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Use of Doehlert Design for Optimizing the Digestion of Beans for Multi-Element Determination by Inductively Coupled Plasma Optical Emission Spectrometry

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Neste trabalho a otimização de um procedimento rápido e de baixo custo para digestão ácida de feijão foi desenvolvida. Elementos essenciais (Ca, Cu, Fe, K, Mg, Mn, Ni, P, Zn) e não essenciais (Al, Ba, Sr) foram determinados por espectrometria de emissão óptica com plasma indutivamente acoplado (ICP OES). Foi utilizado planejamento fatorial de dois níveis fracionário com cinco fatores (volumes de HNO_3 e H_2O_2, tempo, temperatura do bloco e tamanho de partícula). O fator temperatura foi o parâmetro mais importante e o planejamento Doehlert foi aplicado para determinar as condições ótimas, que foram obtidas quando se utiliza 3,5 mL de HNO_3 concentrado e o tempo de 45 min. A exatidão foi demonstrada usando um material de referência certificado (folhas de espinafre NIST 1570a) e método oficial recomendado. Os resultados obtidos para todos os analitos foram concordantes com os valores certificados de acordo com o teste-*t* pareado para um nível de confiança de 95%.

In this work, the optimization of a fast and inexpensive acid wet digestion procedure for beans was developed. Essential (Ca, Cu, Fe, K, Mg, Mn, Ni, P, Zn) and non-essential (Al, Ba, Sr) elements were determined in beans degestates by inductively coupled plasma optical emission spectrometry (ICP OES). Experimental designs for five factors (HNO_3 and H_2O_2 volume, digestion time, block temperature and particle size) were used for optimization of the digestion procedure, adopting a factorial experiment with 2^{5-1} design. The factor block temperature was found to be the most important parameter and Doehlert designs were applied in order to determine the optimum conditions. Decomposition conditions were attained using 3.5 mL of concentrated HNO_3 and lasts 45 min. The accuracy of the results was demonstrated using one certified reference material (spinach leaves NIST 1570a) and comparison with recommended official method. A paired t-test showed a good agreement for all analytes at 95% confidence values.

Keywords: experimental design, essential and non-essential elements, bean, sample digestion, ICP OES, response surface methodology, Doehlert design

Introduction

The mineral composition of food legumes is a more or less variable factor and influenced by a number of interrelated factors, such as genetic diversity, climate, soil characteristics (content of organic matter, pH and clay mineralogy), cropping strategies, transport, storage, and preparation. These factors vary from one region to another and even within the same country.[1-9] Food legumes are crops of the family *Leguminosae*. They are mainly grown for their edible seeds, and are also called grain legumes.[10] Common beans (*Phaseolus* spp.) are an important food in Brazil, being responsible to improve the nutritional value of the Brazilian diet as a good source of protein (17.5 to 28.7%), carbohydrates (58.0 to 74.4%), lipids (1.0 to 4.3%), minerals (2.9 to 4.5%), hydro soluble vitamins, soluble fibers, lectins and other vitamins.[11-14] They are also considered staple food in many developing countries having great socio-economic importance and particularly in Brazil as they are cultivated and consumed in all regions. Other bean plants are also cultivated in Brazil, such us *Vigna unguiculata*, L., known popularly as cowpea, rope bean, brown bean, stick bean, cow bean and thus are

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also named *caupi* or *macaçar* bean in Brazil. As well the *Cajanus cajan*, L., know as pigeon pea and also called popularly *andu, guando* or *guandu* in Brazil.

Iron, copper, iodine, zinc, chromium, cobalt, molybdenum, manganese, nickel, selenium and fluorine are considered essential elements vitally important for human health. Lead is considered by the European and Brazilian legislations as the most important pollutant in foods and its poisoning effects are well known, as well as those of mercury, barium and aluminum.[15,16] Some minerals essential for the normal metabolic functional of the human body can be found in beans. The most important ones among them are the macro-elements Ca, K, Mg and P, the micro-elements Cu, Fe, Mn and Zn, and the trace elements Mo, Cr and Co.

Dry ashing at 450-600 ºC has been used extensively as sample preparation procedure for the determination of the mineral composition of grain legume,[17-20] and it is among the procedures approved by Official Methods of Analysis of AOAC.[21] However, the disadvantages of this technique are numerous, it is very time consuming, there is the risk of losses of volatile analytes such as Zn, Cu, Fe, As, Cd, Hg and Pb, as well as a high risk of contamination.[22,23] Wet ashing has also been described for the determination of minerals in bean seeds using mixtures of acids and other agents as oxidation aids.[7,10,24-30] Mubarak,[2] using wet ashing by concentrated nitric and perchloric acid (1:1, v/v), determined Na, K and Ca by flame photometry, Mg, Mn and Fe using atomic absorption spectrometry, and P was determined photometrically through the phosphorus molybdenite complex.

