



Microwave-assisted diluted acid digestion for trace elements analysis of edible soybean products



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ABSTRACT

A new method for the decomposition of soybean based edible products (soy extract, textured soy protein, transgenic soybeans, and whole soy flour) was developed to essential (Co, Cr, Cu, Fe, Mn, Ni, Se, V, and Zn) and non-essential (As, Ba, Cd, Pb, and Sr) trace elements determination by ICP OES and ICP-MS respectively. Effects related to the concentration of HNO_3 ($2.1\text{--}14.5\text{ mol L}^{-1}$) and the use of hydrogen peroxide on the efficiency of decomposition was evaluated based on the residual carbon content (RCC). It was demonstrated that $2.1\text{ mol L}^{-1}\text{ HNO}_3$ plus $1.0\text{ mL H}_2\text{O}_2$ was suitable for an efficient digestion, since RCC was lower than 18% and the agreement with certified values and spike recoveries were higher than 90% for all analytes. The concentrations of analytes in the samples (minimum–maximum in mg kg^{-1}) were: The concentrations of analytes in the samples (minimum–maximum in mg kg^{-1}) were: As ($<0.007\text{--}0.040$), Ba ($0.064\text{--}10.6$), Cd ($<0.006\text{--}0.028$), Co ($0.012\text{--}102$), Cr ($0.56\text{--}5.88$), Cu ($6.53\text{--}13.9$), Fe ($24.9\text{--}126$), Mn ($16.4\text{--}35.2$), Ni ($0.74\text{--}4.78$), Se ($<2.90\text{--}25$), Sr ($2.48\text{--}20.1$), Pb ($<0.029\text{--}0.11$), V ($<0.027\text{--}20$), and Zn ($30.1\text{--}47.3$). Soy-based foods investigated in this study presented variable composition in terms of essential and potentially toxic elements, which can be attributed to different methods of processing.

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1. Introduction

Soybeans are grown for their seeds that are rich in nutritional ingredients, such as proteins (40%), oils (20%), carbohydrates (35%) and essential elements. Due to the increased production of soy-based foods, the determination of trace elements is important in view of toxicity and nutritional assessments (Llorent-Martinez, Fernandez de Cordova, Ruiz-Medina, & Ortega-Barrales, 2012; Yip, Chan, Cheung, Poon, & Sham, 2009). There are previous studies related to the contents of essential and non-essential elements in different species of soybeans and related products in some countries (Fernandez et al., 2002; Hseu, 2004; Karr-Lilienthal, Griesshop, Merchen, Mahan, & Fahey, 2004; Kazi, 2009; Liu, Wang, Liu, Zhao, & Zhan, 2004; Sola-Larranaga & Navarro-Blasco, 2009; Souza, 2005; Wei, Shen, Rui, & Jiao, 2008; Yip et al., 2009). However, despite the large consumption, there are few literature reports regarding the trace elements contents in soybeans produced in Brazil (Carvalho et al., 2011; Gonzalez et al., 2009; Gris

et al., 2008; Mataveli, Pohl, Mounicou, & Arruda, 2010; Sussulini, Souza, Eberlin, & Arruda, 2007; Zhou et al., 2011).

The determination of trace elements in food requires sensitive analytical techniques such as inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma optical emission spectrometry (ICP OES). However, results obtained employing these techniques may be affected by matrix and/or spectral interferences when working with solutions containing elevated concentrations of dissolved organic compounds. Furthermore, usually before performing measurements by ICP-MS or ICP OES, food samples must be decomposed with suitable methods leading to digests containing reduced amounts of residual carbon compounds and low residual acidity (Korn et al., 2008; Nardi et al., 2009).

Microwave-assisted digestion procedures using closed vessels and various acid mixtures have been employed to decompose organic matrices of biological samples at elevated temperatures and/or pressure. It was already demonstrated that microwave-assisted sample digestion accelerates and minimises contamination and losses of volatile elements (Bakkali, Ramos Martos, Souhail, & Ballesteros, 2009; Cindrić et al., 2012; Guo et al., 2012;

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Millour et al., 2011; Phan-Thien, Wright, & Lee, 2012; Poitevin, 2012; Shaltout et al., 2011, Zanariah, Ngah, & Yahya, 2012).

