Food Anal. Methods (2013) 6:872–880
DOI 10.1007/s12161-012-9497-0

Evaluation of Sample Preparation Procedures for Trace Element Determination in Brazilian Propolis by Inductively Coupled Plasma Optical Emission Spectrometry and Their Discrimination According to Geographic Region

Maria Graças Andrade Korn · Mauro Agildo Barbosa Guida · José Tiago Pereira Barbosa · Ednildo Andrade Torres · Andréa Pires Fernandes · Josué Carinhanha Caldas Santos · Kelly das Graças Fernandes Dantas · Joaquim Araújo Nóbrega

Received: 15 May 2012 / Accepted: 23 August 2012 / Published online: 9 September 2012

Abstract Propolis is a complex mixture of substances collected by honeybees from buds or exudates of plants, beeswax, and other constituents, as pollen and sugars. The main purpose of this study was to evaluate two digestion procedures for determination of major, minor, and trace elements (Ba, Ca, Cd, Cr, Co, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, and Zn) in natura propolis samples by inductively coupled plasma optical emission spectrometry (ICP OES). The first procedure studied was an open-vessel digestion using HNO_3 + H_2SO_4 + H_2O_2 in a heating block and the second one was a microwave-assisted concentrated acid digestion using HNO_3 + H_2O_2. Both digestion procedures led to similar results and quantitative recoveries. The residual carbon contents (RCCs) for propolis sample digests were 0.269±0.012 % when using the first procedure with conventional heating and 0.458±0.023 % by microwave-assisted closed vessel digestion, demonstrating high efficiency of both procedures. Accuracy of the results was demonstrated using a certified reference material and by comparison with a recommended official method. The *t* test (unpaired) at 95 % confidence level showed that there was no significant difference between determined and certified values of all analytes under investigation, except Ca concentration employing conventional procedure. The optimized microwave-assisted digestion procedure led to recoveries around 89–103 % and precision better than 5 % for most samples. The second procedure was faster, safer, and more accurate than the one based on conductive heating. Additionally, principal component analysis (PCA) was applied for checking if there was correlation between inorganic composition and source of propolis samples collected around Bahia State in the Northeast of Brazil.

Keywords Propolis · Trace elements · Digestion · Microwave radiation · ICP-OES

In memoriam of Mauro Agildo Barbosa Guida

M. G. A. Korn (✉) · M. A. B. Guida · J. T. P. Barbosa
Instituto de Química, Universidade Federal da Bahia,
Campus de Ondina, Ondina,
40170-290, Salvador, Bahia, Brazil
e-mail: korn@ufba.br

E. A. Torres
Escola Politécnica, Universidade Federal da Bahia,
Campus de Ondina, Ondina,
40170-290, Salvador, Bahia, Brazil

A. P. Fernandes · J. C. C. Santos
Instituto de Química e Biotecnologia,
Universidade Federal de Alagoas,
Campus A.C. Simões, Tabuleiro dos Martins,
57072-970, Maceió, Alagoas, Brazil

K. d. G. F. Dantas
Faculdade de Química, Instituto de Ciências Exatas e Naturais,
Universidade Federal do Pará,
66075-110, Belém, Pará, Brazil

J. A. Nóbrega
Departamento de Química, Universidade Federal de São Carlos,
13560-970,
São Carlos, São Paulo, Brazil

Introduction

Propolis is a product from resinous substances, gummy and balsamic, collected by bees from buds, flowers, and exudates of plants, in which the bees add salivary secretions,

wax, and pollen for preparation of final product which has important roles in the hive. Propolis protects against pathogenic agents that can exterminate the hive [Burdock 1998; Bankova et al. 2000].

The chemical composition of propolis is affected by climate conditions and type of bee flora. Depending on the source, propolis may contain up to 400 different chemicals substances. These chemical substances reflect the environment because propolis is produced from nature products and at the same time, it is exposed to the surroundings. The chemical composition of propolis is varied, with 55 % of resins and balsams, 30 % of wax, 10 % of volatile oils, and 5 % of pollen. It is composed also of several organic acids and considerable amount of minerals, including Ca, Cu, Mn, and Zn. Vitamins B1, B2, B6, C, and E and nicotinic, pantothenic, and amino acids are also present (Castaldo and Capasso 2002; Bankova et al. 2000; Buriol et al. 2009).

Due to its antibiotic and antifungal activities, propolis has gained popularity and used extensively in health drinks and foods to improve health and prevent diseases, such as inflammation, heart disease, diabetes, aging, and cancer (Castaldo and Capasso 2002; Bankova et al. 2000; Banskota et al. 2001; Buriol et al. 2009; Sforcina and Bankova 2011).

Evaluation of essential and toxic element contents enables one to assess nutritive quality of propolis originating from different regions and also helps in tracking and judging its authenticity according to certain geographical and biological origins. However, limited information on trace element propolis composition is currently available (Gonzáles-Rodríguez et al. 1999; Conti and Botre 2001; Dogan et al. 2006; Sales et al. 2006; Cvek et al. 2008; Lima et al. 2009; Cantarelli et al. 2011; Roman et al. 2011; and Gong et al. 2012).