Several means can be used for the optimization of the extraction of metals from different matrices, but some of them are time-consuming: for instance, the study of each variable separately. Procedures for optimization of factors by multivariate techniques have been encouraged, as they are faster, more economical and efficient, and allow more than one variable to be optimized simultaneously.[31,32] In order to determine the real function established among the analytical response and the significant factors, second order designs are used such as Central Composite, Box-Behnken or Doehlert matrix.[33,34] Central Composite is the approach used most frequently but Doehlert and Box-Behnken designs require a smaller number of experiments (N). For Doehlert ($N = k^2 + k + 1$) and Box-Behnken designs ($N = 2k\ (k - 1) + 1$), where k is the number of variables under study. Another advantage of the Doehlert designs over the Central Composite approach is its higher efficiency value (ϕ), which is determined by dividing the coefficient number of the quadratic equation (p) by the number of experiments (N) required for the design.[35] For Doehlert designs with $k = 3$, N is equal to 13 and p is 10 yielding $\phi = 0.77$. In turn, for central composite designs with the same $k = 3$, N is 15, p is 10 and then $\phi = 0.67$. The optimization procedures based on these approaches are nowadays being applied to optimize sample pre-treatment and operating conditions for several analytical techniques.[36-42]

The aim of this study was to develop a multi-element analytical method for bean samples using inductively coupled plasma optical emission spectrometry (ICP OES) after an appropriate wet digestion procedure with an open vessel technique. Fractional factorial and Doehlert design were applied for optimization of the experimental variables for Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Ni, P and Zn determination in bean, using block digestion.

Experimental

Instrumentation and operating conditions

An inductively coupled plasma optical emission spectrometer with axially viewed configuration (VISTA PRO, Varian, Mulgrave, Australia) equipped with solid state detector, cyclonic spray chamber, and concentric nebulizer was employed for Al, Ba, Ca, Cd, Cr, Co, Cu, Fe, K, Mg, Mn, Ni, Pb, P and Zn determinations. The operating conditions (Table 1) were optimized in order to obtain both plasma robustness and greater signal-to-background ratios, using Mg^{II}/Mg^{I} intensity ratio. The emission lines were selected in order to include the most interesting elements for food analysis.

An aluminum heating block (Tecnal, Piracicaba, Brazil), a programmable muffle furnace (Quimis, São Paulo, Brazil) and hot plate have been used for sample

Table 1. Optimized instrumental conditions for multi-element ICP OES determinations in digested bean samples

RF incident power / W	1300
Plasma gas flow rate / (L min^{-1})	15
Auxiliary gas flow rate / (L min^{-1})	1.5
nebulizer argon gas flow rate / (L min^{-1})	0.70
Replicate read time / s	1
Instrument stabilization delay / s	15
Pump rate / rpm	15
Spectral lines / nm	Al^{I} 396.152; Ba^{II} 455.395; Ca^{II} 396.847; Cd^{II} 226.502; Co^{II} 230.786; Cr^{II} 267.716; Cu^{I} 327.398; Fe^{II} 259.940; K^{I} 766.465; Mg^{II} 279.551; Mn^{II} 257.610; Ni^{II} 231.302; P^{I} 213.618; Pb^{II} 220.352 and Zn^{I} 213.857

^{I}atomic line; ^{II}ionic line.

mineralization and ashing. A Parr bomb (Model 4746, Parr Instrument Company, USA) and drying oven have been used for pressure digestion.

Dry beans were milled in knife micro-mill MA 630 (Marconi, Piracicaba, Brazil). Green and humid grains were triturated in a domestic processor and lyophilized in TERRONI FAUVEL LT 1000/8 lyophilizer (São Carlos, São Paulo) previous to digestion.

Reagents and solutions

Nitric acid 65% (m/m) and hydrogen peroxide 30%, v/v (Merck, Darmstadt, Germany) were used. Ultrapure water of Milli-Q quality (Millipore, Bedford, USA) with 18.2 MΠ cm resistivity (25 °C) was used throughout. A mixed working standard solution containing 50 mg L^{-1} of Al, Ba, Ca, Cu, Cd, Co, Cr, Fe, K, Mg, Mn, Ni, Pb, P and Zn was prepared from 1000 mg L^{-1} stock solutions (Merck).

Reference materials and samples

The reference material Spinach Leaves 1570a from NIST (Gaithersburg, Maryland, USA) was employed for quality control. The samples of *Phaseolus vulgaris L.* (white, black and *carioca*), *Vigna unguiculata L.* (green cowpea) and *Cajanus cajan* L. (pigeon pea) were purchased from different local markets of Salvador, Bahia, Brazil. The dried beans after being grinded were homogenized and sieved in nylon sieves of 100, 300 and 500 πm mesh size, respectively. Fresh beans were washed with deionized water, dried and triturated in a domestic food processor resulting in a homogeneous mass. Part of this material was lyophilized. After pre-treatment, all samples were stored in plastic bags and kept in a freezer.

