



Determination of calcium and magnesium in hydroethanolic extracts of propolis by atomic absorption flame spectrophotometry

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ABSTRACT

Propolis is a natural product collected by honeybees and has a large range of pharmacological activity, including antimicrobial, antitumoral, antioxidant and anti-inflammatory. Its use as a popular medicine is increasing all over the world, creating a need for quality control of the commercial products. In this study the levels of calcium and magnesium in commercial hydroalcoholic propolis extracts from various states of Brazil were determined by atomic absorption flame spectrophotometry and different values were obtained for northern and southern states. This study can be extended to the analysis of metals that are harmful to health. The results showed that the calibration curves were linear over a wide concentration range (0.5-4.0 $\mu\text{g}\cdot\text{mL}^{-1}$ for calcium and 0.05-0.4 $\mu\text{g}\cdot\text{mL}^{-1}$ for magnesium) with good correlation coefficients (0.999 and 0.988, respectively). Good analytical recovery (94%) was obtained. The proposed method showed adequate precision and relative standard deviation lower than 2%. The method is accurate and precise as well as having advantages such as simplicity and speed.

Keywords: hydroalcoholic propolis extract; mineralization; analysis; calcium; magnesium.

Most propolis preparations are based on ethanolic extracts. In this form, propolis has been used extensively in folk medicine for many years and there is substantial evidence indicating that it has antiseptic, antifungal, antibacterial, antiviral, anti-inflammatory and antioxidant properties (Kujumgiev et al., 1999; Banskota et al., 2001; Banskota et al., 2002; Buratti et al., 2007; Sosa et al., 2007; Seidel et al., 2008).

For the determination of metals in any organic matrix such as plants and propolis, the samples first have to be mineralized. Various mineralization processes have already been described for different matrices, such as alcohol, alcoholic beverage, honey, beeswax, food, vegetables and seeds (Campos & Limaverde Filho, 1996; Gonzáles et al., 1999; Okada et al., 1997). Cadmium, chromium and lead have been determined in bee products (including raw products) by atomic absorption spectrometry with a graphite oven (Conti & Botrè, 2001). However, a procedure of mineralization for the hydroethanolic extract of propolis has not yet been published.

The purpose of the present study was to optimize and validate a method to analyze calcium and magnesium in hydroethanolic extract of propolis, consisting of microwave mineralization of the sample and determination of the elements by atomic absorption flame spectrophotometry (AAFS).

INTRODUCTION

Propolis is a resinous mixture collected by honeybees from leaf buds and cracks in the bark of various trees and plants, and it is 50% resin (composed of flavonoids and related phenolic acids), 30% wax, 10% essential oils, 5% pollen and 5% other organic compounds. Bees mix the original propolis with beeswax and β -glucosidase they secrete during its collection. The resulting material is used by bees to seal holes in the hives, exclude draught, protect against external invaders and mummify their carcasses (Pietta et al., 2002).

MATERIAL AND METHODS

Chemicals and apparatus

Standard stock solutions of metallic calcium and magnesium 1,000 $\mu\text{g}/\text{L}$ of (Carbo-Erba and Merck) were dissolved in deionized water and used to provide control solutions.

The analytical curves were linear throughout the concentration range investigated. Quantitation of calcium and magnesium were achieved by regression analysis of the absorbance against concentration.

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The nitric acid used for the mineralization procedure and for glass washing was analytical reagent grade (Merck). The water used to dilute mineralized samples was obtained from a Millipore Simplicity 185 ultrapure water purification system. Wet mineralization of these samples was performed in a microwave oven (DGT-100 from Provecto, Brazil). Quantitative determination of calcium and magnesium was performed with a GBC AA 932 atomic absorption spectrometer. All samples were analyzed in triplicate.

Propolis samples

Twenty-three commercial propolis samples in the form of hydroethanolic extracts were obtained from apiaries in different geographical regions of Brazil (São Paulo, Paraná, Piauí and Pernambuco).

Methods

Mineralization

The hydroethanolic propolis extracts (0,6g) were transferred to teflon reaction tubes. After the addition of 4,0mL of a 65% aqueous solution of nitric acid, the tubes were stoppered and then subjected to mineralization in the microwave.

The following heating program was applied: 250W/5min.; 500W/2min.; 800W/4min. and ventilation for 4min. The samples were then transferred to polyethylene bottles, washed with 3.0mL of milli-Q water and the volume was completed to 10.0mL of deionized water.

Analytical parameters

To check the quality and usefulness of the optimized method for determining the calcium and magnesium contents of hydroethanolic propolis extracts, analytical parameters of the method were established (linearity, detection limit, precision and accuracy).

Determination of calcium and magnesium

The concentrations of metals were determined in an atomic absorption flame spectrophotometer (GBC AA 932). The equipment was calibrated with a standard solution at the concentration of $5 \mu\text{g}\cdot\text{mL}^{-1}$, as recommended by the manufacturer. In the absence of a propolis matrix free of metals, two controls were prepared containing 65% nitric acid, the same as used in the preparation of all mineralized samples in all the analytical procedures of the analysis. All the samples had been analyzed in triplicate.