Nowadays, one of the most important trends in solid sample preparation for elemental analysis is the search for environmentally and friendly processes. Based on green chemistry recommendations, the use of diluted acids for digestion leads 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 demonstrated the potential of diluted nitric acid solutions in microwave-assisted digestion (Araujo, Gonzalez, Ferreira, Nogueira, & Nóbrega, 2002; Bizzi, Flores, Picoloto, Barin, & Nóbrega, 2010; Bizzi and Barin et al., 2011; Bizzi and Flores et al., 2011; Bressy, Brito, Barbosa, Teixeira, & Korn, 2013; Castro et al., 2009; Gonzalez et al., 2009; Trevizan, Donati, Nogueira, & Nóbrega, 2006). This approach was recently applied for digestion of black, white, and kidney beans followed by determination of Ba, Ca, Co, Cu, Fe, K, Mg, Mn, Mo, Ni, P, Pb, Se, Sr, and Zn by ICP OES (Santos, Santos, Fernandes, Castro, & Korn, 2013). However, no additional study was performed for evaluating the use of diluted nitric acid for samples containing relatively high oil and protein contents such as soybeans. Castro et al. (2009) proposed a microwave-assisted digestion procedure using diluted solutions ($2 \text{ mol L}^{-1} \text{ HNO}_3$ and H_2O_2) for multielement determination of macro and micro-elements in coffee samples by ICP OES. In another work, the use of diluted nitric acid associated with pressurised oxygen atmosphere was proven to be feasible for milk powder digestion, reducing the volume of reagents and the amount of digestion residues (Bizzi and Barin et al., 2011; Bizzi and Flores et al., 2011). More recently, a procedure using diluted nitric solutions for the decomposition of bean samples was applied for the determination of Ba, Ca, Co, Cu, Cr, K, Mg, Mn, Mo, Ni, P, Pb, Se, Sr, and Zn by ICP OES (Santos et al., 2013). In this work, three experimental factors (acid concentration, temperature, and processing time) were used to select the best conditions for digestion. Results were consistent with previous studies reported in literature where the digestion time and temperature had a significant effect on the residual concentration of nitric acid (Saavedra, Gonzalez, Fernandez, & Blanco, 2004). In all these works, digestion performance was evaluated taking into account the recovery of the analytes, residual carbon content (RCC) and residual acidity (RA) in the final solutions compared to reference values obtained after microwave-assisted acid digestion using concentrated acid.

Taking into account the difficulties involved in soybean digestion due to the high content of proteins and fats, the aim of this study was to optimise and validate a microwave-assisted digestion procedure for determination of essential (Co, Cr, Cu, Fe, Mn, Ni, Se, V, and Zn) and non-essential (As, Ba, Cd, Pb, and Sr) trace elements in soybeans and their products (soy extract, textured soy protein, and whole soy flour) using diluted nitric acid solution.

2. Materials and methods

2.1. Instrumentation

For microwave-assisted acid digestion using concentrated and diluted acid, a closed-vessel microwave digestion system with control of sensor pressure and temperature (ETHOS EZ, Milestone, Sorisole, Italy) was used. This microwave system was equipped with 10 vessels made of perfluoroalcoxi polymer (PFA) with a volume of 100 mL.

An inductively coupled plasma optical emission spectrometer with axial viewing – ICP OES (Vista Pro, Varian, Mulgrave, Australia) equipped with solid state detector, cyclonic spray chamber, and concentric nebulizer was employed for analytes (Ba, Cr, Cu, Fe, Mn, Ni, Sr, and Zn) and RCC determination. The analytes determination was carried out under manufacturer recommended

conditions for radiofrequency applied power (1.3 kW), plasma (15.0 L min^{-1}), auxiliary (1.5 L min^{-1}) and nebulizer (0.7 L min^{-1}) gas flow-rates. The emission lines less affected by spectral interferences and with high intensities and background ratios were selected. The analytical wavelengths (nm) chosen were: Ba II 455.403, Cr II 267.716, Cu I 324.754, Fe II 238.204, Mn II 257.611, Ni II 231.605, Sr II 407.771, Zn I 213.857, and C I 193.025 nm.