Experimental design and data analysis

In order to establish the optimum conditions for a routine digestion in a heating block, a factorial experiment with 2^{5-1} design (Table 2) with triplicate in the central point was carried out, taking into account that five variables - temperature of the digester block, heating time, volumes of 65% (m/m) HNO_3 and 30% (v/v) H_2O_2 and particle size - might affect sample digestion. A sample mass of 0.50 g of the lyophilized green cowpea (*Vigna unguiculata*, L.) bean sample was used for all the experiments.

The response for experimental design was obtained by evaluating the recovery percentages of the elements using as reference the values obtained after acid-bomb digestion. It generally accepted that the bomb digestion is usually better than simple open-vessel digestions. However, these systems are more expensive and dangerous. For the determination of the critical conditions a Doehlert design with two factors was developed. The statistically significant effects of the variables were studied and interactions among them evaluated by applying analysis of variance (ANOVA) using the software Statistica 7.[43]

Table 2. Selected variables and levels for the 2^{5-1} design

	(–)	(+)
Volume of HNO_3 / mL	2.0	5.0
Volume of H_2O_2 / mL	0	2.0
Heating block temperature / ºC	100	140
Heating time / min	15	75
Particle size / πm	< 100	< 500

Investigation of matrix effects

In order to evaluate the single and combined effects of reagents and major elements on the analytical signal of Cu, Fe, Mn and Zn, for each solution of the analytical procedure, the following media were prepared, containing the analytes, at various concentrations: (*i*) diluted nitric acid solution; (*ii*) diluted nitric acid solution containing the major elements; (*iii*) solution containing digested bean sample. The concentration of major elements in solution (*ii*) was fixed at Ca: 30 mg L^{-1}, Mg: 40 mg L^{-1}, P: 100 mg L^{-1} and K: 400 mg L^{-1}, in accordance with previously determined values, while the concentration of trace metals was according to values occurring in real samples.

In addition, operational parameters, such as radio-frequency (rf) incident power and nebulizer argon gas flow rate were optimized. The Mg^{II}/Mg^{I} intensity ratio is a practical criterion to evaluate plasma robustness and analytical performance.

Digestion procedures

Three different digestion procedures were applied to digest the bean samples. A set of digestion blanks was prepared together with each batch of samples.

Procedure A: dry ashing

A 2.0 g aliquot of sample was weighed into a porcelain crucible and transferred into a muffle furnace. The temperature was increased to 450 ºC at a rate of about 50 ºC h^{-1}, and this temperature was kept constant overnight. After cooling, the ash was mixed with 1 mL of water and evaporated on a hot plate. Then the flasks with the ash were returned to the oven for incineration at 450 ºC for additional 1-2 h. The procedure was repeated until sample was completely ashed, *i.e.*, the ash should be white/grey or slightly colored. The ashes were dissolved with 5.0 mL of 6 mol L^{-1} HCl under careful heating on a hot plate and the solution was transferred quantitatively to a 25.0 mL volumetric flask and diluted to volume with Milli-Q water.

Procedure B: acid-bomb digestion

Closed vessel steel-jacketed Teflon PTFE bombs have been traditionally employed in this laboratory for sample digestion in a conventional oven. About 0.25 g of sample was accurately weighed into dry, clean PTFE digestion vessels, and 2.0 mL of concentrated HNO_3 and 1.0 mL 30% (v/v) H_2O_2 were added. The bombs were closed and put in an oven at 110 ± 10 °C for 12 h, which was the optimized time to achieve complete dissolution. After cooling to room temperature the bombs were opened, the digested solution transferred to a 25.0 mL volumetric flask and diluted to volume with Milli-Q water. This procedure was used as reference for to obtain the response for experimental design, expressed as recovery percentages of de analytes.

Procedure C: acid wet digestion on heating block

After the optimization of the digestion conditions, the selected factors were applied to beans samples. About 0.50 g of sample was accurately weighed into glass vessels and 3.5 mL of concentrated HNO_3 was added. The block temperature was adjusted to 150 ºC and the sample digested for 45 min. As the digestion was developed using only nitric acid the reaction mixture temperature was limited by boiling point of the concentrated acid at atmospheric pressure (*ca.* 122 degrees). After cooling the solution was filtered in quantitative filter paper and diluted to 25.0 mL with Milli-Q water.

Results and Discussion

Optimization of digestion factors

The recoveries obtained in the experiments of the factorial plan 2^{5-1} for the digestion procedure in the heating block was: Cu (74 to 115%), Mn (78 to 86%), Fe (58 to 100%), Ni (76 to 90%), Zn (102 to 117%), Ca (89 to 117%), K (98 to 119%), Mg (88 to 107%) and P (75 to 91%).

The results were evaluated using analysis of variance (ANOVA) and Pareto charts. The factor block temperature was statistically significant only for Zn, being affected by a positive sign (Figure 1). Also, the interactions between temperature and particle size were significant for Mg, as shown in Figure 2. This comportment was similar for Cu, K and P and the interaction between volume of nitric acid and particle size was significant only for Cu. The interactions between volumes of nitric acid and peroxide and between temperature and time digestion were statistically significant for Mg and P.

