Geographical Characterization of Beans Based on Trace Elements After Microwave-Assisted Digestion Using Diluted Nitric Acid

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Abstract In this paper, multivariate optimization was applied for the development of microwave-assisted digestion using diluted nitric acid for the determination of essential (Ca, Co, Cr, Cu, K, Mg, Mn, Mo, Ni, P, Se, and Zn) and nonessential (Al, As, Ba, Pb, and Sr) elements in bean samples. Three variables (nitric acid concentration, temperature, and processing time) were regarded as factors in the optimization study. The optimum working conditions were as follows: 4.0 mol L^{-1} nitric acid, 190 °C, and a 15-min reaction time for step 4, which required a microwave heating program. The proposed procedure was then applied to bean samples from small producers of Bahia and to samples acquired from markets of Salvador, Bahia, Brazil. Pattern recognition techniques were applied to the data sets to characterize samples in relation to their geographical origin, species, and production mode. Some differences were found in the samples taken from the northeastern, central, and southern parts of Brazil.

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Introduction

Leguminous plants are very important internationally for nutritional and socioeconomic reasons, especially in Third World countries where they present a relatively accessible source of nutrients, such as Ca, Fe, and Zn.

Brazil is the largest world producer and consumer of beans, reaching a production of 3.79 million tons in 2010/2011. The main bean-producing states are Parana, Minas Gerais, São Paulo, Goiás, and Bahia, which account for more than 65 % of national production (Richetti et al. 2011).

The common bean (*Phaseolus vulgaris* L.), cowpea bean (*Vigna unguiculata* L. Walp), the pigeon pea (*Cajanus cajan* L.), and Mangalô bean (*Lablab purpureus* L. Sweet) are of great importance for producers, especially in the northeastern part of Brazil. However, the quantification of elements in vegetal samples, such as bean seeds, can disclose the presence of inorganic contaminants that can lead to human health problems (Parengam et al. 2010).

Additionally, the determination of the geographical origin of commodities and food products is becoming an increasingly active research area, focused on both

geographical authenticity and adulteration of foods (Anderson and Smith 2002). Chemical analyses in conjunction with pattern recognition methods provide interesting tools for the study of the quality, geographical origin, and genetic characteristics of food products (Patras et al. 2011; Arlorio and Brandolini 2011; Meziane-Assami et al. 2012). Recent studies have employed these methods to classify foods using mineral composition in terms of major and trace elements (Sola-Larrañaga and Navarro-Blasco 2006; Conti et al. 2007; Tormen et al. 2011).

High variability is observed in the mineral composition of legume seeds (Pinheiro et al. 2010). On the other hand, since the concentrations of some important elements are low, it is advisable to use sensitive techniques, such as inductively coupled plasma optical emission spectrometry (ICP OES), which allows the simultaneous determination of major and minor constituents, without any changes in the operational parameters, as well as providing the desirable sensitivity (Montaser and Golightly 1992). So, the use of pattern recognition tools combined with multielement techniques is advantageous.

Nowadays, one of the most important trends in solid sample preparations is the search for environmentally friendly processes. Based on green chemistry principles, the use of diluted acids for digestions led to relevant advantages, such as cost reduction, production of smaller amounts of residues, and reduction of blank values. In this sense, a number of recent applications demonstrate the potential of the use of diluted acid in microwave-assisted digestions (Araujo et al. 2002; Gonzalez et al. 2009; Castro et al. 2009; Bizzi et al. 2010, 2011a, b; Brito et al. 2012). In another work, the use of diluted nitric acid associated with pressurized oxygen atmosphere was proven to be feasible for milk powder digestion, reducing the volume of reagents and the amount of digestion residues. Using digestion vessels under oxygen pressures ranging from 7.5 to 20 bar, it was possible to digest sample masses of up to 500 mg with an amount equivalent to only 0.86 mL of concentrated nitric acid (Bizzi et al. 2011a, b). Recently, a procedure using diluted nitric solutions for the decomposition of three tomato species (Khaki, Cherry, and Italy) was proposed for the determination of Al, Ba, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Se, Sn, Sr, V, and Zn by ICP OES and inductively coupled plasma mass spectrometry (Bressy et al. 2012). In all these works, the digestion efficiency was established by measuring residual carbon content (RCC) and residual acidity (RA).

In this work, microwave-assisted digestion using the diluted nitric acid procedure for trace elements determination in bean samples by ICP OES was investigated in a multivariate way. Three experimental factors (acid concentration, temperature, and processing time) were used to determine the optimization of the digestion procedure. A factorial experiment was adopted to obtain the elements' concentrations at the lowest possible working conditions with good precision and accuracy; these factors were determined in the shortest possible time frame and with the smallest viable consumption of reagent to implement the method at routine food quality control laboratories. Afterwards, similarities and differences between the elements were highlighted by principal component analysis (PCA) and hierarchical cluster analyses (HCA) to a preliminary exploratory data approach with the objective of characterizing samples in relation to their geographical origin and species.

Materials and Methods

Sample Collection

A total of 103 samples (fresh or dried beans) for the species *P. vulgaris* L. (black bean, white bean, and kidney bean), *V. unguiculata* L. Walp (cowpea), *C. cajan* L (pigeon pea), and *L. purpureus* L. Sweet (Mangalô) were collected.

