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Original Research Article

Development and comparison of two analytical methods to quantify the mercury content in honey

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ABSTRACT

The traditional method routinely used in laboratories to analyze mercury (Hg) in honey includes decomposition of samples, cold vapor generation and atomic absorption detection. Methods that avoid sample digestion could be applied in these laboratories and would represent an innovation relevant to the control of Hg in honey. In this paper, two methods were developed to determine Hg concentration in honey samples: one utilized cold vapor atomic absorption spectrometry (CVAAS), and the other used a direct mercury analyzer (DMA). The CVAAS method consisted of preparing solutions containing 5.0% (w/v) honey, 4.0% (v/v) H_2O_2 and 6.0% (v/v) HNO_3. Hg determination was accomplished by treatment with 0.6% (w/v) $NaBH_4$ in 0.5% (w/v) NaOH and 6.0 mol/L HCl. In the DMA method, measurements were made using up to 100 mg of honey sample without any prior treatment. The drying and decomposition times along with drying temperature were each optimized. After validation, the methods were used to quantify Hg in 35 honey samples collected from several cities in Minas Gerais, Brazil. All honey samples showed Hg concentrations below 2.5 ng/g. The proposed methods were rapid, simple and did not require sample decomposition using concentrated acids at high temperatures, which is advantageous considering the high volatility of Hg.

1. Introduction

Mercury (Hg) is a toxic metal when it comes into contact with humans by ingestion, skin absorption or air inhalation. It is known that the metal accumulates in living organisms, and mild poisoning is characterized by anemia, depression, dermatitis, headaches, hypertension, insomnia, irritability and impaired hearing and vision. Chronic poisoning can lead to various neurodegenerative diseases and death (Counter and Buchanan, 2004; Rooney, 2007). Because the accumulation of Hg in humans is due to contaminated food, water and air, methods are necessary to assess the presence and concentration of Hg in these sources.

Honey is a food susceptible to the accumulation of metals and is recognized as an environmental indicator. This is because honeybees are continuously exposed to many substances, including contaminants, present in an area of approximately 7 km^2 surrounding the apiary (Bilandžić et al., 2011; Madejczyk and Baralkiewicz, 2008). In addition, some people use honey as a sugar substitute, which has led to an increase in its worldwide consumption. Thus, the monitoring of inorganic contaminants, such as Hg, in honey has become necessary.

Methods for the determination of mineral constituents in honey can employ different procedures of sample preparation, such as ashing followed by dissolution in acid (Baroni et al., 2009), wet digestion in opened or closed systems such as a microwave oven (Millour et al., 2012) and the direct analysis of slurries (Ajtony

Abbreviations: α, intercept; β, slope; CCD, central composite design; CNPq, Conselho Nacional de Pesquisa e Desenvolvimento Tecnológico; *d*, Durbin–Watson's test statistic; DMA, direct mercury analyzer; EC, European Commission; *F*, variance ratio; FAPEMIG, Fundação de Pesquisa do Estado de Minas Gerais; Hg, mercury; CVAAS, cold vapor atomic absorption spectrometry; IAEA, International Atomic Energy Agency; IMA, Instituto Mineiro de Agropecuária; INMETRO, Instituto Nacional de Metrologia, Normalização e Qualidade Industrial; J_{ei}, Jackknife residuals; LOD, limit of detection; LOQ, limit of quantification; *N*, number of observations; OLSM, ordinary least squares method; *p*, probability; *R*, correlation coefficients; R_c, critical *R*; RSD, relative standard deviation; t_L, Levene's test statistic; X_i, Hg concentration; Y_i, instrument response.

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et al., 2007; Ioannidou et al., 2005). Thermal decomposition procedures using hot plates permit the destruction of the organic matrix but may also cause errors due to the loss of volatile analytes such as Hg. The use of microwave ovens minimizes losses through volatilization because the constituents of interest in the sample are extracted in a closed system. However, the use of microwave ovens is still inaccessible to many laboratories. Therefore, direct analysis of slurries is simple and more economical than conventional procedures. In addition to reducing the contamination of samples by using less acid, slurry methods also reduce sample preparation time and can be performed at room temperature, avoiding the loss of analytes by volatilization (Cal-Prieto et al., 2002).

The direct determination of Hg in food samples can be accomplished using cold vapor atomic absorption spectrometry (CVAAS). The main advantage of this technique is its ability to separate the analyte from the matrix because few elements are capable of forming volatile compounds under normal circumstances (Dědina and Tsalev, 1995).

Another technique for rapid Hg determination is the direct mercury analyzer (DMA), which consists of an oven where the sample is burned and its vapors are passed through a gold trap that retains Hg. The trap is then heated and the released Hg is measured by atomic absorption (Boylan and Kingston, 1998). DMA allows for the analysis of liquid or solid samples without any dilution or dissolution, thus, the detection limit can be up to 100 times better than conventional techniques and is directly dependent on the amount of sample used.