A quadrupole ICP-MS XseriesII (Thermo, Germany) equipped with a hexapole collision cell (CC) was used for the determination of analytes in lower concentrations (As, Cd, Co, Cr, Pb and V). The instrument software allows a rapid switch between the standard mode (no gas cell vented to mass analyser chamber) and the CC mode, whilst continuously aspirating the sample. The pre-mixed gases H_2 (7% v/v) in He (H_2O and other impurities < 5 mg/kg) were admitted into CC under rigorous flow control. The measurements were made using nickel sampler and skimmer cones (1.0 mm and 0.7 mm diameter orifices) and standard concentric nebulizer. A glass impact bead spray chamber cooled to 4 °C by a Peltier device and a shielded Fassel torch was used to minimise the plasma potential and thereby obtain a low and narrow initial ion energy distribution. The operating conditions were: radiofrequency applied power of 1.3 kW, nebulizer gas flow-rate of 0.87 L min^{-1} , plasma gas flow-rate of 13 L min^{-1} , auxiliary gas flow-rate of 0.70 L min^{-1} , CCT mode gas flow-rate, 6.5 L min^{-1} ; dwell time 10 ms, sweeps 100, and three replicate readings. Internal standards (Tl, In, Bi, Ge, Rh and Sc) were added to compensate acid effects and instrument drift (calibration concentrations were $0.1\text{--}25 \mu\text{g L}^{-1}$). Measurements were performed using ^{75}As , ^{111}Cd , ^{59}Co , ^{52}Cr , ^{208}Pb , and ^{51}V isotopes.

2.2. Reagents and standards

All reagents used in this work were of analytical grade (Merck, Darmstadt, Germany). Water obtained from a Milli-Q system (Millipore, Billerica, MA, USA) was used for preparing all reagents and standard solutions. Reference analytical solutions for ICP OES and ICP-MS analysis were prepared before use by serial dilution of stock reference solutions containing 1000 mg L^{-1} of each analyte (Multielement solution, Fluka, Buchs, Switzerland) in water.

The RCC determination was performed using yttrium as internal standard (Spex CertiPrep) in a final concentration of 1 mg L^{-1} in digests. Reference solutions for the calibration curve of C were prepared in the range of $25\text{--}500 \text{ mg L}^{-1}$ through serial dilution of a carbon stock reference solution (citric acid in water, $10,000 \text{ mg L}^{-1}$) (Bizzi et al., 2010; Gouveia, Silva, Costa, Nogueira, & Nóbrega, 2001).

2.3. Samples

Commercial soybean non-transgenic samples (named SG, $n = 4$), transgenic samples (named SGT, $n = 2$) and respective products (soy extract (named ES, $n = 3$), textured soy protein (named PTS, $n = 11$), and whole soy flour (named FS, $n = 2$) were purchased from different local markets of Salvador, Bahia, Brazil. All samples were ground in a ball mill, model 8000 M (Spex Sample Prep, Metuchen, NJ, USA) with a set of tungsten carbide vial and ball and sieved in Nylon® sieves to particle sizes lower than $500 \mu\text{m}$.

A certified reference material from National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) of rice flour (NIST 1568a) was used for the evaluation of accuracy since there is no CRM with similar matrix composition available with certified values for metals in soybeans.

2.4. Microwave-assisted digestion of soybeans and derivatives

Microwave-assisted digestion was employed using 250 mg of sample previously ground in a ball mill (model 8000 F, Sample

Prep Spex, USA), homogenised and weighed directly into PFA bottles with a capacity of 100 mL. The following nitric acid solutions were evaluated: 2.1, 4.2, 7.3 and 14.5 mol L⁻¹, considering the dilution of sub-boiled nitric acid (16.2 mol L⁻¹) and H₂O₂. In all vessels it was added 1 mL of 30% H₂O₂ so the total volume of reagents was 8 mL in all experiments. The heating program was performed in four successive steps. In the first step, the temperature was linearly increased to 120 °C in 5 min with a maximum power of 1000 W; in the second step, the temperature was kept at 120 °C for 2 min; the third step consisted of increasing the temperature linearly to 210 °C in 10 min, whereas in the fourth step, the temperature was kept at 210 °C for 15 min. After the end of the heating program, vessels were cooled down to room temperature and carefully open since they still might be pressurised. Solutions were transferred to 20 mL volumetric flasks and volumes were made up with water. The digests were further 10-fold diluted prior to determination by ICP-MS.

2.5. Determination of the acidity and residual carbon contents of digests

Acid-base titrations were performed in order to determine the final acidity in digests. Titration was carried out with a standardised solution of sodium hydroxide (0.09970 mol L⁻¹) and phenolphthalein (1%, m/v, in ethanol).

The RCC was determined in soybeans digests by ICP OES with axial configuration, using the line emission for C I at 193.025 nm (Gouveia et al., 2001).

2.6. Statistical analysis

Data were reported as mean ± standard deviation of triplicate measurements. All statistical calculations were performed using Statistica 7.0 software (StatSoft®, Tulsa, OK, USA). A significance level of $P < 0.05$ was adopted for all statistical evaluation.