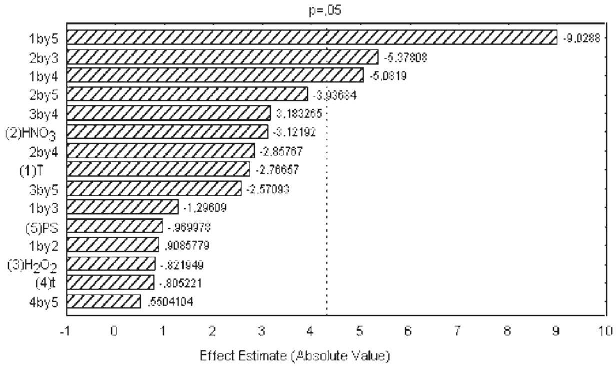

Figure 1. Pareto chart standardized effects of variable zinc (recovery %). (1)T = temperature of the digester block (ºC); (2)HNO_3 = volume of 65% (m/m) nitric acid (mL); (3)H_2O_2 = volume of 30% (v/v) hydrogen peroxide (mL); (4)t = heating time (min) ; (5)PS = particle size.

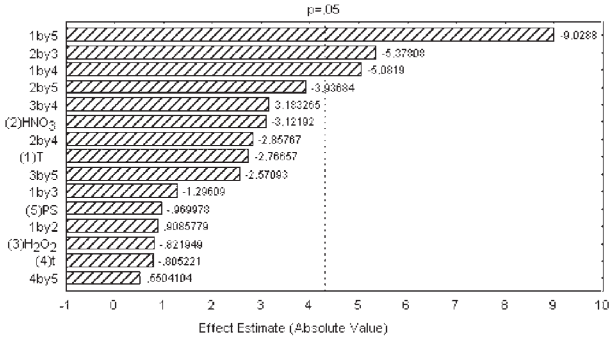

Figure 2. Pareto chart standardized effects of variable magnesium (recovery %). (1)T = temperature of the digester block (ºC); (2)HNO_3 = volume of 65% (m/m) nitric acid (mL); (3)H_2O_2 = volume of 30% (v/v) hydrogen peroxide (mL); (4)t = heating time (min) ; (5)PS = particle size.

Final optimization by Doehlert design

The factorial design demonstrated that the variables at the levels studied need final optimization. A Doehlert matrix design was developed for the optimization of the heating block temperature and of the volume of nitric acid, with the particle size fixed at <300 πm and the heating time in the central point (45 min) of the factorial design plan. H_2O_2 was not used since this variable was not significant as demonstrated by the analysis of the factorial design. The nine experiments

Table 3. Doehlert matrix and recoveries obtained for the analytes after acid digestion in heating block

T^a / ºC	V^b / mL	Recovery %										
		Ni	Zn	Fe	Mn	Cu	Al	Ba	P	K	Mg	Ca
135	5.0	118	111	79.0	89.0	95.2	45.9	89.9	114	84.6	119	86.5
165	5.0	124	113	43.7	90.2	96.7	59.6	87.9	111	81.9	116	90.2
120	3.0	142	116	81.0	90.4	116	33.0	88.2	109	79.6	114	89.9
150	3.0	125	122	87.1	94.3	106	75.7	91.5	115	86.1	121	104
150	3.0	129	115	86.6	91.6	111	90.0	88.2	115	85.2	119	99.4
150	3.0	126	118	83.7	91.2	110	84.4	89.3	114	85.9	119	104
180	3.0	115	115	84.1	91.0	109	69.8	102	112	81.8	115	91.4
135	1.0	97.2	107	74.1	82.6	85.6	38.7	91.7	101	75.9	107	87.8
165	1.0	104	111	78.5	88.0	95.2	46.7	95.2	111	81.8	114	87.5

T^a = block temperature ; V^b = volume of concentrated nitric acid.

required by the Doehlert design are described in Table 3. The temperature of the heating block and volume of nitric acid varied from 120 to 180 ºC and 1.0 to 5.0 mL, respectively.

Critical points were obtained in the maximum area for micro-elements such as Al, Mn, Ni and Zn, and the macro-elements Ca, K, Mg and P. The application of Langrange's criterion indicated a saddle point for Ba, Cu and Fe; the results for these elements did not show a maximum within our experimental limits.

The data obtained for Al, Ca, K, Mg, Mn, Ni, P and Zn were used in the Doehlert matrix and Table 4 illustrates the relationship between the heating block temperature (T), the volume of nitric acid (V) and recovery % for these elements. The best conditions for Al, Ca, K, Mg, Mn, Ni, P and Zn were obtained within the range from 146 to 158 ºC and 3.0 to 4.0 mL of acid. This range is considered narrow could make possible a multi-element determination within the defined experimental condition of 150 ± 10 ºC and 3.5 mL of concentrated nitric acid. The corresponding surface response for Mn, Ca and P is shown in Figures 3 to 5; the profile of the surface response estimated for most of the elements was comparable to that of calcium.