Graphite furnace atomic absorption spectrometry (GFAAS), inductively coupled plasma optical emission spectrometry (ICP OES), and neutron activation analysis (NAA) are the main techniques used for determination of trace element contents in propolis and similar samples (Cantarelli et al. 2011; Castaldo and Capasso 2002; Conti and Botre 2001; Cvek et al. 2008; Gong et al. 2012). However, to determine trace element contents using atomic spectrometry techniques, food samples typically need to be digested to convert solid samples to a solution for measurements. Traditional techniques for sample preparation are time consuming and require large amounts of reagents, which are expensive, generate hazardous waste, and might contaminate samples (Korn et al. 2008; Cantarelli et al. 2011; Conti and Botre 2001). Advances in sample preparation over the last few decades have been propelled by development of microwave-assisted acid digestion and extraction (Korn et al. 2008). Microwave-assisted digestion using HNO_3 plus H_2O_2 has proved an effective, fast, and simple method to determine trace elements in foods (Castro et al. 2009; Khajeh and Sanchooli 2010; Millour et al. 2011; Reis et al. 2012).

Therefore, our study was designed to: (1) develop and critically compare two digestion methods for digestion of in natura propolis samples followed by determination of trace elements by ICP OES and (2) explore principal component analysis (PCA) to evaluate similarities and differences among propolis samples from different geographic regions of Bahia State (Brazil).

Materials and Methods

Samples

Forty-eight propolis samples were collected directly from beekeepers from different regions of Bahia State, in the Brazilian northeast (Fig. 1). These regions correspond to six phytogeographic regions: North Coast (NC, n=27), West (WE, n=4), Chapada Diamantina (CD, n=5), South (SO, n=2), Reconcavo (RE, n=5), and Southwest (SW, n=5). Three different samples (green, brown, and red Brazilian propolis) were used as experimental materials to evaluate the best procedure for digestion of the samples. Prior to the digestion, all propolis samples were ground and homogenized.

Apparatus

A Varian (Mulgrave, Australia) Vista simultaneous ICP OES instrument with axial viewing and a charge-coupled device (CCD) detector was used in all measurements. The ICP OES instrument was calibrated with a multielement stock solution and optical alignment was adjusted using a solution containing 5.0 mg L^{-1} Mn. Emission lines were selected according to absence of spectral interferences and adequate sensitivity for determination of elements at low and high concentrations. Optima values for instrumental parameters were: radio frequency applied power (1.3 kW), plasma gas flow rate (15 L min^{-1}), auxiliary gas flow rate (1.5 L min^{-1}), nebulizer gas flow rate (0.7 L min^{-1}), replicate reading time (1 s), instrument stabilization delay (15 s), replicates (n=3), and pump rate (15 rpm). Analytical wavelengths (nm) chosen were: Ba II 455.403, Ca II 396.847, Cd II 226.502, Co II 238.892, Cr II 267.716, Cu I 327.398, Fe II 238.203, K I 766.468, Mg II 280.267, Mn II 257.611, Na I 589.592, Ni II 231.604, Pb II 220.354, and Zn I 213.858. The residual carbon content (RCC) was determined using ICP OES by measuring the carbon atomic emission line at 193.025 nm. An aluminum heating block (Tecnal, Piracicaba, SP, Brazil), a programmable muffle furnace (Quimis, São Paulo, SP, Brazil) and a closed-vessel microwave digestion system with sensors for controlling pressure and temperature

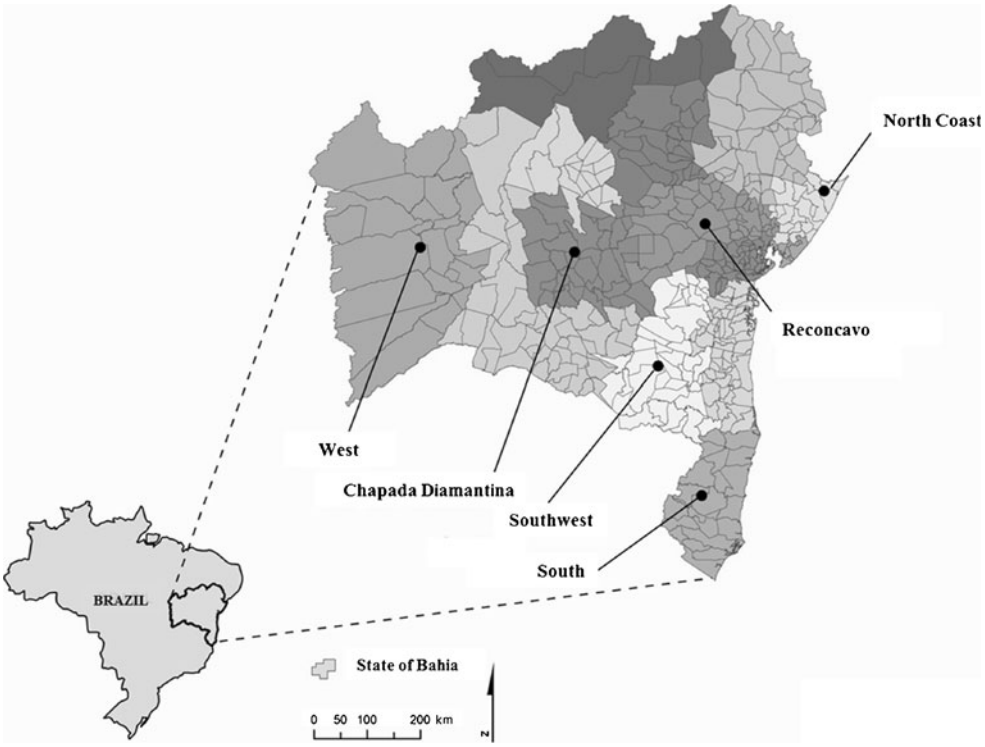

Fig. 1 Sampling points in the State of Bahia (Brazil) for in natura propolis samples

(ETHOS EZ, Milestone, Sorisole, BG, Italy) were used for sample digestion.