3. Results and discussion

3.1. Effect of nitric acid concentration

The use of nitric acid for organic matrix oxidation is the most usual approach for sample pre-treatment before the determination of trace elements using atomic spectrometric methods. Ideal digestion should lead to the complete decomposition of organic material using minimum amount of acids. On the other hand, acid solutions should be as diluted as possible in order to decrease residual acidity, RA (Korn et al., 2008).

Initially, a sample of ground and homogenised soybeans was used to evaluate the efficiency of digestion using nitric acid solutions (2.1, 4.2, 7.3 and 14.5 mol L⁻¹) and 1.0 mL hydrogen peroxide. The efficiency of the digestion was evaluated by determining RCC. The RA and analytes recoveries were also evaluated.

The RA obtained after the digestions with 2.1, 4.2, 7.3 and 14.5 mol L⁻¹ HNO₃ were 2.38 ± 0.02, 4.08 ± 0.07, 7.38 ± 0.02 and 14.1 ± 0.1 mol L⁻¹, respectively. As demonstrated in previous works, these results confirm that nitric acid can be regenerated in closed vessels heated by microwave radiation. This effect has been attributed to the temperature gradient during the beginning of the heating program. In this step of digestion, the soluble gases as well as those formed by evaporation and chemical processes are transferred to the gas phase, which remains at low temperature and suffers condensation. This temperature gradient also acts to improve the reaction between NO and O₂ leading to NO₂ production. The formed NO₂ is reabsorbed in the acid solution and regenerates HNO₃ (Castro et al., 2009; Trevizan et al., 2006).

The RCCs for digests of soybean and its products ranged from 18 ± 3 to 10 ± 2% ($n = 3$). According to RCC values, the use of concentrated solutions of HNO₃ allowed a slightly better oxidation of organic matter as previously reported (Kubrakova, Formanovskii, Kudinova, & Kuz'min, 1999; Araujo et al., 2002). In all experimental conditions, clear solutions were always observed.

Table 1 shows the concentration of trace elements in digests obtained using different nitric acid solutions. Concentrations of Ba, Co, Cr, Cu, Fe, Mn, Ni, Sr and Zn in soybeans and its products following digestion using diluted nitric acid solutions did not differ significantly (ANOVA) when compared to those obtained using digestion with concentrated nitric acid. Thus, 2.1 mol L⁻¹ HNO₃ solution was selected for further analysis of soybeans and its products.

3.2. Analytical performance

The limits of detection (LOD) and quantification (LOQ) of each analyte were calculated as the analyte concentration that corresponded to three and ten times, respectively, the standard deviation of ten independent measurements of the blank, divided by the slope of the calibration curve. The LODs for As, Cd, Co, Cr, Pb and V (elements determined by ICP-MS) were 0.007, 0.006, 0.0021, 0.32, 0.03, and 0.027 µg g⁻¹, respectively. When ICP OES was employed, the LODs for Ba, Fe, Mn, Ni, Se, Sr and Zn determinations were 0.46, 1.95, 0.76, 0.41, 2.90, 0.89, and 1.70 µg g⁻¹, respectively.

In order to evaluate the accuracy of the proposed procedure, a CRM sample (NIST 1568a, Rice Flour), was digested by the proposed procedure and analytes were determined by ICP OES and ICP-MS. This botanical material was selected since it showed similarity to soybean matrix. The comparison between determined and certified values for four analytes is presented in Table 2. The unpaired *t* test at 95% confidence level showed that there was no significant difference between determined and certified values for these analytes. Spiked experiments were performed by adding standard reference solutions containing 0.1, 0.5, 1, and 2 mg L⁻¹ of each analyte in the five types of investigated samples. Recoveries for all analytes were considered acceptable (90–116%) and relative standard deviations (RSD) were in the range of 3–15%.

Furthermore, soybean samples and their products were digested using diluted acid microwave-assisted system (MW-DA) and results were compared with microwave-assisted concentrated acid digestion (MW-CD) (Table 3). Based on Student's *t*-test, the determined concentrations in digests using both sample preparation procedures were not significant at 95% confidence level for Ba, Co, Cr, Fe, Mn, Ni, Sr and Zn.

3.3. Analytical application in soybeans samples

The selected analytical conditions were applied for the determination of As, Ba, Cd, Co, Cr, Fe, Mn, Ni, Pb, Se, Sr, V and Zn in five

Table 1

Concentrations (µg g⁻¹) of analytes in soybeans after microwave-assisted digestion with HNO₃ and H₂O₂ (mean ± standard deviation, $n = 3$) and ICP OES determinations.

