017	0.182±0.019^{c}
SCH	(3.77±0.87)x10^{-3b}	(0.367±0.067)x10^{-3b}	(0.383±0.031)x10^{-3b}	(3.31±0.15)x10^{-3b}
SMW	(6.18±0.12)x10^{-3b}	(0.815±0.052)x10^{-3b}	(0.06±0.008)x10^{-3b}	(1.59±0.36)x10^{-3b}
Mangalô IN	0.611±0.006^{a,c}	0.0696±0.0011	0.0146±0.0005	0.2137±0.0027^{a,c}
Mangalô CH	0.550±0.015^{a}	0.0667±0.0022^{b}	0.0143±0.0014	0.1918±0.0010^{a,b}
Mangalô MW	0.570±0.011^{c}	0.0728±0.0030^{b}	0.0153±0.0016	0.2042±0.0036^{b,c}
SCH	(6.42±0.58)x10^{-3b}	(0.232±0.087)x10^{-3}	(0.220±0.30)x10^{-3}	(2.92±0.53)x10^{-3b}
SMW	(3.40±0.49)x10^{-3b}	(0.125±0.063)x10^{-3}	(0.169±0.31)x10^{-3}	(1.45±0.48)x10^{-3b}

Note: "a" means that there is a significant difference between IN and CH;"b" means that there is a significant difference between CH and MW; “c” means that there is a significant difference between IN and MW

The cooking treatments (ordinary cooking, pressure-cooking and microwave cooking), in addition to improving digestibility, lead to a considerable decrease in antinutrients.

Microwave cooking could be recommended for legume preparation, since it reduces cooking time and improves nutritional quality, that is, better retention rates of both B-vitamins and minerals, reduction in the level of antinutritional factors, besides an increase in *in vitro* protein digestibility.[33]

The heating of beans can increase the protein and starch digestibility from 25-60 % (raw grains) to 85 % (cooked grains), depending on the species and cooking procedure.[34]

For comparison of mean concentrations, the t-test was used at 95 % confidence level. Significant differences ($p < 0.05$) between concentrations of the evaluated elements for fresh samples and samples treated by conductive heating and by microwaves are described in Tables 2 and 3.

Considering the three species studied, copper was the trace element that most frequently showed significant differences between the concentrations obtained in the fresh samples and sample submitted to thermal treatments. On other hand, for major constituent magnesium frequently showed significant differences between the concentrations obtained for fresh samples and samples submitted to thermal treatments.

Considering the pigeon pea legume, significant differences ($p < 0.05$) were observed between the mean concentrations of the trace elements Cu, Ni and Zn in fresh samples (IN) and heat-treated samples with conductive heating (CH) (Table 2). Cu, Fe and Zn showed a significant difference ($p < 0.05$) in the fresh samples (IN) and those treated in microwave oven (MW).

For major constituent elements P and Mg, the concentrations obtained in CH samples were significantly lower for IN sample when compared with CH treated samples (Table 3). However, for the major elements no significant differences in concentrations were observed after microwave treatment, which suggests lower leaching effect of these elements with the microwave assisted heating.

In general, the concentration of all evaluated elements decreased or showed not significant variations after thermal treatments. Only Fe had a different behavior, with significant increase of concentration after microwave thermal treatment, indicating greater retention in the legume beans when compared to other analytes or an artifact from cooking recipient. Silva *et al.* evaluated the mineral composition of different cultivars of raw and cooked beans. It was observed that the contents of P, K, Mg, S, Mn did not vary for two varieties of beans. However, the contents of Ca, Na, Fe increased and Cu, Zn reduced after cooking.[35]

For the cowpea specie, significant differences ($p < 0.05$) were observed for the minor elements Cu, Mn and Ni and for all major elements (P, Ca, Na and Mg) when comparing the mean concentrations for fresh samples (IN) and heat-treated samples by conductive heating (CH). The concentration of Na was the only one significantly higher after conductive heating treatment and with slight increase after microwave heating, so this can be related with extraction of this element from heating vessel. Similar significant differences were observed comparing Cu and Ni levels for fresh samples (IN) and those treated in microwave oven (MW) as well as for Ni comparing samples treated by conductive heating (CH) and by microwaves (MW). The soluble chemical elements of high molecular weight species present in the fresh pea were completely removed by boiling. On the other hand, low molecular weight species (eg nickel, zinc and molybdenum) were more stable to heat treatment.[36]

Considering the *mangalô* specie, for IN and CH treated samples, concentrations of Cu, Mo and Zn exhibited significant difference ($p < 0.05$) and similar behavior were observed for P and Mg. For MW samples, Cu was the only trace element that showed significant differences ($p < 0.05$) in concentration, when compared to IN samples but for major constituent this pattern was also observed for P and Mg. Only the concentrations of the larger elements Ca and Mg and the trace elements Cu and Zn presented significant differences between CH and MW, with lowest concentrations for CH samples, which may indicate higher leaching effect by this last thermal processing.