Part of the samples (56 samples) was collected from markets of Salvador City. The samples had its origin identified directly with market suppliers. The bean samples were found to originate from the following Brazilian states: Minas Gerais (MG), Paraná (PR), Goiás (GO), Bahia (BA), and São Paulo (SP). The region defined by the code ARG indicated that the samples originated from Argentina. These are distributed in the following regions: 7 (MG), 10 (PR), 4 (GO), 16 (BA), 7 (SP), and 7 (ARG). Also, the geographical origins of five collected bean samples (kidney bean) are unidentified.

The other 47 samples were collected from small farms of 8 economic regions of Bahia, Brazil: Metropolitan Region of Salvador; Recôncavo (Amargosa City); northeast of Bahia (Jeremoabo, Paulo Afonso, and Santa Brígida cities); Paraguaçu (Feira de Santana City); southwest of Bahia (Jequié and Vitória da Conquista cities); Chapada Diamantina (Rio de Contas and Seabra cities); the middle of São Francisco River (Carinhanha City); and Serra Geral (Caculé and Guanambi cities) (Fig. 1).

Samples collected from the producers of the States of Bahia are distributed as follows: 2, Metropolitan Region of Salvador; 3, Recôncavo; 4, northeast of Bahia; 7, Paraguaçu; 10, southwest of Bahia; 6, Chapada Diamantina; 4, the middle of São Francisco River; and 10, Serra Geral. Upon collection, all bean samples (1,000 g) were placed into clean plastics bags, labeled, and stored at 4–8 °C until the sample preparation step for a period of 12 to 48 h.
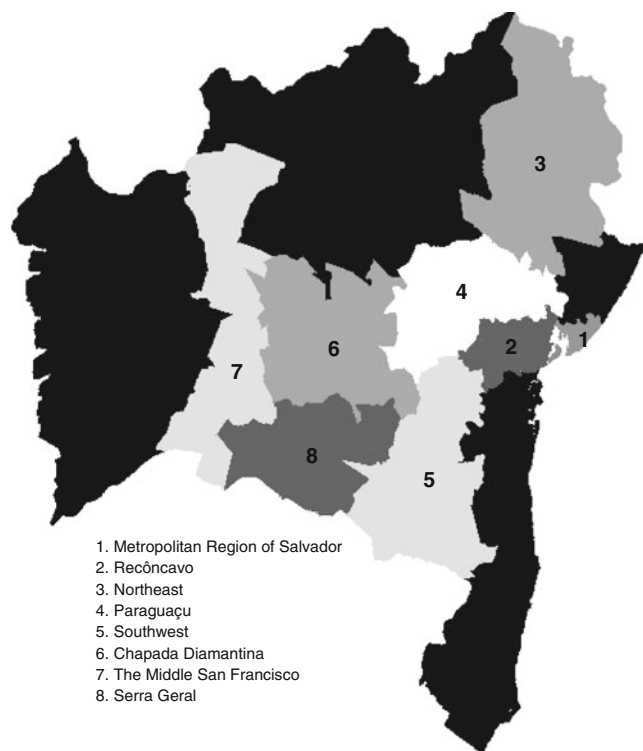
Fig. 1 Map of Bahia, Brazil, showing the geographical location of the eight study regions

1. Metropolitan Region of Salvador
2. Recôncavo
3. Northeast
4. Paraguaçu
5. Southwest
6. Chapada Diamantina
7. The Middle San Francisco
8. Serra Geral

Reagents, Standard Solutions, and Reference Materials

In the experimental design, nitric acid 65 % *w/w* and hydrogen peroxide (30 % *w/w*; Merck, Darmstadt, Germany) were used. All solutions were prepared using analytical grade reagents and deionized water (Milli-Q water, 18.2 MΩ cm; Millipore, Bedford, MA, USA). All glassware and polypropylene flasks were washed with neutron soap, soaked in 10 % *v/v* nitric acid (Merck), and rinsed with deionized water prior to use. High-purity analytical stock solutions of 1,000 mgL^{-1} (Titrisol®, Merck) of each element were used daily to prepare the multi-element reference analytical solutions. Trace elements determination by ICP OES was performed with external calibration. The acidity of the standard solutions at different concentration levels were matched to the acidity of the final solution obtained after digestion. The comparison of the slopes of the analytical calibration curves obtained using the external calibration and analyte addition methods indicated no significant differences at the 95 % confidence level, suggesting no detectable matrix effects for Ba, Ca, Co, Cr, Cu, K, Mg, Mn, Mo, Ni, P, Pb, Se, Sr, and Zn determinations.

Because of the lack of certified bean reference materials or similar certified reference material (CRM), the accuracy of the proposed procedure was verified by the analysis of CRMs: apple leaves NIST 1515 and spinach leaves NIST 1570a (National Institute of Standards and Technology, Gaithersburg, MD, USA).

Instrumentation

ICP OES with an axially viewed configuration (VISTA PRO, Varian, Mulgrave, Australia) was used for the determination of elements (Ba, Ca, Co, Cu, Fe, K, Mg, Mn, Mo, Ni, P, Pb, Se, Sr, and Zn). The conditions of the instrumental parameters used were as follows: RF generator (40 MHz), power (1.3 kW), plasma flow (15 Lmin^{-1}), auxiliary flow (1.5 Lmin^{-1}), and nebulizer flow (0.7 Lmin^{-1}). The V-groove nebulizer and Sturman–Masters chamber were used to put the system solutions in the spectrometer, while Ba II, 493.408 nm; Ca, 422.673 nm; Co II, 230.786 nm; Cu II, 213.598 nm; Fe II, 238.204 nm; K I, 766.491 nm; K, 769.897 nm; Mg II, 280.270 nm; Mn II, 257.610 nm; Mo II, 202.032 nm; Ni II, 221.645 nm; P I, 213.618 nm; Pb II, 220.353 nm; Se I, 196.026 nm; Sr II, 421.552 nm; and Zn II, 202.548 nm were used as atomic emission line (I) and ionic emission line (II). A ball mill (model 8000M; Spex Sample Prep, Metuchen, NJ, USA) with a tungsten carbide vial set and tungsten carbide ball was used for grinding samples. An analytical balance (Sartorius Ag Gottiengen, Germany) was used to weigh the bean samples, and a microwave oven (model Ethos EZ; Milestone, Sorisole, Italy) equipped with 120 mL Teflon TFM vessels was used for the digestion of the bean samples.