Element	HNO ₃ concentration (mol L ⁻¹)			
	2.1	4.2	7.3	14.5
Ba	10.6 ± 0.4	9.5 ± 0.6	9.6 ± 0.1	11.3 ± 0.8
Cr	1.0 ± 0.1	0.7 ± 0.1	0.802 ± 0.002	1.12 ± 0.03
Cu	11.0 ± 0.2	11 ± 1	11.5 ± 0.1	13.3 ± 0.9
Fe	84 ± 4	77 ± 4	77 ± 2	81 ± 4
Mn	35.2 ± 0.4	33 ± 1	31.5 ± 0.3	33.3 ± 0.8
Ni	4.1 ± 0.5	4.7 ± 0.8	4.9 ± 0.3	4.4 ± 0.5
Sr	5.10 ± 0.09	5.0 ± 0.4	6.4 ± 0.1	5.8 ± 0.7
Zn	42.6 ± 0.9	40 ± 2	37.3 ± 0.6	38 ± 2

Table 2

Concentrations of analytes in certified reference material NIST 1568a (Rice flour) after microwave-assisted digestion with 2.1 mol L⁻¹ HNO₃ and H₂O₂ (mean ± standard deviation, *n* = 3, values in mg kg⁻¹ dry weight) and ICP OES determinations.

Element	Determined values (mg kg ⁻¹)	Certified values (mg kg ⁻¹)
Cu	2.25 ± 0.13	2.4 ± 0.3
Fe	6.7 ± 0.3	7.4 ± 0.9
Mg	500 ± 20	560 ± 20
Mn	22.1 ± 0.7	20 ± 2
Zn	20.8 ± 0.5	19.4 ± 0.5

types of the most consumed commercial soybeans products in Brazil. Samples were purchased from different suppliers, depending on market availability (Tables 4 and 5).

The average concentrations obtained for the analytes considering the 22 samples analysed were (values in bracket are in µg g⁻¹): As (<0.007–0.040), Ba (0.064–10.6), Cd (<0.006–0.033), Co (0.012–102), Cr (0.56–5.88), Cu (6.53–13.9), Fe (24.9–130), Mn (16.4–35.2), Ni (0.68–4.78), Pb (<0.029–0.062), Se (<2.90–25.0), Sr (2.48–20.1), V (<0.027–20.0) and Zn (29.3–47.3). Soy-based food investigated in this study presented variable composition in terms

of essential and potentially toxic elements, which can be attributed to different methods of processing. Brazilian legislation establishes maximum levels for the presence of potential inorganic contaminants in food (BRASIL, 1998). For the evaluated samples, the determined concentrations were below the maximum values (MV) allowed for the elements: As (MV = 1.0 mg g⁻¹), Cd (MV = 1.0 mg g⁻¹), Ni (MV = 5.0 mg g⁻¹), Pb (MV = 1.0 mg g⁻¹) and Zn (MV = 50 mg g⁻¹), except for Cr and Se which were above the established limits: Cr (VM = 0.1 mg g⁻¹) and Se (VM = 0.05 mg g⁻¹).

In recent works, also made in South America, similar results for Cd (0.02–0.11 µg g⁻¹), Fe (69.8–77.4 µg g⁻¹), Cu (11.18–12.65 µg g⁻¹), and Zn (33.9–37.5 µg g⁻¹) were obtained for soybeans seeds harvested in Uruguai (Morales, Knochen, & Pistón, 2013; Pistón, Pioda, Dol, & Torre, 2011). The results obtained in this work are in similar levels to those obtained for other samples of soybeans as shown by Kaar-Lilienthal et al. that evaluated samples from five countries with special concern to Fe, Mn and Zn.

In this study a comparative evaluation was also carried out between the multielement composition of two samples of soybeans cultivated in the city of Barreiras, Bahia, one involving

Table 3

Results obtained for microwave-assisted digestion with concentrated acid (MW-CD) and microwave-assisted digestion using diluted acid (MW-DA) procedures of soybeans and related products (mean ± standard deviation for *n* = 3, values in mg kg⁻¹) and ICP OES determinations.