Reagents and Analytical Solutions

All solutions were prepared from analytical reagent-grade chemicals. Ultrapure water was supplied by Milli-Q® water purification (18.2 MΩ cm, Milli-Q, Millipore, Bedford, MA, USA). Mineral acids and oxidizing agents [65 % (w w^{-1}) HNO_3 (d=1.40 kg L^{-1}), 30 % (w w^{-1}) H_2O_2 (d=1.11 kg L^{-1}), 97 % (w w^{-1}) H_2SO_4 (d=1.84 kg L^{-1})] were used. High purity analytical stock solutions of 1,000 mg L^{-1} (Titrisol, Merck, Darmstadt, Germany) of each element were used daily to prepare multielement analytical solutions.

Sample Preparation

Two digestion procedures were developed to digest powdered propolis samples. A set of digestion blanks was prepared together with each batch of samples. An official method (AOAC No. 999.11–1999.19) using dry ashing was performed to compare the accuracy of the proposed procedures (AOAC 2000).

Conventional Heating Procedure

About 1.0 g of ground and homogenized raw propolis was accurately weighed into glass vessels and 2.0 mL of concentrated H_2SO_4, 5.0 mL of concentrated HNO_3, and 1.0 mL H_2O_2 were added. The block temperature was adjusted to 100 °C and the sample was digested for 30 min. Further, volumes of 3.0 mL of concentrated HNO_3 and 1.0 mL H_2O_2 were added. The block temperature was adjusted to 150 °C and the sample digested for 30 min. Then, more than 2.0 mL of concentrated HNO_3 and 1.0 mL H_2O_2 were added. The block temperature was kept at 230 °C for 1 h. When the solution became limpid, it was cooled down and diluted to 10 mL with ultrapure water.

Microwave-Assisted Procedure

About 250 mg of each sample was accurately weighed into dry, clean microwave-closed vessels made of perfluoroalcoxi polymer (PFA) with a volume of 100 mL. Volumes of 6.0 mL of concentrated HNO_3 were added. Predigestion at room temperature was performed for 1 h and then 1.0 mL of H_2O_2 (30 %, w w^{-1}) was added and vessels were gently shaken and sealed. The heating program was performed in five steps: in the first step, the temperature was linearly increased up to 90 °C in 4 min with maximum applied power of the magnetron set at 500 W. In the second step, the temperature was kept at 90 °C for 2 min. In the third step, the temperature was linearly increased up to 180 °C in 6 min, and in the fourth step, the temperature was kept at 180 °C for 10 min. The fifth step was applied just for cooling down. After digestion and cooling, digests were transferred to plastic flasks and made up to a final volume

of 20 mL with ultrapure water. The digestion heating program was performed in 42 min. Temperature and pressure sensors were used in all digestions.

Reference Procedure: Dry Ashing

Approximately 2.0 g of sample was weighed into a porcelain crucible and transferred into a muffle furnace. The temperature was increased to 450 °C at a rate of about 50 °C h^{-1}, and this temperature was kept constant overnight. After cooling, ash was mixed with 1 mL of water and evaporated on a hot plate. Then, the flasks with ashes were returned to the oven for incineration at 450 °C for additional 1–2 h. The procedure was repeated until samples were completely ashed, i.e., ashes should be white/gray or slightly colored. The ashes were dissolved with 5.0 mL of 6 mol L^{-1} HCl solution under careful heating on a hot plate. Resulting solutions were transferred quantitatively to 20-mL volumetric flasks and diluted to volume with ultrapure water (AOAC 2000).

Determination of the Acidity and Residual Carbon of the Digests

To determine final acidity, acid–base titrations of the digests were made for the above-mentioned procedures. Titrations were carried out with a standardized solution of sodium hydroxide (9.972×10^{-2} mol L^{-1}) and phenolphthalein (1.0 %m/v in ethanol). The reference solutions were prepared with the same acid concentration for each digestion procedure. Residual carbon was also determined by ICP OES using urea for preparing reference solutions (Gouveia et al. 2001).

Investigation of Matrix Effects

Matrix effect studies were carried out by spiking some metals of the original undigested samples with variable amounts of standard solutions of the analytes. Spiked samples were then mineralized using the same digestion procedures as those applied to the nonspiked samples. All digestions were performed in triplicate. For all reference solutions and sample digests, yttrium was used as internal standard at the final concentration of 1.0 mg L^{-1} before the ICP OES determination. Digests were analyzed by ICP OES using external calibration and internal standard calibration.

Principal Component Analysis

Metal concentrations (Ba, Cu, Fe, K, Mg, Mn, Ni, and Zn) in 48 propolis samples were submitted to PCA with autoscaling pretreatment (Santos et al. 2008; Liu et al. 2008), resulting, initially, in a 48×10 data matrix. After selection of variables exploring discrimination power, a new 48×8 data matrix was proposed. Since PCA is a well-known chemometrics technique of multivariate analysis, which makes it easier to visualize grouping similarities, this tool was employed to grouping tendencies of in natura propolis samples from different geographic regions of Bahia State. The Unscrambler 8.0 (CAMO, Norway) chemometrics package was employed for PCA calculations.

Results and Discussion

Comparison of Sample Preparation Procedures

The efficiency of the digestion procedure using open-vessel conductively heated, so-called conventional procedure, and closed-vessel microwave-assisted procedure, named as microwave procedure was evaluated by determining RCC, residual acidity, and analytes recoveries. Conditions and analytical characteristics for each procedure are shown in Table 1.