Sample Preparation

The fresh beans were washed with deionized water and triturated in a domestic food processor, which resulted in a homogeneous mass. Then, samples were stored in plastic bags and kept in a freezer (−10 °C). After at least 72 h of freezing, the material was lyophilized (Terroni Fauvel LT 1000/8, São Carlos, SP, Brazil).

The dried beans were grinded in a ball mill. The first aliquot of grinded samples was discarded. Three cycles of 2 min were used to grind samples. Aliquots of grinded samples were sieved in nylon sieves of 300 μm. The bean flour obtained was stored in decontaminated dried plastic bottles. These bottles were stored in desiccators under room temperature (22–25 °C) for later analysis of the samples.

Digestion Procedure

After grinding and screening the samples, 500 mg of each sample was digested via a microwave-assisted procedure. Concentrated acid, HNO$_3$ 65 % (*w/w*) (7.0 mL), and 1.0 mL of H$_2$O$_2$ 30 % (*v/v*) in TFM closed vessels were used for the digestion. The heating program was performed in four successive steps. In the first step, the temperature was linearly increased to 90 °C in 4 min with a maximum power of 1,000 W; in the second step, the temperature was kept at 90 °C for 2 min; the third step consisted of increasing the

temperature linearly to 180 °C in 8 min, whereas in the fourth step, the temperature was kept at 180 °C for 15 min. The analytes in the final solution were determined by ICP OES.

Experimental Design for the Applied Digestion

The following three variables and levels were chosen to optimize conditions for trace elements recovery: (1) nitric acid concentration (2.0, 4.0, and 6.0 mol L^{-1}); (2) temperature (170, 190, and 210 °C) for the microwave heating program; and (3) time (12, 15, and 24 min). The following parameters were based on previous studies (Santos et al. 2008): volume of H$_2$O$_2$ 30 % v/v (1.0 mL), sample mass of beans (500 mg), particle size (300 μm), volume of the nitric acid solution (7.0 mL), and final volume of the solution (15.0 mL).

The responses were obtained by evaluating the recovery of the analytes, RCC, and RA in the final solutions compared to reference values obtained after the microwave-assisted acid digestion using concentrated acid. The overall response (OR) was adopted for establishing suitable experimental conditions for the simultaneous extraction of all analytes (Santos et al. 2009). The statistical significance of the variables was studied, and the interactions among them were evaluated by applying the analysis of variance (ANOVA) using the Statistica 7.0 software package (StatSoft®, Tulsa, OK, USA).

Data Analysis and Pattern Recognition Tools

Fifteen elements (Ba, Ca, Cu, Co, Fe, K, Mg, Mn, Mo, Ni, P, Pb, Se, Sr, and Zn) were selected as chemical descriptors. Each bean sample was considered as an assembly of 17 variables, which constituted their chemical descriptors. For bean samples acquired in markets, a data matrix of 16 columns and 170 rows was built; the mean of each duplicate analysis was considered one sample. Another data matrix of 139 rows and 17 columns was built for bean samples directly acquired from producers. These matrices were used for the chemometric calculations.

Data analysis was performed using the software Statistica 7.0. Pattern recognition methods were applied to the data sets according to the discussion in the succeeding paragraph. Autoscaling was used as a data preprocessing step in this study, where each variable (each column of the data matrix) was mean-centered and then divided by its standard deviation.

Pattern recognition methods were applied to the data collection: PCA as an unsupervised classification method and HCA as an unsupervised learning method. As preprocessing, the data were autoscaled because of the differences in the order of magnitudes between the variables. The measurement of the similarity is based on the squared Euclidean distance (Daszykowski et al. 2001; Smoliński et al. 2002).

The clustering method used was Ward's method, which considers in each step the heterogeneity or deviance of every possible cluster that can be created by linking two existing clusters (Meloun et al. 1992). PCA results were validated using the cross-validation method. As the methods used are nonparametric, they do not make suppositions about the statistical distribution behind the data set, and any evaluation of the normal distribution of the data (Gaussian) is then necessary (Sharaf et al. 1986).

Results and Discussion

Optimization of the Procedure

The extraction efficiency, expressed as percentage, was calculated as the ratio between the contents of the elements obtained by microwave-assisted digestion using diluted acid (MAD) and those obtained by microwave-assisted acid digestion using concentrated acid (MAC), based on the following equation:

$$\%\text{Recovery} = [\text{MAD}]/[\text{MAC}] \times 100$$

Recoveries reached the following ranges for trace elements (in percent): Ba, 99 to 111; Cu, 94 to 119; Mn, 92 to 101; Fe, 81 to 93; Mo, 75 to 92; Ni, 78 to 91; Sr, 115 to 125; V, 91 to 104; and Zn, 93 to 106. For the macroelemental recoveries, percentages varied in the following ranges: Ca, 91 to 111; Mg, 97 to 112; K, 94 to 111; and P, 96 to 112.