Element	Soy extract		Whole flour soy		Textured protein soy		Soybeans	
	MW-CD	MW-DA	MW-CD	MW-DA	MW-CD	MW-DA	MW-CD	MW-DA
Ba	3.4 ± 0.3	2.9 ± 0.1	9.1 ± 0.1	8.9 ± 0.3	3.4 ± 0.5	3.7 ± 0.1	11.3 ± 0.8	10.6 ± 0.4
Cr	0.58 ± 0.06	0.63 ± 0.03	0.70 ± 0.05	0.73 ± 0.04	0.81 ± 0.09	0.71 ± 0.02	1.12 ± 0.03	1.04 ± 0.03
Cu	13 ± 1	12.9 ± 0.3	12.6 ± 0.3	11.1 ± 0.2	11 ± 2	11.5 ± 0.3	12.1 ± 0.9	11.0 ± 0.2
Fe	58.3 ± 0.2	56.0 ± 2.0	70.8 ± 0.7	72.4 ± 0.9	81 ± 3	77 ± 1	81 ± 4	84 ± 4
Mn	24 ± 2	22 ± 1	25 ± 1	23.5 ± 0.4	29 ± 2	24.4 ± 0.1	33.3 ± 0.8	35.2 ± 0.4
Ni	3.2 ± 0.3	3.3 ± 0.2	1.98 ± 0.05	2.07 ± 0.04	1.4 ± 0.1	1.7 ± 0.1	4.3 ± 0.5	4.1 ± 0.5
Sr	2.3 ± 0.1	2.5 ± 0.1	2.3 ± 0.2	2.1 ± 0.5	5.9 ± 0.4	5.8 ± 0.1	5.8 ± 0.7	5.1 ± 0.1
Zn	43 ± 3	40 ± 1	36.1 ± 0.8	34 ± 1	44 ± 1	47 ± 1	39.2 ± 0.2	42.6 ± 0.9

Table 4

Results obtained for soybeans and its products after digestion using microwave-assisted digestion using diluted acid and detection by ICP OES (mean ± standard deviation for *n* = 3, mg kg⁻¹).

Samples		Elements							
		Ba	Cu	Fe	Mn	Ni	Se	Sr	Zn
Soy extract ^a	ES1	290 ± 0.6	11.5 ± 0.3	56 ± 2	21.9 ± 0.4	2.3 ± 0.2	<2.90	2.48 ± 0.04	40.0 ± 0.5
	ES2	1.46 ± 0.03	9.6 ± 0.1	47.8 ± 0.9	19.0 ± 0.1	1.87 ± 0.09	<2.90	3.47 ± 0.03	29.3 ± 0.2
	ES3	2.75 ± 0.07	8.6 ± 0.1	24.9 ± 0.7	17.8 ± 0.5	2.55 ± 0.07	<2.90	5.34 ± 0.09	39.3 ± 0.8
Whole Soy flour ^b	FS1	8.85 ± 0.03	11.1 ± 0.2	72.4 ± 0.9	23.5 ± 0.4	2.07 ± 0.04	<2.90	20.1 ± 0.5	33.9 ± 0.7
	FS2	3.38 ± 0.05	10.6 ± 0.1	46 ± 1	24.8 ± 0.3	2.34 ± 0.05	<2.90	4.17 ± 0.03	35.5 ± 0.4
Textured soy protein ^c	PTS1	2.66 ± 0.04	11.5 ± 0.3	77 ± 1	26.4 ± 0.3	1.0 ± 0.2	<2.90	5.29 ± 0.05	45.2 ± 0.6
	PTS2	1.71 ± 0.01	7.6 ± 0.2	85 ± 5	25.4 ± 0.1	0.68 ± 0.03	<2.90	5.78 ± 0.01	46.7 ± 0.2
	PTS3	6.95 ± 0.09	12.0 ± 0.4	130 ± 4	29.3 ± 0.6	1.8 ± 0.1	<2.90	9.5 ± 0.2	45.1 ± 0.6
	PTS4	1.70 ± 0.05	7.6 ± 0.2	86 ± 3	24.0 ± 0.6	0.91 ± 0.09	<2.90	5.7 ± 0.1	46 ± 1
	PTS5	5.0 ± 0.2	11.7 ± 0.5	72 ± 3	23.5 ± 0.8	1.54 ± 0.04	<2.90	9.2 ± 0.3	44 ± 2
	PTS6	1.57 ± 0.03	7.34 ± 0.08	88 ± 2	23.4 ± 0.3	0.81 ± 0.02	<2.90	5.8 ± 0.1	45.5 ± 0.5
	PTS7	1.57 ± 0.01	6.53 ± 0.05	78 ± 2	26.1 ± 0.1	0.82 ± 0.08	<2.90	5.52 ± 0.02	46.4 ± 0.7
	PTS8	6.0 ± 0.2	12.2 ± 0.4	116 ± 10	27.3 ± 0.8	0.74 ± 0.03	<2.90	9.2 ± 0.2	46 ± 1
	PTS9	3.05 ± 0.01	9.4 ± 0.3	102 ± 1	28.1 ± 0.2	1.03 ± 0.08	<2.90	8.53 ± 0.02	45.9 ± 0.2
	PTS10	7.8 ± 0.2	10.7 ± 0.1	126 ± 3	30.6 ± 0.4	0.87 ± 0.04	<2.90	9.5 ± 0.2	43.7 ± 0.7
	PTS11	6.27 ± 0.05	13.2 ± 0.1	112 ± 6	33.6 ± 0.2	1.21 ± 0.02	<2.90	10.2 ± 0.1	47.3 ± 0.6
Soybeans ^d	SG1	10.6 ± 0.4	11.0 ± 0.2	84 ± 4	35.2 ± 0.4	4.1 ± 0.6	21 ± 3	5.1 ± 0.1	42.6 ± 0.9
	SG2	3.5 ± 0.2	10.6 ± 0.4	124 ± 3	25 ± 1	3.4 ± 0.2	25 ± 2	7.5 ± 0.3	35 ± 2
	SG3	5.5 ± 0.2	7.0 ± 0.1	46 ± 2	19.3 ± 0.3	2.5 ± 0.2	14 ± 2	6.22 ± 0.07	30.8 ± 0.3
	SG4	0.31 ± 0.09	7.4 ± 0.2	58 ± 5	17.2 ± 0.4	2.84 ± 0.03	18 ± 1	4.1 ± 0.2	41 ± 2
Transgenic soybeans ^e	SGT1	0.064 ± 0.001	6.90 ± 0.02	72.8 ± 0.1	16.4 ± 0.1	1.33 ± 0.02	4.39 ± 0.03	2.69 ± 0.01	31.4 ± 0.2
	SGT2	2.91 ± 0.05	13.9 ± 0.6	94 ± 4	24.7 ± 0.3	4.78 ± 0.05	15.5 ± 0.3	5.8 ± 0.1	42 ± 1