Optimization of ICP OES parameters

Once the digestion conditions were fixed, the next step was to optimize the instrumental conditions for ICP OES. It is important that these parameters are defined in the presence of the sample matrix because the intensity of the element signals clearly depends on the matrix. In this study the instrumental conditions were usually adjusted to accommodate the particular sample type, *i.e.* organic materials and high dissolved solids, rather than to obtain the best limits of detection. Particularly the radio-frequency (rf) incident power and nebulizer argon gas flow rate were optimized using digested bean matrix rather than aqueous standard solutions. The rf incident power was optimized as it affects seriously the plasma temperature and improves atom excitation performance. It was studied in the range between 800 and 1400 W. The results indicated that the sensitivity and the linearity are better at 1300 W for almost all analytes with a few exceptional cases, and the stability of plasma was also improved. The effect of nebulizer argon gas flow rate was studied from 0.5 to 0.9 L min^{-1} and maximum intensity was observed at 0.7 L min^{-1} for almost all analytes. Thus, a 0.7 L min^{-1} nebulizer argon gas flow rate was adopted throughout this study, which provided

Table 4. Surface response function for percent element recoveries and critical values of the heating block temperature (T) and volume of nitric acid (V) in optimized conditions

Element	Surface response function (Recovery %)	Critical values	
		Temperature / ºC	HNO_3 / mL
Zinc	$R_{Zn} = 14.5 + 1.07 T + 14.1V - 0.017T^2 - 0.0033TV - 1.81V^2$	153	3.2
Manganese	$R_{Mn} = 15.1 + 0.704T + 13.1V - 0.035T^2 - 0.002TV - 1.13V^2$	158	3.4
Aluminun	$R_{Al} = -844 + 11.05T + 37.0V +0.047T^2 - 0.035TV - 6.91V^2$	158	3.2
Nickel	$R_{Ni} = -208 + 3.85T +12.3V - 0.013T^2 + 0.034TV - 2.862V^2$	150	3.2
Phosphorus	$R_P = -63.8 + 1.79T + 24.4V - 0.11T^2 - 0.005 TV - 1.09V^2$	148	3.8
Potassium	$R_K = -89.7 + 1.94T + 17.0V -0.072T^2 - 0.006TV - 0.856V^2$	150	3.7
Magnesium	$R_{Mg} = -67.1 + 2.00T + 20.8V -0.083 T^2 - 0.006TV -1.09V^2$	146	4.0
Calcium	$R_{Ca} = -207 + 3.84T + 12.3V + 0.033T^2 - 0.013TV - 2.86V^2$	151	3.0

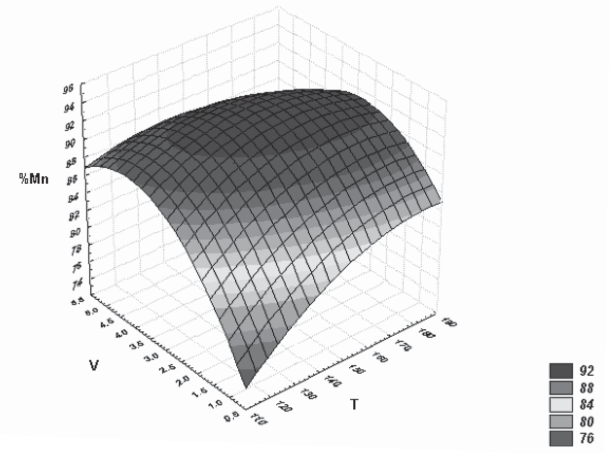

Figure 3. Surface response estimated from manganese recuperation (%). T: temperature (ºC); V: volume of 65 % (m/m) HNO_3.

high sensitivity and good precision. The optimum values for instrumental parameters are shown in Table 1.

Matrix effect and calibration techniques

The comparison of the slopes obtained with the usual analytical curves and the curves using analyte addition indicated no significant differences at the 95% confidence level, suggesting no detectable matrix effect for Al, Ba, Cu, Fe, Mn, Sr and Zn. As the experiments for attainment of the analytical curve they had been carried through in five levels of concentration and in third copy, it was possible to get the estimate of the random error and to calculate the lack of adjustment of the linear model. On the basis of the ANOVA it had been gotten the values of p for the lack of adjustment of the regression for the curves of calibration of the elements of Table 5. All the values of p for the adjustment lack had been greaters that 0.05, showing that the linear model is adjusted. The regression equations and determination coefficient (r^2) of the regression lines was described in Table 5.

To evaluate the effects caused by macro-elements on the micro-elements analytical intensities, the spectral line profiles of analytes were measured on solutions containing various concentrations of the elements encountered in bean matrix such as Ca, Mg, P and K. The obtained results indicated that significant interferences were not observed on the analytical intensities ($P>0.05$).