The efficiency of the digestion was evaluated in terms of RCC and RA. The RCC values varied from 11 to 41 % and the RA values varied from 0.4 to 2.0 mol L^{-1}. The use of more concentrated HNO$_3$ solutions led to a slightly better oxidation of the organic matter; as expected, this result showed that the amount of acid influenced the oxidation process. In general, 2 to 50 % of the original carbon compounds that did not decompose may have remained in the open systems when the oxidizing agent was nitric acid.

In experiments 1, 8, 13, and 20, high levels of RCC were obtained. These values were similar to those obtained in systems with conductive heating at atmospheric pressure (Santos et al. 2008). The efficiencies to reduce acidity (ERA) and carbon concentration (ERC) were calculated according to Eqs. 1 and 2, where C_i is the initial acid concentration:

$$\text{ERA} = 100(C_i - \text{RA})/C_i \tag{1}$$

$$\text{ERC} = 100 - \text{RCC} \tag{2}$$

Greater values of ERC were obtained in experiments 3, 5, 6, 7, and 9, when compared to experiments 1, 2, 4, and 8 (Fig. 2).

According to the ANOVA for the response variable RCC, the concentration of the nitric acid solution (3) and temperature (2) of steps 3 and 4 of the microwave heating program were significant at 95 % confidence level (MQep^2=5.3 %; r^2=0.9710). Furthermore, the curvature factor was significant (coefficient of curvature= 5.98±1.28), which is visible on the contour graph shown in Fig. 3.

The interactions between time and temperature and between time and concentration were also significant. Thus, it can be argued that RCC was strongly dependent on the initial acid concentration. A reduction of 26 % for RCC with an increasing digestion time for samples of biological materials digested in a microwave cavity, with 2.0 mL HNO$_3$ 65 % and 1.0 mL of H$_2$O$_2$ 30 %, was reported in the literature (Gouveia et al. 2001). However, a stronger effect on RCC with reductions of up to 45 % was attributed to the increase in temperature.

The variables concentration and temperature were significant ($P<0.05$) for RA, interaction time, and temperature (MQep mol^2=0.0013 L^{-2}; r^2=0.9988). The interaction of three variables (1×2×3) was significant. However, the curvature factor was not significant, indicating that the linear model fits the experimental data set best. The concentration of nitric acid was the most significant parameter and the increase in temperature and time had a relatively small influence on the reduction of acidity. Similar results were observed for the interaction of time and temperature, i.e., a longer duration of the heating program combined with a higher temperature resulted in lower RA.

The equations obtained from the regression with significant terms were calculated (Eqs. 3 and 4); the

variable C represented the nitric acid concentration (in moles per liter); t, time (in minutes); and T, temperature (in degrees Celsius).

$$RCC = 22.3 \pm 0.6 - 7.76 \pm 0.57C - 5.97 \pm 0.57T \quad (3)$$
$$- 1.78 \pm 0.57tT + 1.65 \pm 0.57tC + 3.45$$
$$\pm 0.57TC$$

$$RA = 1.39 \pm 0.01 + 0.02 \pm 0.01t + 0.073 \pm 0.01T \quad (4)$$
$$+ 0.86 \pm 0.01C + 0.04 \pm 0.01tT + 0.02$$
$$\pm 0.01tTC$$

The results were also analyzed by considering the OR. Temperature and time were significant ($P<0.05$) and associated with a positive value. The curvature factor was also evaluated and was not significant (MQep= 0.045; r^2=0.8610). Equation 5 was obtained by the regression that contained the significant terms and the estimated standard errors of coefficients considered by RG, where t is a time variable (in minutes) and T is the temperature (in degrees Celsius).

$$RG = 12.9 \pm 0.1 + 0.207 \pm 0.053t + 0.271$$
$$\pm 0.053T - 0.229 \pm 0.053tT \quad (5)$$

Temperature and heating time closer to the upper level resulted in more quantitative recoveries. This result was consistent with previous studies reported in the literature, where the digestion time and temperature had a significant effect on the residual concentration of nitric acid (Saavedra et al. 2004). The interaction between time and concentration also significantly enhanced the result observed for the effect of the time variable and indicated that the recovery of analytes, in general, was favored when using longer heating times.

However, recoveries above 95 % were obtained for most analytes at the midpoint of the design; this condition was recommended when taking the security and the lifetime of materials and equipment into account. Moreover, lower relative standard deviations (RSD; <2 %) in the experiments of the central point of the factorial design (experiment 9) for all elements (except Ni, 2.8 %) indicated an adequate repeatability at this experimental condition for the extraction of the analytes.