In the conventional procedure, concentrated nitric and sulfuric acids were added to the samples, and the addition of hydrogen peroxide completed the digestion. Concentrated nitric acid is the most common acid for oxidation of organic matrices. However, the limitation in the use of this acid is its low boiling point at atmospheric pressure, around 120 °C. To facilitate digestion in open flasks that operate at atmospheric pressure, the addition of an aliquot of sulfuric acid is recommended (which has a boiling point of 330 °C), thereby increasing the oxidative efficiency of the medium and making the decomposition of fat globules possible (Momen et al. 2007; Korn et al. 2008; Korn et al. 2010). In this procedure, it is possible to use a block with only 20 samples being prepared simultaneously, since it is necessary to manipulate the tubes for addition of reagents during the digestion.

In the microwave procedure, complete digestion of ten samples per run was performed using concentrated nitric acid and hydrogen peroxide. During the course of the digestion, the reaction conditions inside the control vessel were compared to the performance-based protocol. The microwave power was adjusted based on the difference between prescribed settings and measured conditions. For safety reasons and in order to ensure complete digestion, the microwave digestion program was chosen in agreement with manufacturer recommendations and earlier studies on microwave-assisted digestion optimization (Castro et al. 2009; Korn et al. 2010; Nunes et al. 2011).

The RCCs for digests of propolis samples were determined using axial view ICP OES and mean values and standard deviations (SDs) (n=3) were 0.269±0.012 % and 0.458±0.023 % for conventional and microwave procedures, respectively, demonstrating the high efficiency of

Table 1 Conditions and analytical characteristics of the analytical procedures investigated

Parameters	Digestion procedure	
	Conventional	Microwave
Sample mass (g)	1.00	0.200
Volume of reagents (mL)	HNO_3 (10)	HNO_3 (5.0)
	H_2O_2 (5.0) H_2SO_4 (2.0)	H_2O_2 (3.0)
RCC (%)	0.269±0.012	0.458±0.023
Residual acidity (moL L^{-1})	2.20	4.40
Heating program (min)	150	42
Analytical throughput (sample/150 min)	20	30

both. The slightly lower RCC obtained using the conventional procedure is related to the higher temperature reached using sulfuric acid, but it should be remembered that this acid may cause transport interferences.

The final acidities of the digests were determined using acid–base titration with a standardized solution of NaOH. Residual acidities were 2.20 and 4.40 mol L^{-1} for conventional and microwave procedures, respectively. The lower acidity observed with the conventional procedure is related with evaporation of acid vapors in open vessels at the set temperatures for the digestion block. On the other hand, it was demonstrated that nitric acid can be regenerated in a closed vessel heated by microwave radiation due to the temperature gradient during the beginning of the heating program. In this step of digestion, the soluble gases as well 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 O_2 leading to NO_2 production. The formed NO_2 is reabsorbed in the acid solution and regenerates HNO_3 (Castro et al. 2009; Arruda 2006).

The comparison of the slopes of the analytical calibration curves obtained using external calibration and the analyte additions method indicated no significant differences at the 95 % confidence level, suggesting no detectable matrix effects for Ba, Ca, Cd, Cr, Co, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, and Zn. The limits of detection (LOD) and limits of quantification (LOQ) were estimated using the SD obtained for ten independent experimental blanks. The obtained LOQs (μg g^{-1}) for the conventional procedure and microwave procedure were, respectively: Ba (0.50 and 0.32), Ca (15.2 and 11.1), Cd (0.83 and 0.45), Cr (0.15 and 0.13), Co (0.50 and 0.61), Cu (0.39 and 0.25), Fe (5.9 and 2.8), K (4.2 and 3.6), Mg (3.8 and 0.75), Mn (1.2 and 0.85), Na (1.7 and 1.2), Ni (0.16 and 0.14), Pb (3.2 and 2.2), and Zn (2.90 and 3.9).

Since certified reference materials were unavailable for trace elements in propolis, the accuracies of both procedures were verified and analyzed using the certified reference material NIST SRM 1570a Spinach Leaves (Gaithersburg, MD, USA). The comparison between experimental and certified values for some analytes is presented in Table 2. The *t* test (unpaired) at 95 % confidence level showed that there was no significant difference between determined and certified values of all analytes under investigation, except for Ca, Cu, Cd and Mn concentrations employing conventional procedure .

Furthermore, three propolis samples have been digested using the proposed open-block digestion, microwave-assisted digestion, and dry ashing using the official method AOAC No. 999.11–1999.19 and the results are shown in Table 3. Based on an analysis of variance (ANOVA) test, the determined concentrations among the three sample preparation procedures were not significant at the 95 % confidence level for microelements Ba, Cr, Cu, Fe, Mn, Ni, and Zn. The concentrations determined for Cd, Co, and Pb were lower than the respective LOQ. In addition, results for conventional and microwave procedures were evaluated by spike recovery tests. Propolis samples were enriched in order to reach concentrations of 0.5, 1.5, and 3.0 mg L^{-1} of each analyte. All recoveries were acceptable (81 to 108 %) with relative SDs in the 1–9 % range.

The conventional procedure in heating block can be considered a low cost alternative for quantification of macro- and microelements in natura propolis samples. However, the microwave procedure was preferred instead of the open system with conventional heating procedure for three reasons: it required less time (42 min); it used lower volume and types of reagents, minimizing the risks of contamination; and it led to lower LOD and LOQ for most metals evaluated. Throughput is limited by the capacity of the microwave system, but overall efficiency is improved because the run time is short and digestion does not require supervision.