RESULTS

Calibration curves for calcium and magnesium were obtained from triplicate tests ($n=3$) on known amounts of the corresponding standards. Linearity was in the range

$0.5\text{-}4.0 \mu\text{g}\cdot\text{mL}^{-1}$ for calcium and $0.05\text{-}0.4 \mu\text{g}\cdot\text{mL}^{-1}$ for magnesium. Least-squares linear regression analysis was used to determine the slope, y- intercept and correlation coefficients $Y= 0.05533X + 0.01844$ for calcium and $Y= 1.04690X + 0.06683$ for magnesium, where Y and X are the absorbance and concentration, respectively. The correlation coefficients were 0.999 and 0.988 for calcium and magnesium, respectively. The detection limits were $0.02 \mu\text{g}\cdot\text{mL}^{-1}$ for calcium and $0.003 \mu\text{g}\cdot\text{mL}^{-1}$ for magnesium. The analytical parameters obtained were 94% recovery and a precision of less than 2% standard deviation in both cases). The statistical data were analyzed by Student's t-test (95% confidence level) and showed no significant difference between these results.

Determination of calcium and magnesium in hydroethanolic propolis extract

The efficiency of the mineralization procedure was tested through the possibility of analyzing of the macro elements calcium and magnesium in samples of hydroethanolic propolis extract. Table 1 shows the results obtained from the samples of hydroethanolic extracts from apiaries in Brazilian states of Paraná, Pernambuco, Piauí and São Paulo.

Table 1 - Calcium and Magnesium concentrations in hydroethanolic extracts of propolis analyzed by AAFS.

State of origin and source (number of samples)	Calcium ($\mu\text{g}/\text{mL}$)	Magnesium ($\mu\text{g}/\text{mL}$)
Piauí		
Apiary 1 (2)	7.293 ± 0.027	ND
Apiary 2 (3)	91.412 ± 0.804	10.605 ± 0.468
Pernambuco		
Apiary 3 (2)	4.325 ± 0.139	ND
Apiary 4 (2)	7.279 ± 0.066	ND
Parana		
Apiary 5 (3)	57.218 ± 5.253	16.025 ± 0.074
Apiary 6 (4)	57.403 ± 1.171	15.610 ± 0.068
São Paulo		
Apiary 7 (3)	105.204 ± 0.936	16.965 ± 0.120
Apiary 8 (4)	139.127 ± 4.688	16.844 ± 0.027

ND = Not detectable

DISCUSSION

A procedure for mineralization of raw propolis and other bee products has been describe and evaluated for the risk of environmental contamination (Gonzales et al., 1999) and revealed to be efficient. However, mineralization of

hydroethanolic extracts of propolis, has not been described before and preliminary experiments in our laboratory led to ejection of all samples by sudden expansion (rapid volatilization) during the process of mineralization, probably because of the presence of its active components in ethanol (this does not occur with samples of ethanol alone).

The optimization were done on the amount of extract to be mineralized. The results showed that the optimal aliquot was defined as 0.6g hydroethanolic extract per 4,0mL of nitric acid, diluted in 10mL water for analysis by AAFS.

The results of determination of calcium and magnesium showed high concentrations in São Paulo samples and low concentrations in Pernambuco. It can be observed that calcium appears in higher concentration than magnesium in all samples and when the concentration of calcium is less than 7.293 $\mu\text{g/mL}$, magnesium is not detectable by this methodology. Piauí samples showed two very different values. It can be caused by different sources of the crude propolis.

These metals are not harmful to humans in these amounts. The method adopted for mineralization of hydroethanolic propolis extract proposed in this work has been validated in this study. In view of the wide popular use of propolis in Brazil, specially in the form of hydroethanolic extracts the lack of control of metals in propolis products in Brazilian law, this study may contribute to quality control of propolis, mainly for analysis of toxic metals such as Pb.

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RESUMO

Determinação de cálcio e magnésio em extratos hidroalcoólicos de própolis por espectrofotometria de absorção atômica

A própolis possui ampla utilização farmacológica, dentre as quais se destacam as ações: antimicrobiana, antiviral, antiparasitária, antiinflamatória. O seu uso na medicina popular está aumentando em todo o mundo, sendo necessária uma metodologia de controle de qualidade de produtos comerciais, inexistente até o momento. A proposta desse trabalho foi a determinação de cálcio e magnésio em extratos hidroalcoólicos de própolis oriundos de diferentes estados do Brasil, através de espectrofotômetro de absorção atômica (EAA-chama), como base de estudo posteriores para metais nocivos à saúde. Os resultados mostraram que as curvas de calibração foram lineares num largo intervalo de concentração (0,5–4,0 $\mu\text{g}\cdot\text{mL}^{-1}$ para o cálcio e 0,050-0,4 $\mu\text{g}\cdot\text{mL}^{-1}$ para o magnésio) com ótimo coeficiente

de correlação (0,999 e 0,988 respectivamente). O método proposto apresentou precisão adequada com desvio padrão relativo < 2 %. Os resultados mostraram que a exatidão do método apresentou valores de recuperação dentro dos limites recomendáveis. O método é exato, preciso e apresenta vantagens tais como: simplicidade e rapidez.

Palavras-chave: extrato hidroalcoólico própolis; cálcio; magnésio; análise; mineralização.

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