The influence of the acidity of the solution obtained after the acid extraction on the analytical signal was also evaluated, considering that the volume of nitric acid was a factor studied in all of the accomplished experimental

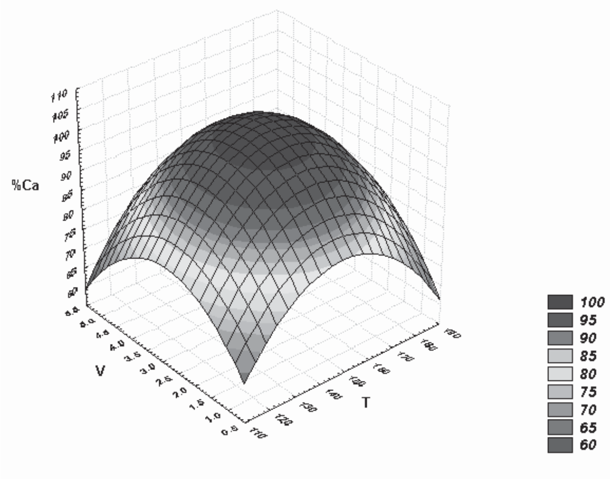

Figure 4. Surface response estimated from calcium recuperation (%). T: temperature (ºC); V: volume of 65% (m/m) HNO_3.

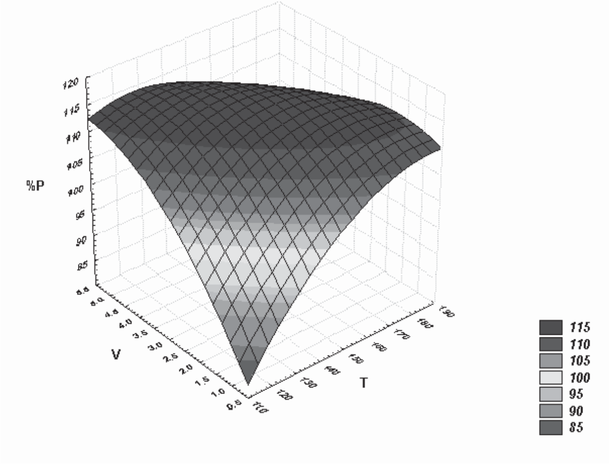

Figure 5. Surface response estimated from phosphorus recuperation (%). T: temperature (ºC); V: volume of 65% (m/m) HNO_3.

Table 5. Comparison of the regression data using standard in nitric acid and standard addition solutions of digested bean matrix

Element	Standard in nitric acid Regression equation	r^2	Standard addition solutions of matrix Regression equation	r^2
Al	y = 7783.1x + 290.95	0.9999	y = 7953.5x + 2896.5	0.9998
Ba	y = 991954x + 3883.9	0.9998	y = 993808x + 103314	0.9984
Cu	y = 13834x + 210.64	0.9998	y = 13461x + 1517.2	0.9958
Fe	y = 18312x + 245.72	0.9999	y = 18328x + 17347	0.9967
Mn	y = 168511x + 1932.2	0.9998	y = 167764x + 53471	0.9961
Sr	y = 747527x + 3076.7	0.9996	y = 727860x + 54525	0.9995
Zn	y = 59813x + 1145.3	0.9998	y = 60760x + 46950	0.9945

design. The residual acidity for the solution corresponding to the experiment using minimum volume and temperature and maximum time was 0.22 mol L^{-1}. For the experiment where was used maximum volume and time and minimum temperature, the residual acidity was 1.8 mol L^{-1}. Finally, for the experiment where was used maximum volume and minimum time and temperature, the residual acidity was 2.5 mol L^{-1}.

The *t*-test at 95% confidence level was used to evaluate the results. The analytical signal obtained for the analytes did not differ significantly ($P>0.05$) in the interval of acidity studied. Therefore, the analytical curve established with 1.5 mol L^{-1} of nitric acid was chosen.

Accuracy

Due to the lack of beans certified reference materials or more similar CRM, the accuracy of the proposed procedure was verified by the analysis of the certified reference material NIST 1570a (spinach leaves), which has been treated by the proposed procedure as well as by pressure bomb digestion, and analyzed by ICP OES. The mean values of analytes determined are shown in Table 6. The paired *t*-test at 95% confidence level showed that there was no significant difference between the means of the certified and obtained values for almost all analytes under investigation.

Moreover, all samples have been digested using the proposed open block digestion, the acid-bomb digestion and dry ashing using the official method AOAC No. 999.11-1999.[19] The results are showed in Table 7. On the basis of an analysis of variance (ANOVA) test, the differences between the three sample preparation procedures, were found to be not significant at the 95% confidence level, considering the concentrations obtained for micro-elements Al, Cu, Fe, Mn and Ni and for the macro-elements Ca, K, Mg and P. The signals obtained for Cr, Cd, Co and Pb, with one exception, were lower than the LOD.