^a Three soy extract samples.

^b Two whole soy flour samples.

^c Eleven textured soy protein samples.

^d Four non-transgenic soybeans samples.

^e Two transgenic soybeans samples.

Table 5
Results obtained for soybeans and its products after digestion using microwave-assisted digestion using diluted acid and detection by ICP-MS (mean \pm standard deviation for $n = 3$, mg kg⁻¹).

Samples		Elements					
		As	Cd	Co	Cr	Pb	V
Soy extract ^a	ES1	<0.007	<0.0059	0.0229 \pm 0.0005	0.69 \pm 0.03	0.038 \pm 0.007	<0.027
	ES2	<0.007	0.0176 \pm 0.0001	0.093 \pm 0.005	3.0 \pm 0.1	0.013 \pm 0.002	<0.027
	ES3	<0.007	0.028 \pm 0.002	0.14 \pm 0.02	3.4 \pm 0.3	<0.029	<0.027
Whole Soy flour ^b	FS1	<0.007	<0.0059	0.012 \pm 0.008	0.73 \pm 0.04	0.062 \pm 0.004	<0.027
	FS2	<0.007	0.022 \pm 0.005	0.29 \pm 0.07	3.00 \pm 0.03	0.015 \pm 0.001	<0.027
Textured soy protein ^c	PTS1	<0.007	<0.006	3.3 \pm 0.1	0.71 \pm 0.02	<0.029	0.47 \pm 0.04
	PTS2	<0.007	<0.006	9.8 \pm 0.9	0.663 \pm 0.006	<0.029	0.45 \pm 0.02
	PTS3	0.0076 \pm 0.0003	<0.006	6.4 \pm 0.2	0.67 \pm 0.02	0.036 \pm 0.008	0.47 \pm 0.01
	PTS4	<0.007	<0.006	8.7 \pm 0.8	0.70 \pm 0.05	<0.029	0.45 \pm 0.03
	PTS5	<0.007	<0.006	3.0 \pm 0.2	0.56 \pm 0.03	0.05 \pm 0.01	0.09 \pm 0.01
	PTS6	<0.007	<0.006	7.9 \pm 0.8	0.691 \pm 0.006	0.11 \pm 0.02	<0.027
	PTS7	<0.007	<0.006	6.9 \pm 0.1	0.6 \pm 0.1	<0.029	0.54 \pm 0.01
	PTS8	0.013 \pm 0.002	0.017 \pm 0.003	18 \pm 2	0.56 \pm 0.01	<0.029	<0.027
	PTS9	<0.007	0.023 \pm 0.003	18.1 \pm 0.3	3.0 \pm 0.3	<0.029	<0.027
	PTS10	0.011 \pm 0.003	<0.006	8 \pm 1	2.4 \pm 0.4	<0.029	<0.027
	PTS11	0.0148 \pm 0.0004	0.033 \pm 0.005	29 \pm 4	4.2 \pm 0.2	0.039 \pm 0.007	<0.027
Soybeans ^d	SG1	0.018 \pm 0.001	0.0185 \pm 0.0002	46 \pm 1	5.88 \pm 0.03	<0.029	9.8 \pm 0.5
	SG2	0.040 \pm 0.007	0.019 \pm 0.004	100 \pm 10	10 \pm 1	0.035 \pm 0.001	20 \pm 3
	SG3	0.011 \pm 0.001	<0.006	21.0 \pm 0.2	8 \pm 1	<0.029	4.3 \pm 0.3
	SG4	0.017 \pm 0.001	0.016 \pm 0.001	44 \pm 2	5.5 \pm 0.5	<0.029	9.4 \pm 0.5
Transgenic soybean ^e	SGT1	0.011 \pm 0.002	0.014 \pm 0.001	12 \pm 2	4.7 \pm 0.2	<0.029	<0.027
	SGT2	0.019 \pm 0.005	0.018 \pm 0.004	32 \pm 9	5.00 \pm 0.06	0.057 \pm 0.009	6.3 \pm 0.9