Analytical Application in Propolis Samples

The microwave procedure was applied to determine trace elements in 48 propolis samples from different regions of the State of Bahia, Brazil. The range of concentrations, i.e.,

Table 2 Concentrations (mean ± standard deviation, n=3) of trace elements obtained by conventional and microwave-assisted digestion procedures for NIST standard reference material 1570a (spinach leaves)

Analyte	Digestion procedure		Certified values (1570a NIST)	t test values*	
	Conventional (CONV)	Microwave (MW)		t_{cal} CONV vs. reference	t_{cal} MW vs. reference
Concentration (%, mg g^{-1})					
Ca	1.378±0.014	1.500±0.031	1.527±0.041	6.14	0.50
K	2.740±0.041	3.044±0.035	2.903±0.052	2.29	2.32
Concentration (μg g^{-1})					
Cu	9.86±0.15	10.9±0.7	12.2±0.6	9.00	1.07
Cd	2.13±0.05	2.45±0.06	2.89±0.07	8.77	4.23
Mn	59.0±2.0	72.9±1.0	75.9±1.9	4.88	1.73
Ni	1.72±0.15	2.07±0.29	2.14±0.10	1.62	0.14
Zn	75.4±2.3	79.8±8.1	82±3	1.66	0.16

* $t_{critical}$=4.30 at 95 % confidence level

minimum and maximum values, for each region on propolis samples are shown in Table 4.

All samples presented high contents of Ca and K. Higher contents of Mg were found in propolis samples from areas closer to the coast. The concentrations of Mg found in this work varied from 276 to 350 μg g^{-1} for samples collected in Chapada Diamantina region, from 162 to 364 μg g^{-1} for the North Coast, and from 178 to 232 μg g^{-1} for samples from Southwest.

Table 3 Concentration determined using three digestion procedures: conventional, microwave, and dry ash. Mean values (n=3), μg g^{-1}, and standard deviations (SD) of trace elements in natura propolis samples

Analyte	Procedure	Concentration (μg g^{-1})		
		Sample A	Sample B	Sample C
Ba	Conventional	3.32±0.09	1.19±0.09	2.96±0.17
	Microwave	3.35±0.08	1.22±0.04	2.81±0.14
	Dry ash	3.25±0.29	1.18±0.16	2.71±0.06
Cr	Conventional	1.17±0.19	1.57±0.13	4.08±0.18
	Microwave	1.13±0.02	1.53±0.15	4.39±0.21
	Dry ash	1.14±0.12	1.57±0.12	4.08±0.18
Cu	Conventional	2.52±0.18	1.72±0.01	2.81±0.10
	Microwave	2.54±0.08	1.62±0.09	2.96±0.09
	Dry ash	2.01±0.42	1.55±0.09	2.72±0.10
Fe	Conventional	163±15	86±2	497±12
	Microwave	162±12	91±2	489±10
	Dry ash	165±12	88±4	495±12
Mn	Conventional	15.4±1.3	23.0±1.9	25.6±2.1
	Microwave	16.5±0.9	21.0±1.7	26.6±1.5
	Dry ash	16.2±1.2	22.8±0.3	25.4±1.1
Ni	Conventional	0.70±0.09	0.71±0.06	1.79±0.16
	Microwave	0.98±0.15	0.98±0.15	1.69±0.01
	Dry ash	0.92±0.12	0.98±0.12	1.71±0.05
Zn	Conventional	81±3	48±2	76±4
	Microwave	79±1	47±1	87±3
	Dry ash	79±1	51±2	88±5

The concentration range of Mn and Zn in propolis varied from 1.92 to 13.3 μg g^{-1} and from 1.32 to 139 μg g^{-1}, respectively. Higher Zn values were found in Croatia (80.14–9,325 μg g^{-1}) (Cvek et al. 2008), in Turkey (176–6,760 μg g^{-1}.) (Dogan et al. 2006) and in China (35.1–386.4 μg g^{-1}) (Gong et al. 2012); however, similar levels of Zn were detected in propolis samples collected from bee colonies in the industrialized region of Wroclaw, Poland (Roman et al. 2011) and from Argentina (Cantarelli et al. 2011; Lima et al. 2009). Nickel and Cr concentrations varied from 0.40 to 3.33 μg g^{-1} and from <LOQ–8.02 μg g^{-1}, respectively. These results are comparable to those previously reported in literature for some other countries such us China (Gong et al. 2012), Argentina (Cantarelli et al. 2011), and Croatia (Cvek et al. 2008). All samples presented Zn, Cr, and Ni concentrations above the maximum allowed in Brazil's regulations for food, except for Zn in the propolis samples collected in SO.

The concentrations detected for Cu ranged from 0.33 to 2.63 μg g^{-1}. The results of Cu concentrations are comparable to, or lower than, the values reported by Cvek et al. (2008), Dogan et al. (2006), Gong et al. (2012), and Roman et al. (2011). All samples had Cu concentrations below the maximum allowed in Brazil's regulations for food. For Fe, the concentration range was 21 to 356 μg g^{-1} for samples collected in Southwest and North Coast, respectively. The high contents of Fe in North Coast region can be explained by intense industrial activity. The concentrations of Fe were lower than those reported in literature for some other countries such as China (Gong et al. 2012), Argentina (Lima et al. 2009), and Croatia (Cvek et al. 2008). Concentrations

Table 4 Mean concentrations and standard deviations of trace elements (n=3, μg g^{-1}) in natura propolis samples from beekeepers located in different geographic regions of the State of Bahia (Brazil): North Coast (NC, n=27), West (WE, n=4), Chapada Diamantina (CD, n=5), South (SO, n=2), Reconcavo (RE, n=5), and Southwest (SW, n=5)