This study aimed to develop two analytical methods to determine Hg in honey samples that could be implemented in food surveillance centers, because the traditional method used by these laboratories utilizes decomposition of samples before CVAAS. Newer methods that avoid sample digestion could be applied in these laboratories, and they would represent an important innovation in terms of potential control of Hg in honey. The developed methods consist of the use of slurry analysis followed by quantification by CVAAS and direct analysis of the sample by DMA.

2. Materials and methods

2.1. Apparatus

Mercury determination was performed using a direct mercury analyzer (DMA-80, Milestone Srl, Sorisole, BG, Italy) and an atomic absorption spectrometer (SpectrAA 220FS, Varian Inc., Mulgrave, Vic., Australia) equipped with a vapor generation accessory (VGA77, Varian Inc., Mulgrave, Vic., Australia). Solutions of $NaBH_4$ and HCl (Merck, Darmstadt, Germany), were used for cold vapor generation, with a flow rate of 1.3 mL/min. The sample and standard solutions flow rate was 8.0 mL/min. A hollow cathode Hg lamp (Varian Inc., Mulgrave, Vic., Australia) and a low-pressure Hg vapor lamp (Milestone Srl, Sorisole, BG, Italy) were used in SpectrAA 220FS and DMA-80, respectively, operating at 253.7 nm. Oxygen (99.8%) and argon (99.998%) (White Martins, São Paulo, SP, Brazil) were used as carrier gases in DMA-80 and VGA77, respectively.

2.2. Reagents, calibration standards and certified reference materials

All reagents used were analytical grade. Solutions were prepared using ultrapure water obtained using a Milli-Q purification system, 18.2 $M\Omega\ cm^{-1}$ (Millipore Corporation, Milford, MA, USA). A stock solution containing 1000 mg/L Hg was prepared by diluting a Hg ampoule (Titrisol, Merck, Darmstadt, Germany) in a solution of 1.0% (v/v) HNO_3 (65.0% w/w, Merck, Darmstadt, Germany). A 1000 μg/L of Hg solution was prepared from the stock solution and was used to prepare further dilutions. HCl, $NaBH_4$, NaOH and H_2O_2 solutions were prepared with Merck reagents (Darmstadt, Germany). All pieces of glassware were decontaminated before use by washing with detergent (Extran, Merck, Darmstadt, Germany), rinsing with tap and distilled water and soaking in 10% (v/v) HNO_3 for at least 16 h. The glassware was then rinsed with ultrapure water and dried at room temperature in a clean and protected place.

The certified reference materials used to check the accuracy of the DMA method were SRM-8415 (Whole Egg Powder, National Institute of Standards and Technology, Gaithersburg, MD, USA), IAEA-336 (Trace Elements in Lichens, International Atomic Energy Agency, Vienna, Austria) and GBW-08301 (River Sediment, Institute of Environmental Chemistry Academia Sinica, Nankang Taipei, Taiwan, ROC) with actual results of Hg concentrations of (4.0 ± 3.0) ng/g, (200 ± 40) ng/g and (220 ± 40) ng/g, respectively.

2.3. Sample collection

Honey samples were purchased from markets and farmers' fairs in several cities in Minas Gerais, Brazil. Some of the samples were also obtained in collaboration with the Food Safety Laboratory of the IMA (Instituto Mineiro de Agropecuária). Some of the honey samples were produced on an industrial scale, while others were home-produced. The 35 honey samples were stored at room temperature and kept under light.

2.4. Method development

The determination of Hg in honey was carried out by dilution in an acidic solution without heating followed by quantification by CVAAS. In the case of DMA, sample preparation was not necessary.

In the CVAAS method, central composite design (CCD) was employed to optimize the concentration of reagents and sample. The parameters studied were hydrogen peroxide and nitric acid concentrations and sample mass (Table 1). The optimization was performed with honey samples spiked with Hg at a final concentration of 10 μg/L. The central point of the experimental design was conducted in quintuplet, requiring a total of 19 experiments.

DMA calibration was performed following the United States Environmental Protection Agency: method 7473 (2007). The Hg measurements were made using up to 100 mg of sample weighed directly into small containers of nickel, which were automatically transported to the oven where the sample was first dried and then thermally decomposed under a continuous oxygen flow. The drying temperature and the drying and decomposition times were also optimized by CCD (Table 1) to obtain accurate results in a short time frame.

Table 1
Factors and levels studied in the optimization of mercury determination in honey by central composite design (CCD).

Optimization of mercury determination in honey by CVAAS[a].					
Factors	−1.68	−1	0	+1	+1.68
H_2O_2% (v/v)	0.64	2.00	4.00	6.00	7.36
HNO_3% (v/v)	0.64	2.00	4.00	6.00	7.36
Mass sample (to 100 ml)	2.64	4.00	6.00	8.00	9.36
Optimization of mercury determination in honey by DMA[b].					
Factors	−1.68	−1	0	+1	+1.68
Drying time (s)	10	30	60	90	110
Decomposition time (s)	10	30	60	90	110
Drying temperature (°C)	150	190	250	310	350

[a] Cold vapor atomic absorption spectrometry.
[b] Direct mercury analyzer.