The variables investigated in the factorial design study were significant ($P<0.05$) for the recoveries of Mn and Zn, whereas for the extraction of Ni, Ca, and Mg, these variables were not significant. The variable temperatures of steps 3 and 4 showed greater significance for Ba, Cu, Fe, S, and Zn. The time variable of the baseline in step 4 was significant for Fe, K, and P. The interaction of the acid concentration and heating time was significant for Ba, Fe, Mn, Mo, Sr, K, P, and Zn.
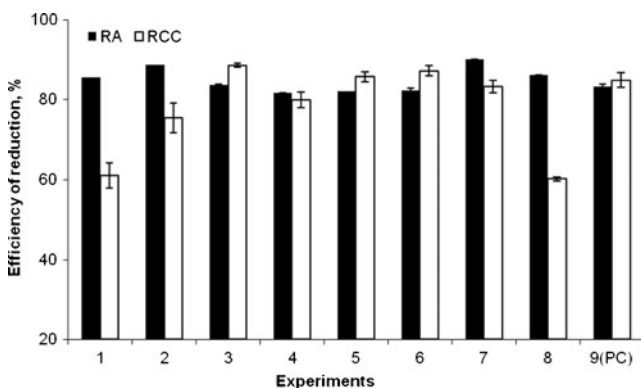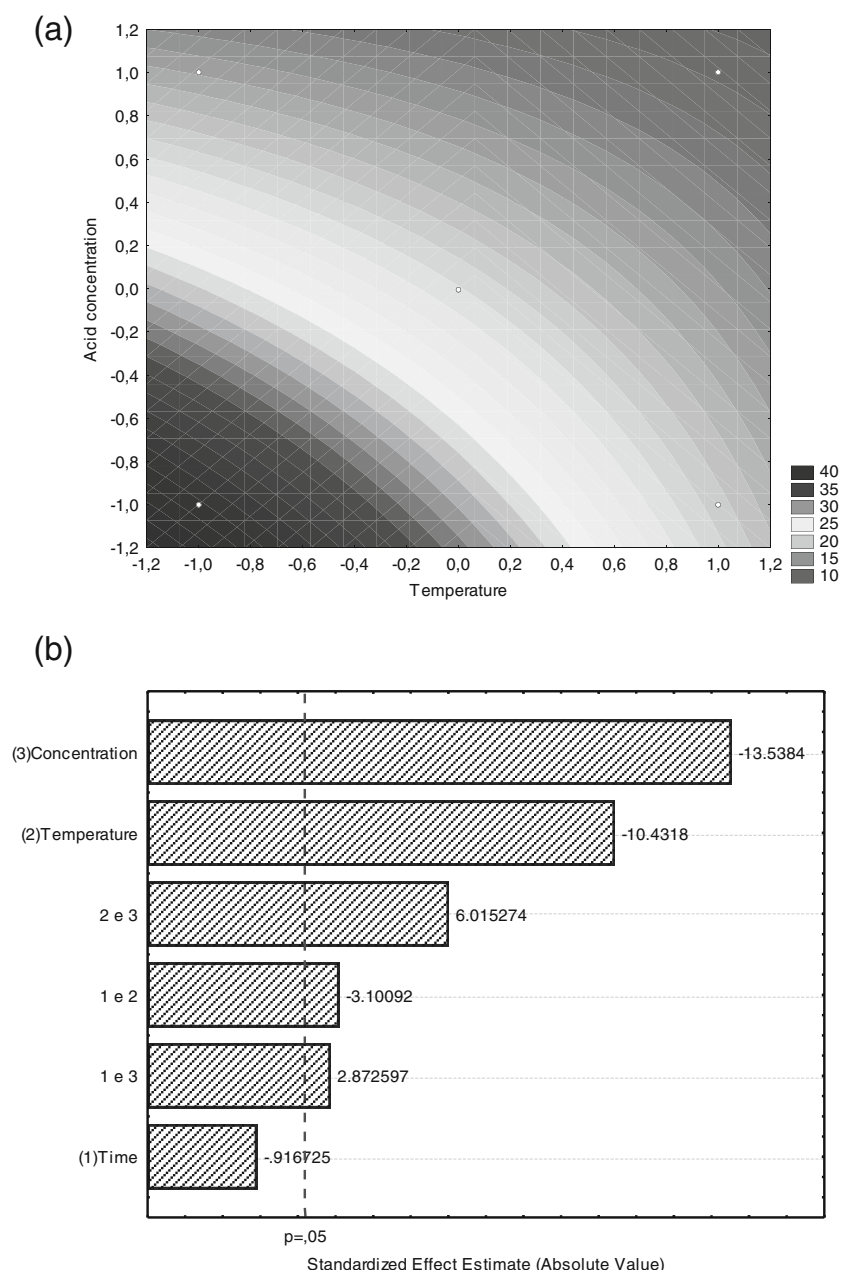

Fig. 2 Efficiency of acid and carbon reduction (in percent) for each test of factorial design applied to the digestion in a microwave oven cavity

Fig. 3 Contour (**a**) and Pareto diagram (**b**) compared to RCC (percent, mass), obtained by factorial design applied to the digestion in a microwave oven cavity

The working conditions were established as the optimum values determined for each analyte, where the robustness of the procedure was taken into consideration. Finally, the optimized experimental conditions consisted of 7.0 mL of 4.0 molL^{-1} nitric acid and 1.0 mL of hydrogen peroxide 30 % (*w/v*). The heating program was performed in four successive steps: (1) 4 min to reach 90 °C; (2) 3 min at 90 °C; (3) 10 min to reach 190 °C; and (4) 15 min at 190 °C.

Analytical Figure of Merit

The limits of quantification (LOQ) were estimated using the RSD for ten consecutive measurements of the blank and the background equivalent concentration (Thomsen et al. 2003). The LOQ values were determined as follows (in micrograms per gram): Ba, 0.22; Ca, 9.0; Co, 0.56; Cu, 0.58; Cr, 0.61; K, 3.7; Mg, 0.58; Mn, 0.08; Mo, 0.79; Ni, 0.99; P, 2.8; Pb, 2.2; Se, 4.1; Sr, 0.14; and Zn, 4.0.