Similar behavior obtained for all legume species can be described by lower concentrations of elements frequently obtained by CH treatment which suggest that this heating process promotes higher leaching of elements. The leaching effect due to heating seems to be more pronounced for pigeon pea compared to cowpea and *mangalô*; the latter was the least affected.

In general, a reduction in micronutrient concentration was observed after heat treatments (Table 2). However, a significant increase in Fe, Na and Mg concentrations was observed for pigeon pea and cowpea, respectively, after CH, which can suggest that the leaching effect also affects the heating vessel as a potential contamination source for these elements.

As for major elements (Table 3), variations in analyte concentrations after thermal treatments were observed, when compared to the fresh sample. The highest variation was observed in Mg concentrations for cowpea after CH and MW.

A reduction in the levels of minerals Ca, Cu, Fe, K, P and Mg in *mangalô* and cowpea samples processed thermally was reported in a recent study.[37] However, higher concentrations of Ni and Ba were observed in processed *mangalô* samples.In pigeon pea samples processed in a microwave oven, an increase was observed in Ca, Mg, Mn, P, and Zn. Similar behavior was observed in another study, in which boiling caused an increase in Mn and P concentrations in beans and chickpeas, however there was a significant loss of K and Mg.[38]

In cowpea samples processed in a microwave oven, the Ca content was 19 % higher at the longest cooking time. The thermal process caused loss of Ca (0.75 %) content in cowpea.[39] The loss of divalent metals may be due to their binding to proteins and formation of phytate-cation, in addition to leaching.[40]

Mosisa and Tura determined the effect of processing (traditional cooking and pressure cooking) on the mineral composition of *Lablab purpureus* L., which is an indigenous legume in Ethiopia.[41] The results showed that the processing methods caused a significant ($p < 0.05$) difference in all minerals (Ca, P and Zn), except the iron (Fe) content, which was retained during processing. The loss of this element was due to their binding to proteins and formation of phytate-cation, besides leaching during the cooking treatment.[37]

On the other hand, Cu, P, Ca, Na and Mg in the cooking liquid were determined in the two cooking conditions for the three bean species. The presence of Mo was not verified in the cooking liquid for pigeon pea and cowpea, in the two thermal procedures. However, Mo was determined in *mangalô* samples. The element Fe was detected in the cooking liquid of *mangalô* in the two thermal procedures, and cowpea in the cooking procedure using a microwave. However, it was below the limit of quantification for the pigeon pea species in the two procedures and, in the cowpea, for the conductive thermal cooking procedure. The element Zn was detected only in the cowpea species, and Mn, in pigeon pea for the microwave thermal procedure.

3.2. Chemometric data analysis

Figure 1 shows PCA score plot, where each point represents a sample. Evaluating this Figure, it is possible to visualize that the samples pigeon pea, *mangalô* and cowpea beans tend to form distinct groups. From this graph, it is also possible to infer that the groups of beans with greater differences in mineral composition were pigeon pea and *mangalô*, while cowpea beans occupy an intermediate position.

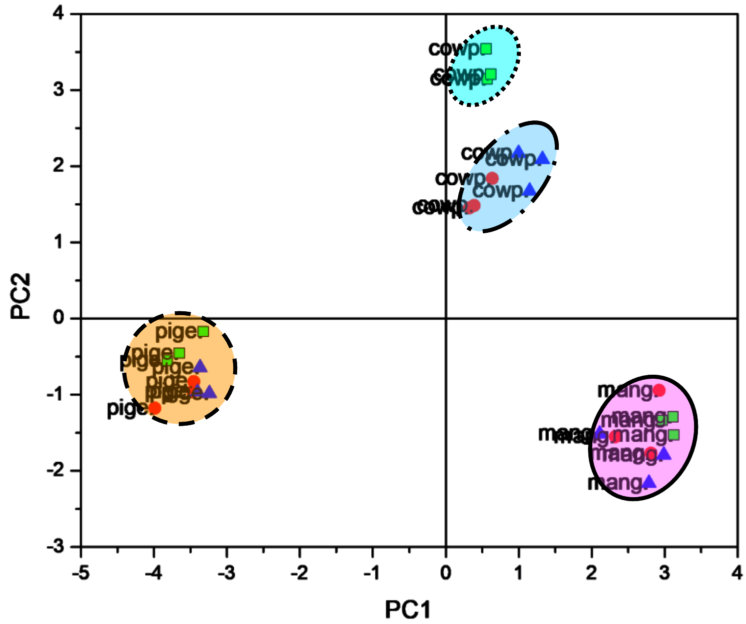

Figure 1. PCA score plot of evaluated elements in fresh legume beans of cowpea, pigeon pea, mangalô beans (green), after conductive heating (red) and cooked by microwaves (blue)