Element	Geographic region					
	NC (mean ± SD)	WE (mean ± SD)	CD (mean ± SD)	SO (mean ± SD)	RE (mean ± SD)	SW (mean ± SD)
Ba (Min–max)	1.11±0.42 (0.42–1.46)	0.56±0.09 (0.43–0.62)	0.62±0.04 (0.55–0.66)	1.54±0.34 (1.20–1.72)	0.82±0.41 (0.58–1.54)	1.01±0.28 (0.54–1.22)
Ca (Min–max)	370±83 (267–545)	530±21 (499–545)	500±62 (423–587)	713±546 (327–1099)	468±81 (412–615)	466±92 (313–567)
Cr (Min–max)	2.40±2.32 (0.11–8.02)	0.72±0.51 (0.17–1.20)	0.54±0.08 (0.42–0.72)	<LOQ	1.02±0.91 (0.19–2.48)	0.37±0.06 (0.31–0.43)
Cu (Min–max)	0.95±0.82 (0.33–2.63)	0.69±0.21 (0.59–0.96)	0.74±0.08 (0.45–0.78)	1.12±0.72 (0.62–1.63)	0.64±0.14 (0.42–0.79)	0.69±0.07 (0.56–0.78)
Fe (Min–max)	206±71 (118–356)	26±4 (21–29)	47±14 (28–59)	274±127 (185–364)	74±20 (45–96)	94±19 (71–115)
K (Min–max)	1,440±373 (776–1892)	735±41 (690–787)	829±40 (759–859)	1,531±168 (1412–1650)	240±6 (231–248)	231±29 (199–279)
Mg (Min–max)	247±58 (162–364)	319±58 (234–362)	331±32 (276–350)	309±110 (231–387)	197±28 (157–245)	206±20 (178–232)
Mn (Min–max)	3.68±1.19 (1.92–6.42)	6.64±3.22 (2.01–9.02)	6.82±2.10 (3.41–8.68)	8.1±7.0 (3.09–13.3)	4.58±1.51 (2.61–6.64)	4.41±0.68 (3.41–5.52)
Na (Min–max)	69±32 (5.23–97)	23±1 (22–24)	20±4 (15–24)	93±5 (89–96)	24±12 (11–40)	24±8 (12–35)
Ni (Min–max)	1.81±0.62 (0.71–3.17)	1.44±0.37 (1.00–1.90)	1.64±0.47 (1.10–1.64)	1.43±0.12 (1.32–1.41)	0.68±0.23 (0.50–1.12)	1.24±1.10 (0.40–3.33)
Zn (Min–max)	83±17 (47–112)	70±12 (62–88)	90±6 (84–100)	118±20 (104–132)	73±12 (52–86)	65±10 (52–79)

Max maximum value, *Min* minimum value, *LOQ* Limit of quantification

of Cd and Pb were below LOQs (0.45 and 2.2 μg g^{-1}, respectively), indicating that Bahia propolis are, in general, free of these contaminants. In earlier studies, Roman et al. (2011) showed the mean level of Pb concentration amounted to 5.74 μg g^{-1} in propolis from the industrialized region of Wroclaw area, Poland. Very low concentrations of cadmium were also obtained by Roman et al. (2011) and Gong et al. (2012). According to the Agência Nacional de Vigilância Sanitária (ANVISA 1998), the maximum level allowed for Pb in sugar is 2.0 mg kg^{-1}, while for cadmium, there is no recommendation. The value of 1.0 ppm (mg kg^{-1} or mg L^{-1}) for Cd, suggested for foods by ANVISA, was arbitrarily adopted for this consideration (ANVISA 1998).

Correlation Analysis

Correlation analysis is associated with the relationships among variables (i.e., element concentration). The correlation analysis of Ba, Ca, Cu, Fe, K, Mg, Mn, Na, Ni, and Zn concentrations was performed (Table 5) and from coefficients of values it was possible establish three groups of elements. The coefficient of values ranged from 0 to 1 (or −1), indicating weak to strong correlations between variables (Gong et al. 2012). The first group was formed by Ba and Ca, and the results obtained indicated that Ba is negatively correlated with almost all elements, followed by Ca that present negative correlation with Ba, Cu, Fe, and Mn. The results show that elements Cu, Fe, Mg, and Mn are positively correlated with all other elements, except Ba. These elements formed the second group. Finally, the third group was constituted by K, Zn, Ni, and Na.

Pattern Recognition Tool: Principal Component Analysis Application

The combined analysis of several elements and chemometry has emerged as a promising tool for the classification of food samples in terms of its type, level, provenance, and technological transformation (Grembecka and Szefer 2012; Naozuka et al. 2011; Chudzinska and Baralkiewicz 2011; Chudzinska and Baralkiewicz 2010; Camina et al. 2008; Santos et al. 2008; Silici et al. 2008; Garcia et al. 2006; Souza et al. 2006; Hernandez et al. 2005). Recently, some works were performed by applying chemometric classification in using data relating the mineral composition for propolis samples from Argentina (Cantarelli et al. 2011)

Table 5 Correlation matrix for the element concentrations in natura propolis samples from beekeepers located in different geographic regions of the State of Bahia (Brazil)

	Ba	Ca	Cu	Fe	K	Mg	Mn	Na	Ni	Zn
Ba	–									
Ca	−0.2102	–								
Cu	−0.4022	−0.1202	–							
Fe	−0.3208	0.1020	0.9382	–						
K	−0.2420	−0.2110	0.0931	0.1502	–					
Mg	−0.3274	0.2801	0.7684	0.8442	0.0381	–				
Mn	−0.1484	0.2773	0.6401	0.6434	0.0090	0.7081	–			
Na	−0.3045	0.3421	0.7003	0.4320	0.0759	0.6367	0.0393	–		
Ni	−0.2781	−0.4096	0.1005	0.1652	0.5038	0.0478	0.2352	−0.1643	–	
Zn	0.1602	−0.1848	0.3188	0.1521	0.7473	0.2944	0.0535	−0.1368	0.0911	–

employing eight elements (Br, Fe, Rb, Zn, Sb, Cr, Sm, and Sc) and China (Gong et al. 2012) using 11 elements (Ca, Al, Mg, K, Fe, Na, Zn, Mn, Pb, Sr, and Cd). Thus, it was possible to infer the quality and standardization of the samples about their similarities and differences.