The precision, in terms of repeatability (*n*=10) for the optimized conditions, was evaluated. Values of %RSD were compatible with a conventional digestion method: Ba, 1.6; Ca, 2.1; Cu, 4.3; Co, 16; Cr, 14; Fe, 1.2; K, 2.2; Mg, 1.5; Mn, 1.1; Mo, 6.1; Ni, 4.8; P, 2.1; Se, 18; Sr, 1.5; and Zn, 1.3.

The accuracy of the procedure for the determination of elements was verified by the analysis of the two CRMs.

Table 1 The determination of elements (in micrograms per gram) compared to CRMs

Element	CRM 1515		CRM 1570a	
	Certified value	Found	Certified value	Found
Ca[a]	1.526±0.015	1.508±0.007	1.527±0.041	1.500±0.031
K[a]	1.610±0.02	1.482±0.044	2.903±0.052	3.044±0.035
Mg[a]	0.271±0.008	0.264±0.006	0.89[b]	0.827±0.007
P[a]	0.159±0.011	0.160±0.005	0.518±0.011	0.528±0.005
Al	286±9	237±12	310±11	215±7
As	0.038±0.007	<LOQ	0.068±0.012	<LOQ
Ba	49±2	43.7±0.2	–	3.56±0.15
Co	–	<LOQ	0.39±0.5	<LOQ
Cu	5.64±0.24	5.17±1.59	12.2±0.6	10.9±0.7
Fe	83±5	69.0±1.1	–	246±4
Mn	54±3	49.0±0.5	75.9±1.9	70.9±1.0
Mo	0.094±0.013	<LOQ	–	<LOQ
Ni	0.91±0.12	1.10±0.16	2.14±0.10	2.07±0.29
Pb	0.470±0.024	<LOQ	0.20*	<LOQ
Se	0.050±0.009	<LOQ	0.117±0.009	<LOQ
Sr	25±2	23.34±0.19	55.6±0.8	50.7±0.6
V	0.26±0.03	0.27±0.05	0.57±0.03	0.50±0.13
Zn	12.5±0.3	11.3±0.1	82±3	71.8±8.1

[a] Concentration in percent (w/w)
[b] Reference value not certified

Table 1 shows that the determined values were consistent with the certified values. The paired t test at a 95 % confidence level showed that there was no significant difference between the means of the certified and determined values for most analytes under investigation. The lowest values of extraction efficiency were obtained for Al (69 %) and Fe (83 %) compared to the CRM 1570a reference sample.

Table 2 Comparison of the mineral contents (in micrograms per gram) among bean samples from small farmers of Bahia, Brazil

Element	Common bean		Pigeon		Mangalô	Cowpea		
	Kidney	Brown	Green and fresh beans	Dry and mature beans	Green and fresh beans	White	Brown	Red
Ba	<LOQ–10	1.2–2.0	2.3–13	0.9–36	0.8–4	<LOQ–2.2	0.2–12	<LOQ–0.2
Co	<LOQ–4.6	0.8–2.0	0.1–0.4	<LOQ	<LOQ	<LOQ–1.6	<LOQ–0.7	1.9–2.3
Cu	3.4–9.1	6.4–6.8	10–11	5.7–13	6.7–9.9	3.1–5.8	4.2–5.5	3.7–4.4
Fe	45–65	54–61	31–37	36–57	66–73	41–56	44–69	52–53
Mn	16–20	13–16	11–14	9.7–17	23–34	9.3–21.4	14.3–23.0	11–19
Mo	0.7–5.9	0.9–5.2	<LOQ–1.6	0.3–4.5	0.7–2.6	<LOQ–4.1	<LOQ–3.1	0.8–23
Ni	<LOQ–1.3	<LOQ–0.75	<LOQ–6.7	1–5.4	<LOQ–1.6	<LOQ–2.9	<LOQ–3.4	<LOQ
V	<LOQ	2.9–5.5	1.6–17	1.3–28	1.6–3.1	0.5–4.9	0.9–12	0.8–4.1
Sr	2.4–14	3.5–4.1	2.6–31	3.8–4.8	3.8–4.8	3.3–4.8	3.5–4.0	3.7–4.2
Zn	23–32	32–28	30.2–33.7	31–40	32.7–40.1	38–47	23–44	32–36
Ca[a]	0.051–0.15	0.048–0.10	0.051–0.081	0.067–0.11	0.063–0.069	0.040–0.074	0.038–0.067	0.037–0.10
K[a]	1.3–1.6	1.5–1.6	1.5–1.6	1.5–2.0	1.5–1.8	1.2–1.4	1.3–1.5	1.5–1.6
Mg[a]	0.15–0.19	0.14–0.15	0.10–0.12	0.13–0.20	0.19–0.22	0.15–0.19	0.14–0.17	0.15–0.17
P[a]	0.33–0.53	0.34–0.56	0.21–0.34	0.40–0.44	0.47–0.58	0.35–0.48	0.22–0.43	0.37–0.49

LOQ limit of quantification

[a] Concentration in percent (w/w)

Table 3 Comparison of mineral contents (in micrograms per gram) among bean samples acquired in markets of Salvador, Bahia, Brazil