Figures of merit

The repeatability of the analytical procedure was obtained by taking replicate measurements of the digested bean sample ($n=10$), which yielded relative standard deviations for macro-elements between 1.05 and 2.3%. The estimative of relative standard deviations for trace elements were (%): Al (18), Ba (4.0), Cu (3.7), Fe (17), Mn (2.0), Ni (5.8), Zn (1.7).

The LOD for the block digestion procedure were calculated as the concentration equal to three times the estimative of standard deviation of ten blanc measurements divided by the slope of the calibration line of standard solutions in 1.0 mol L^{-1} HNO$_3$ The values obtained for LOD (πg L^{-1}) were: Al (52), Ba (30), Ca (40), Cd (0.5), Cu (3.2), Cr (1.0), Co (2.5), Fe (56), K (14), Mg (3.6), Mn (0.5), Ni (6.2), P (7.9), Pb (10) and Zn (34).

Analytical application

The optimized procedure was applied to the analysis of black, white and colours beans samples of the species *Phaseolus vulgaris* L. (white, black and kidney), *Vigna unguiculata* L. (green fresh and dry) and *Cajanus cajan* L. The results are showed in Table 7. The analyte contents varied significantly for bean species considering samples of dried grains and lyophilized green grains. In addition to the grains, the hulls of green beans and pigeon pea were analyzed as well. It was noticed that large amount of essential minerals are present in the hull. The content of Mg, Mn, Zn, K, Cu and Ca, respectively, in the lyophilized hull was 15, 25, 25, 30, 70 and 95% higher than in the processed and lyophilized grain for green bean and 50, 60, 25, 50, 30, 95% for pigeon pea, respectively.

The predominant element was K followed by P, Mg and Ca. By using a paired test t it can be concluded that the analyzed bean samples differed significantly ($P<0.05$). The medium content (πg g^{-1}) of Fe and K of pigeon pea is between 15% and 30%, respectively, of the corresponding content of *carioca* and black beans (*Phaseolus vulgaris* L.).

Reported literature values for macro-, micro- and trace element levels in common bean (*Phaseolus vulgaris* L.) are shown in Table 8. The values for Cd, Cu, Mn, Ni, Fe and Zn in πg g^{-1} dry weight range from < 0.0006 to 0.04, 3.0 to 325, 0.85 to 18.2, 0.17 to 1.0, 9.0 to 180, 15.4 to

Table 6. Comparison of the contents (n = 3) of micro- and macro-nutrients obtained by heating block and bomb acid digestion for NIST standard reference material 1570a (Spinach leaves)

Element	Heating block digestion	Acid-bomb digestion	Reference values (1570a NIST)
		Concentration (%, mg g^{-1})	
Ca	1.345 ± 0.016	1.190 ± 0.007	1.527 ± 0.041
P	0.495 ± 0.009	0.524 ± 0.008	0.518 ± 0.011
		Concentration (πg g^{-1})	
Cd	2.23 ± 0.03	2.38 ± 0.07	2.89 ± 0.07
Co	0.31 ± 0.04	0.29 ± 0.01	0.39 ± 0.05
Cu	9.86 ± 0.13	11.1 ± 0.4	12.2 ± 0.6
Mn	56.0 ± 1.0	61.3 ± 0.4	75.9 ± 1.9
Ni	1.62 ± 0.14	2.04 ± 0.04	2.14 ± 0.1
Sr	38.9 ± 0.9	44.2 ± 1.4	55.6 ± 0.8
Zn	65.4 ± 1.7	73.5 ± 1.9	82 ± 3

Table 7. Comparison between the three digestion procedures: heating block, pressurized system (bomb) and dry ash. Mean values (n=3), πg g^{-1}, and standard deviation (SD) of the micro- and macro-nutrients in bean samples