The score plot (Figure 1) also shows that, for the legumes pigeon pea and *mangalô*, in general there are no relevant changes in the profile of elemental constituents, when comparing them fresh with the beans cooked in microwaves or with the use of a conductive heating, as can confirmed by sample overlap in score plot. On the other hand, for cowpea beans there are more expressive differences in the elemental composition profile after the heat treatment (Figure 1). This shows that, except for cowpea, the results of PCA points out that the proportions among the elementary constituents are not extensively changed after the thermal treatments. When comparing the different thermal treatments used, it was not possible to visualize significant differences between these types of samples by PCA.

By analyzing the graph of the scores (Figure 1) with the loadings (Figure 2), it is possible to identify which groups of elements are most strongly associated with the types of beans. In this way, elements of loading plot at the same quadrant of a sample grouping of score plot tend to be at higher concentrations.

Thus, *mangalô* had higher average concentrations and tended to relate more strongly to Mg and Mo, while the pigeon pea beans samples were mostly related to Cu, Ba and Na, and cowpea beans with Zn, Fe, P and Mn (Figure 2).

The chemical elements characteristic of each legume species studied can be used in studies of identity patterns. It is already known that the mineral characteristics of the legume depend on genetic and environmental factors.[42] However, this study adds another factor that contributes to the understanding of the behavior of minerals in the green bean of the legume, which deals with the migration or retention of the mineral when the grain undergoes heat treatment with cooking water and different heating sources.

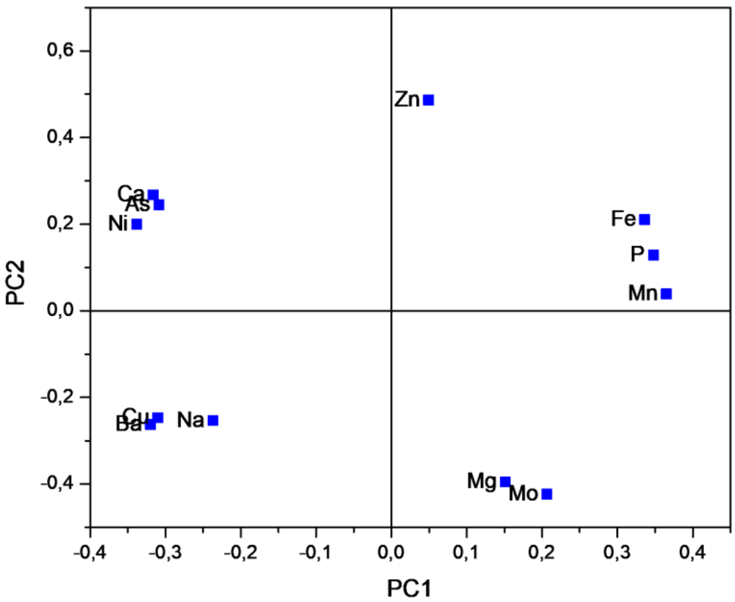

Figure 2. PCA loading plot of evaluated elements in legume beans of cowpea, pigeon pea and *mangalô*

Avanza *et al.* also used the principal component analysis (PCA) for the separation of four different varieties of cowpea, submitted to different thermal treatments (cooking, autoclaving and soaking in alkaline solution). The antinutritional factors (phytic acid and total phenolic compounds) contents contributed importantly to the construction of the PC1. Thermal treatments and soaking in alkaline solution contributed to the decrease of antinutrients in cowpea varieties. The authors also evaluated minerals looses by termal treatment. They observed decrease in K, Mg and Fe content (20 and 50 %) because of thermal treatment, especially autoclaving (20 min and 30 min). Thus, the PCA allowed the characterization of the effect of thermal and non thermal treatments, similar to the results found in this study.[43]

4. Conclusions

Based on the obtained results, it was observed that, in general, cowpea was the specie with largest significant changes of elemental composition when heat treatment is performed. On other hand, *mangalô* was the specie with slight more evident similarities between composition of fresh and heat treated samples while pigeon pea are at an intermediate position near *mangalô*. The elements whose concentrations changed significantly and more frequently, regardless of legume species, were copper and magnesium. The characteristic chemical elements of each of these legume species can be used in studies of the identity patterns of these foods. The chemometry was effective to evaluate mineral retention and separate the three types of beans.

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