Element	Common beans			Cowpea
	White bean	Color bean		Fradinho
		Kidney	Black	
Ba	1–2.2	0.8–2.5	0.2–3.0	<LOQ–2.3
Co	<LOQ–1.4	<LOQ–5.6	<LOQ–3.8	<LOQ–2.1
Cu	6.1–7.2	5.7–9.0	8.4–9.5	4.3–8.0
Fe	51–64	54–61	48–54	40–50
Mn	12–15	9.9–17	11–22	11–18
Mo	1.2–6.4	<LOQ–2.9	<LOQ–2.2	<LOQ–1.1
Ni	1.4–2.8	0.4–2.0	0.3–3.6	0.6–1.7
Pb	<LOQ	<LOQ	<LOQ	<LOQ–0.29
V	2.0–4.5	1.8–4.2	0.5–2.3	<LOQ–2.2
Sr	2.9–3.7	3.0–3.9	3.0–4.0	3.1–3.9
Zn	23–29	23–33	25–30	34–45
Ca[a]	0.041–0.069	0.037–0.057	0.033–0.075	0.034–0.072
K[a]	1.3–1.6	0.96–1.62	1.3–1.7	1.2–1.6
Mg[a]	0.13–0.17	0.14–0.17	0.15–0.17	0.14–0.17
P[a]	0.42–0.58	0.38–0.51	0.37–0.49	0.29–0.55

LOQ limit of quantification

[a] Concentration in percent (*w/w*)

Analysis of Samples

The proposed method was applied for the determination of trace elements in bean samples. The samples were analyzed in triplicate. The values presented in Table 2 show a comparison of mineral element contents among bean samples provided by small farmers ($n=47$) in different cities of Bahia, Brazil. Lead and Cr concentrations were lower than the LOQ in all samples analyzed.

Mineral compositions of foods are a reflection of the compositions of the soil and environment in which the plant grows. Soil–plant systems are highly specific for different elements, plant species, and environmental conditions. The interaction between genetic characteristics (cultivars) and geographical origin has a significant influence on Ca, Fe, Mn, K, N, and Zn concentrations (Moraghan and Grafton 2001). The mineral composition of beans varies and is influenced by a number of factors. These factors (i.e., crop cultivars, management of soil, manure, and climatic conditions of the area) can change in different regions of the same country (Onwuliri and Obu 2002; Shimelis and Rakshit 2005; Mubarak 2005).

The range of concentrations of macroelements, microelements, and trace elements for samples collected in markets of Salvador City were also compared (Table 3). Considering information contained on the package with respect to the geographical origin of the samples (Feira de Santana and Salvador), the mean concentrations of analytes ($n=7$) were compared using the paired *t* test at a 95 % confidence level. The concentrations of P, Mn, Zn, Ba, Fe, and K were significantly different ($P<0.05$) for kidney bean samples.

For black bean samples, Co, Ni, Zn, and Ca presented significant differences in their concentrations. The white bean samples exhibited significant differences in concentrations of the following elements: Ba, P, and Co. Finally, the concentrations of Sr, Zn, Co, and Se were significantly different in cowpea samples. Chromium concentrations were lower than the LOQ in all samples analyzed.
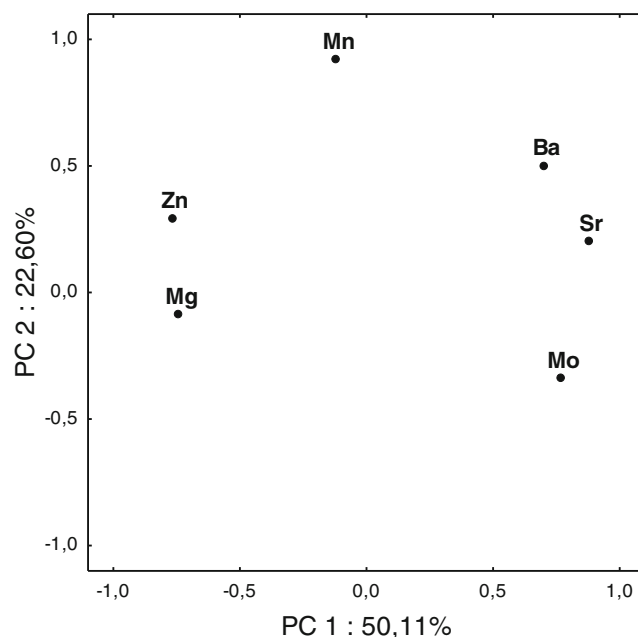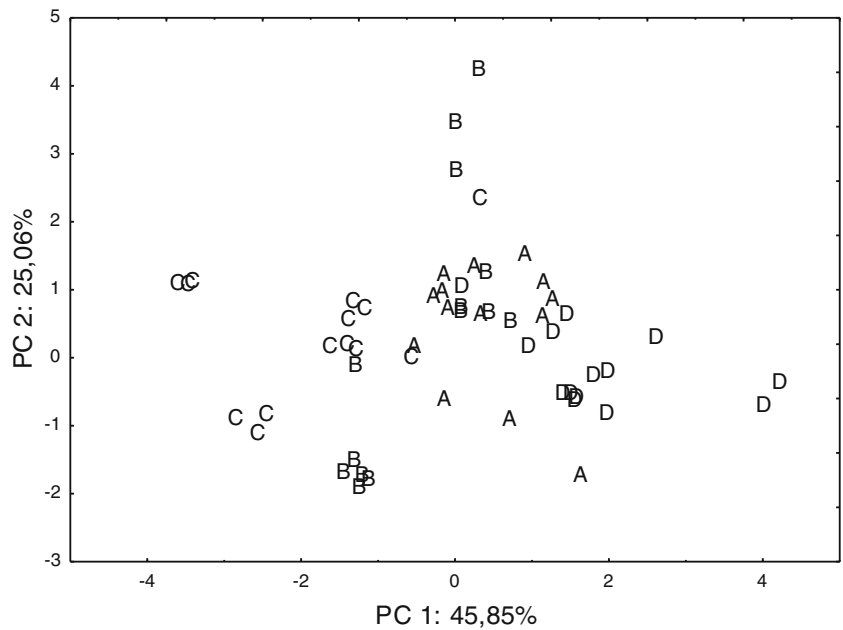