Sample	Procedure	Al	Cu	Fe	Mn	Ni	Zn	Ca	K	Mg	P
White bean											
	Block	10.3	8.30	45.3	12.4	2.06	29.6	1198	18041	1486	4747
	SD	0.6	0.1	3.0	0.3	0.01	0.5	36	434	16	34
	Bomb	13.7	8.09	57.9	12.7	1.97	36.2	919.0	17446	1341	5073
	SD	0.6	0.35	0.62	0.1	0.08	0.4	32.0	202	9	45
	Dry ash	10.9	8.50	50.2	11.7	2.08	33.5	1051	15077	1423	4864
	SD	0.1	0.06	0.69	0.2	0.08	0.5	59	364	36	183
Black bean											
	Block	21.2	7.37	49. 7	12.7	0.450	28.9	1376	17254	1752	4222
	SD	2.2	0.2	4.7	0.2	0.10	1.3	83	323	36	127
	Bomb	34.2	8.61	77.8	13.3	1.13	37.9	1273	14239	1851	4486
	SD	0.4	0.05	0.4	0.1	0.01	0.1	68	330	54	93
	Dry ash	34.4	7.62	69.2	12.3	0.410	34.1	1273	14808	1685	4407
	SD	1.6	0.01	0.1	0.1	0.01	2.1	55	715	70	271
Carioca bean											
	Block	81.3	17.9	50.6	8.85	0.46	35.9	1554	16318	1747	3674
	SD	4.4	0.9	2.5	0.2	0.10	1.3	110	1036	94	197
	Bomb	97.4	17.3	69.9	9.01	-	37.4	1244	16138	1630	4094
	SD	2.8	0.2	0.7	0.04	-	0.6	30	368	14	111
	Dry ash	106	17.8	62.1	8.55	0.34	42.7	1511	14335	1720	3968
	SD	2	0.3	0.6	0.06	0.01	0.7	55	323	43	84
Cowpea											
	Block	6.94	6.38	40.6	16.3	1.86	37.1	831.1	14742	1755	3628
	SD	0.80	0.1	5.6	0.3	0.01	0.8	16.8	336	31	71
	Bomb	15.5	6.39	51.6	17.9	1.82	40.5	679.4	14374	1646	3917
	SD	0.7	0.17	0.50	0.4	0.05	1.2	18.4	105	17	36
	Dry ash	18.4	6.83	49.0	16.5	1.86	46.6	938.3	13682	1782	3920
	SD	0.7	0.22	0.5	0.1	0.03	0.4	36. 9	528	71	184
Green bean											
	Block	4.63	4.37	44.3	11.9	1.93	31.5	564.4	15859	1713	4366
	SD	0.3	0.1	2.0	0.2	0.10	0.60	14.9	450	38	85
	Bomb	5.95	5.34	52.9	13.4	2.57	42.9	566.7	14123	1935	4710
	SD	0.38	0.02	0.0	0.2	0.10	0.2	11.6	232	41	23
	Dry ash	8.04	4.50	45.6	12.0	1.83	37.9	637.3	15237	1768	4799
	SD	0.53	0.14	0.3	0.2	0.05	0.7	7.3	371	23	120
Pigeon pea											
	Block	-	6.31	48.6	12.8	1.27	31.6	630.4	7611	1831	3047
	SD	-	0.31	0.9	0.2	0.01	0.4	17.1	57	79	6
	Bomb	12.3	7.79	39.6	8.77	1.54	43.7	991.9	9327	1410	3342
	SD	0.9	0.21	0.9	0.03	0.19	9.1	138.1	110	13	29
	Dry ash	8.07	6.27	31.7	7.34	1.01	31.7	869.0	10035	1348	3340
	SD	1.59	0.08	0.55	0.21	0.02	0.2	40.4	735	24	196

40.0, respectively. The concentrations of micro-elements found in this work for the common bean (white, black and carioca) were in the interval from 10.3 to 106 for Al, 7.6 to 17.9 for Cu, 1.55 to 8.76 for Ba, 45.3 to 77.8 for Fe, 8.55 to 12.7 for Mn, 0.34 to 2.08 for Ni and 28.9 to 42.7 for Zn. These data demonstrate, as already stated in the Introduction, that the composition of the beans, regarding the micro-elements, varies largely with crop cultivars, soil and climatic conditions of the area, among other factors. For the macro-elements it is observed that the levels don't vary so much, except for P, considering the values in the literature. Therefore, it is important to study the mineral composition of food legumes for each area separately.

Conclusions

The application of a Doehlert matrix was a fast, economical and efficient way for the optimization of the proposed procedure. The developed wet digestion of bean samples, using heating block, for the multi-element

Table 8. Reported concentrations in πg g^{-1} of micro- macro- and trace elements for common bean (*Phaseolus vulgaris* L.)

Element												Reference
Mo	Ni	Co	Pb	Cr	Fe	Mn	Zn	Cu	Al	Ba	Cd	
-	-	-	-	-	53-69	-	24-32	-	-	-	-	7
-	-	-	-	-	9.0-180	-	25.0-40.0	8.0-12.0	-	-	-	17
-	-	-	-	-	66	-	29	-	-	-	-	3
5.59	1.00	0.120	-	-	101	18.2	-	-	-	-	<0.0006	28
-	-	-	-	-	84.6-351	0.85-15.00	24.6-27.5	62.0-325	-	-	-	8
-	-	-	-	-	61.81-83.99	-	15.39-28.22	-	-	-	-	1
-	0.17	-	0.69	0.17	64.40	-	46.90	3.00	19.00		0.007	25

Ca	K	Mg	P	Reference
-	15400-16900	1700-2300	4500-5400	7
2060	14100	1610	3850	28
914-1800	19600-23240	1800-2950	2450-3620	8
1358	-	-	-	3
731.93-1929.77			148.99-174.99	1

*fresh weight.

determination by ICP OES, is inexpensive, employs 3.5 mL of concentrated HNO_3 and lasts 45 min. The proposed method showed good accuracy when applied to CRM. Moreover, there were no significant difference between the results of the proposed method and the well established closed vessel and dry ashing methodologies, when applied the analysis of several commercially available beans.

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