^a Three soy extract samples.

^b Two whole soy flour samples.

^c Eleven textured soy protein samples.

^d Four non-transgenic soybeans samples.

^e Two transgenic soybeans samples.

genetically modified (transgenic, namely SGT) and other conventional (non-transgenic, namely SG). Transgenic soybeans refer to soybeans containing one or more genes transferred by a process of genetic transformation. Because of these characteristics and importance, transgenic soybeans cultures have been greatly developed, so that 60% global cultivated area (about 49 million hectares) is being occupied by soybeans cultivated to be herbicide tolerant (Sussulini et al., 2007). The results are shown in Tables 4 and 5. There was significant difference at 95% confidence level using Student *t*-test between concentrations of analytes studied, except for the elements As, Cd, Cr, and Mn. It may be inferred that the genetic modification in the structure of the plant may cause alterations in the mechanisms of absorption and translocation of some elements (Mataveli et al., 2010). However, these are only preliminary data and none conclusion is possible because the number of samples is not enough and this was not the main goal of this work.

In a recent study developed by Jiao, Si, Zhang, Li, and Crib (2012) the concentration of analytes obtained for different samples of soybeans varieties were (values in $\mu\text{g g}^{-1}$): Fe (22.1–38.8) and Zn (15.8–49.6). In another study, the levels of Fe, Mn, Co, Ni, Cu, Zn and Cd in soybean flour samples, purchased at market of Prague, Czech Republic, were 140 ± 5 , 41.7 ± 0.2 , 0.100 ± 0.002 , 8.85 ± 0.30 , 16.1 ± 0.1 , and 0.106 ± 0.002 , respectively. In general, with the exception of Co, higher concentrations of trace elements were found in this study when compared to the present one. This was expected taking into account differences in soil composition and agriculture practices.

Yamada, Barcelos, Sousa, and De Lima (2003) determined Cu, Fe, Mn and Zn concentrations in four soybeans cultivars from the Campinas Agronomic Institute (IAC), in Campinas, São Paulo State, Brazil. The concentrations of Fe, Mn and Zn were in the range from 84.5 to 110.3, 18.4 to 24.8 and 37.8 to 42.3 mg g⁻¹, respectively. These values were similar to those obtained for four samples of soybeans evaluated in this study.

4. Conclusions

The microwave-assisted digestion procedure using diluted solution of nitric acid (2.1 mol L⁻¹ HNO₃, 1 mL of H₂O₂, 0.25 g of sample mass) was successfully applied for the determination of a wide range of concentrations of essential (Cr, Co, Cu, Fe, Mn, Ni, Se, V, Zn) and non-essential (As, Ba, Cd, Pb, Sr) elements by ICP OES and also ICP-MS. Considering some critical parameters such as cost, green chemistry recommendations, and analyst safety, the proposed procedure has advantages compared to classical microwave-assisted digestion procedures. Additionally, the use of concentrated reagents is always hazardous, involves dilution of the digests prior to the determination step, and may produce large amounts of concentrated acids as residues.

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