Fig. 4 Principal component 1 (*PC 1*) versus principal component 2 (*PC 2*) loadings applied to the elements' concentrations in beans

Fig. 5 PC for score plot (species: *A* kidney bean, *B* black bean, *C* cowpea, and *D* white bean)

Tables 2 and 3 show that the concentration of analytes (major and trace elements) exhibited different orders of magnitude. Therefore, data were autoscaled before HCA and PCA in order to achieve independence on the factors of different scale of the element concentrations, in order to for all the factors to have the same importance (Amorim Filho et al. 2007; Anunciação et al. 2011).

According to their proximity, Fe, K, and Sr provided similar information. The same can be concluded about the descriptors Mo, P, and Pb. The five most discriminating variables were Ba, Mo, Mg, Zn, and Sr. These results indicated that, by using only these five descriptors, the bean

samples could be classified into their corresponding geographical regions.

After preprocessing, we selected the most significant variables in terms of geographical origin, as showed in Fig. 4. PCA demonstrated that a large number of variables dominated the total data variability; the three first principal components (PCs) accounted for 83 % of the total variability. The first component was responsible for only 50 %, the second accounted for 22 %, and the third accounted for 11 % of the total variability. A similar result was observed for analysis in terms of species. A distinct visual cluster appeared when the data were displayed with respect to the

Fig. 6 PC for score plot (geographical origin: *1* MG, *2* PR, *3* BA, *4* GO, *5* ARG, and *6* SP)
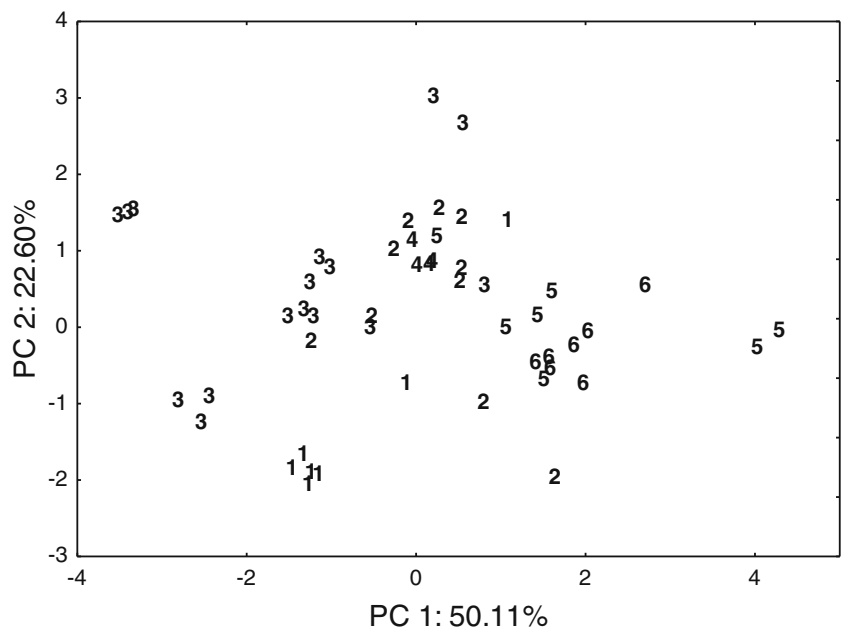
first two PCs; this result was not surprising because the first PC accounted for the maximum possible one-dimensional projection of the total variation of the data points.

The PCA and HCA showed that the correlation between element content and production mode (industrially processed or small producers) in beans did not corroborate with the experimental data. The objective was to correlate the mineral profile with the geographical origin through chemometrics. Some interesting patterns were observed, such as the grouping of cowpea samples (*V. unguiculata*) from producers in the Bahia State, as can be seen in Fig. 5.

The bean samples originating from Bahia received negative scores on PC1 and were separated from other bean samples with null and positive PC1 scores (Fig. 6). Descriptors such as Ba, Mo, and Sr were characterized with the highest positive loading values in PC1, whereas Mg and Zn presented the highest negative loading values in this PC. Thus, beans originating from Bahia were generally well characterized by relatively high values of Mg and Zn and low values of Ba, Mo, and Sr. The opposite result was true for samples from other regions, which were grouped at the right of the plot.

Conclusions

The analytical procedure proposed herein has demonstrated the efficiency of microwave-assisted digestion using the diluted nitric acid procedure for the determination of macroelements and trace elements in bean samples. Taking into account parameters such as economy, environment, and security, the proposed procedure has advantages over classical microwave-assisted procedures based on concentrated acids. Additionally, the use of concentrated reagents is always hazardous, requires dilution of the digests prior to step of determination, and may generate large amounts of concentrated acids as effluents. Multivariate analysis proved to be an efficient strategy for the optimization of microwave-assisted digestion in a closed vessel. Based on the elemental composition and application of tools for pattern recognition, clusters of bean samples were identified and verified the tendency of grouping in relation to their species and their geographical origin.

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