The application of each method depends on the experimental data and the purpose of analysis. Thus, analysis was made by applying PCA to better interpret the analytical data obtained and to evaluate similarities and differences between in natura propolis samples originating from apiaries located in various cities from the State of Bahia. The mineral composition (Ba, Ca, Cu, Fe, K, Mg, Mn, Na, Ni, and Zn) of all in natura propolis samples was used in this evaluation as variables. In this work, the Unscrambler 8.0 (CAMO, Norway) chemometrics package was employed for PCA calculations.

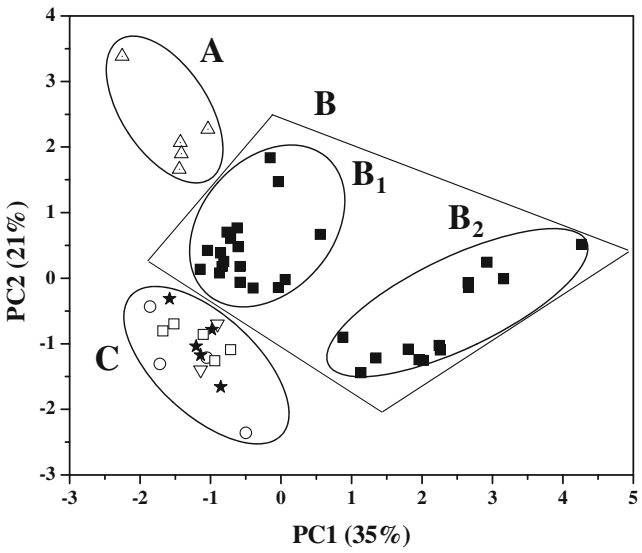

Fig. 2 Scores scatter plot for PCA data of metal concentrations in natura propolis samples for geographic regions of the State of Bahia: (*unfilled triangle*) Chapada Diamantina, (*unfilled circle*) West, (*unfilled square*) Southwest, (*filled star*) Reconcavo, (*unfilled inverted triangle*) South and (*filled square*) North Coast

In the process of variable selection, the statistic tool discrimination power (Oliveira et al. 2004; Sagrado and Cronin 2008; Diaz et al. 2005) observed that Ca and Na concentrations presented lower influence in PCA group separation. Consequently, these variables were excluded from the original data matrix (48×10), and a new data matrix was obtained (48×8) for PCA evaluation.

According to the scores plot (Fig. 2), the formation of three main groups was observed. Group A was formed exclusively by in natura propolis samples from Chapada Diamantina region and based on loadings scores (Fig. 3) and concentrations, Ba and Zn were the variables with major effects on the separation of these samples. The comparison of loadings scores (Fig. 3) and element groups formed by correlation analysis (Table 5) showed similar profiles in variable grouping. Thereby, the elements in the same group showed significant correlation with each other.

Group B was formed by propolis samples from North Coast region, being perceptible to the formation of two subgroups (B1 and B2). The main variables for

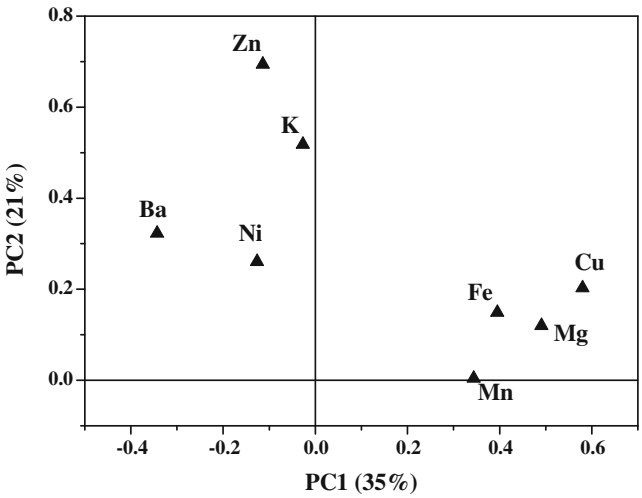

Fig. 3 Loadings plot obtained from PCA data of metal concentrations in natura propolis samples for geographic regions of the State of Bahia

separation of group B2 were Mn, Fe, Cu, and Mg concentrations (Fig. 3). This separation was attributed to the closeness of the sampling points with industrial zone. Group C was composed by propolis samples from West, Southwest, South, and Reconcavo regions indicating similarities in mineral composition.

Conclusions

Both developed procedures proved to be efficient for the determination of metals in propolis with advantages and disadvantages which are characteristic of each process. In general, these procedures are simple, not requiring a great amount of reagents and samples. The microwave procedure offers advantages such as minimization of losses of volatiles and smaller volumes of reagents, which generates less waste and less risk of contamination. Moreover, it is faster when compared to the conventional procedure in heating block. In a general way, Brazilian propolis did not show contamination by potentially toxic species and is a good source of Ca, K, Mg, and Fe. The application of PCA allowed the evaluation of similarities and differences between in natura propolis samples from apiaries located in six regions from the State of Bahia, the formation of three main groups being observed.

Acknowledgments The authors gratefully acknowledge Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for fellowships and financial support. The authors are also grateful to Empresa Baiana de Desenvolvimento Agrícola (EBDA) (Alagoinhas, Bahia), Centro Agrotécnico de Catu, and Leonardo J.S.J de Oliveira for propolis samples.

References

ANVISA. Agência Nacional de Vigilância Sanitária. Portaria Nº 685, de 27/08/1998. Available from. http://www.anvisa.gov.br/legis/decretos/55871_65. Accessed in May/2012

AOAC (2000) Official methods of analysis, 17th ed., Association of Official Analytical Chemist: Washington D.C.

Arruda MAZ (2006) Trends in sample preparation. Nova, New York

Bankova VS, De Castro SL, Marcucci MC (2000) Apidologie 31:3

Banskota AH, Tezuka Y, Kadota S (2001) Phytother Res 15:561

Burdock GA (1998) Food Chem Toxicol 36:347

Buriol L, Finger D, Schmidt EM, Santos JMT, Rosa MR, Quináia SP, Torres YR, Santa HSD, Pessoa C, Moraes MO, Costa-Lotufo LV, Ferreira PMP, Sawaya ACHF, Eberlin MN (2009) Quim Nova 32:296

Camina JM, Cantarelli MA, Lozano VA, Boeris MS, Irimia ME, Gil RA, Marchevsky EJ (2008) J Apicult Res 47:102

Cantarelli MA, Camiña JM, Pettenati EM, Marchevsky EJ, Pellerano RG (2011) LWT - Food Sci Techn 44:256

Castaldo S, Capasso F (2002) Fitoterapia 73:S1

Castro JT, Santos EC, Santos WPC, Costa LM, Korn M, Nóbrega JA, Korn MGA (2009) Talanta 78:1378

Chudzinska M, Baralkiewicz D (2011) Food Chem Toxicol 49:2741

Chudzinska M, Baralkiewicz D (2010) Food Chem Toxicol 48:284

Conti ME, Botre F (2001) Environ Monit Assess 69:267

Cvek J, Medic-Saric M, Vitali D, Vedrina-Dragojevic I, Smit Z, Tomic S (2008) J Apicult Res 47:35

Diaz TG, Merás ID, Cásas JS, Franco MFA (2005) Food Control 116:339

Dogan M, Silici S, Saraymen R, Ilhan IO (2006) Acta Alimentaria 35:127

Garcia JCR, Rodriguez RI, Crecente RMP, Garcia JB, Martin SG, Latorre CH (2006) J Agr Food Chem 54:7206

Gong S, Luo L, Gong W, Gao Y, Xie M (2012) Food Chem 134:583

Gonzáles-Rodríguez E, Blázquez-Abellán G, Orzáez-Villanueva MT (1999) Food Chem 66:15

Gouveia ST, Silva FV, Costa LM, Nogueira ARA, Nóbrega JA (2001) Anal Chim Acta 445:269

Grembecka M, Szefer P (2012) Food Anal Methods 5:250

Khajeh M, Sanchooli E (2010) Food Anal Methods 3:75

Korn MGA, Castro JT, Barbosa JTP, Morte ESB, Teixeira AP, Welz B, Santos WPC, Fernandes AP, Santos EBGN, Korn M (2008) Appl Spectrosc Rev 43:67

Korn MGA, Santos DCMB, Guida MAB, Barbosa IS, Saraiva MLMFS, Lima JLFC (2010) J Braz Chem Soc 21:2278

Hernandez OM, Fraga JMG, Jimenez AI, Jimenez F, Arias JJ (2005) Food Chem 93:449

Lima B, Tapia A, Luna L, Fabani MP, Schmeda-Hirschmann G, Podio NS, Wunderlin DA, Feresin GE (2009) J Agr Food Chem 57:2691

Liu F, He Y, Wang L (2008) Anal Chim Acta 615:10

Millour S, Noel L, Kadar A, Chekri R, Vastel C, Guerin T (2011) J Food Compos Anal 24:111

Momen AA, Zachariadis GA, Anthemidis AN, Stratis JA (2007) Talanta 71:443

Naozuka J, Veiga MAMS, Richter EM, Paixão TRLC, Angnes L, Oliveira PV (2011) Food Anal Methods 4:49

Nunes LS, José TP, Barbosa JTP, Fernandes AP, Lemos VA, Walter NL, dos Santos WNL, Korn MGA, Teixeira LSG (2011) Food Chem 127:780

Oliveira FS, Teixeira LSG, Araujo MCU, Korn M (2004) Fuel 83:917

Reis PA, Valente LMP, Evtyugina M, Almeida MCR (2012) Food Anal Methods 5:82

Roman A, Madras-Majewsa B, Polpiela-Pleban E (2011) J Apic Sci 55:97

Sagrado S, Cronin MTD (2008) Anal Chim Acta 609:169

Sales A, Alvarez A, Areal MR, Maldonado L, Marchisio P, Podriguez M, Bedascarrasbure E (2006) J Haz Mat 137:1352

Santos JS, Santos NS, Santos MLP, Santos SN, Lacerda JJJ (2008) J Braz Chem Soc 19:502

Sforcina JM, Bankova V (2011) J Ethnopharmacol 133:253

Silici S, Uluozlu OD, Tuzen M, Soylak M (2008) J Haz Mat 156:612

Souza RA, Borges Neto W, Poppi RJ, Baccan N, Cadore S (2006) Quim Nova 29:654