Analytical note

Improvement of microwave-assisted digestion of milk powder with diluted nitric acid using oxygen as auxiliary reagent

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ABSTRACT

The feasibility of using diluted HNO_3 solutions under oxygen pressure for decomposition of whole and non-fat milk powders and whey powder samples has been evaluated. Digestion efficiency was evaluated by determining the carbon content in solution (digests) and the determination of Ca, Cd, Cu, Fe, K, Mg, Mn, Mo, Na, Pb and Zn was performed by inductively coupled plasma optical emission spectrometry and Hg by chemical vapor generation coupled to inductively coupled plasma mass spectrometry. Samples (up to 500 mg) were digested using HNO_3 solutions (1 to 14 mol L^{-1}) and the effect of oxygen pressure was evaluated between 2.5 and 20 bar. It was possible to perform the digestion of 500 mg of milk powder using 2 mol L^{-1} HNO_3 with oxygen pressure ranging from 7.5 to 20 bar with resultant carbon content in digests lower than 1700 mg L^{-1}. Using optimized conditions, less than 0.86 mL of concentrated nitric acid (14 mol L^{-1}) was enough to digest 500 mg of sample. The accuracy was evaluated by determination of metal concentrations in certified reference materials, which presented an agreement better than 95% (Student's t test, P<0.05) for all the analytes.

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1. Introduction

Milk could be considered as a staple food that provides essential nutrients (proteins, lipids, and carbohydrates) and micronutrients (minerals, vitamins, and enzymes) [1,2]. On the other hand, milk can also constitute a source of exposure to toxic elements, especially dangerous for infants, such as Cd, Hg and Pb [3,4]. In this sense, it is important to establish appropriate sample preparation methods for subsequent metal determination in order to assure the quality of final products. In addition, these methods should be developed considering lower reagent consumption and suitable digestion efficiency [5]. Most of conventional sample preparation methods for atomic spectrometric techniques involve sample solubilization with complete or partial matrix decomposition generally using oxidant acids [6]. In previous years, some improvements were proposed towards the development of new procedures based on less conventional approaches to organic matter digestion [7–18], which normally present relatively high efficiency of digestion resulting in low values of residual carbon content.

Some procedures based on diluted solutions of nitric acid were also developed for digestion of biological samples [19,20]. The efficiency of diluted nitric acid for oxidation of organic matter can be explained by the regeneration of nitric acid promoted by the combination of the nitrogen oxide species with the oxygen present inside the reaction vessel [20–22]. Based on the reaction mechanism of nitric acid regeneration, which occurs while oxygen is still present in the reaction vessel, digestion procedure has been performed under oxygen pressure [23,24], which allowed digestions with amounts of nitric acid lower than 3 mol L^{-1}.

In the present work, a procedure based on vessels pressurized with oxygen and determination by inductively coupled plasma (ICP)-based spectrometric techniques is proposed for metals determination in milk powder. The determination of essential (Ca, Cu, Fe, K, Mg, Mn, Mo, Na and Zn) and toxic (Cd, Hg and Pb) metals was performed by inductively coupled plasma optical emission spectrometry (ICP OES) and by chemical vapor generation inductively coupled plasma mass spectrometry (CVG-ICP-MS, only for Hg). Certified reference materials (CRM) were used to check the accuracy. In addition, it was tried to reduce as much as possible the amount of nitric acid needed for the digestion process in order to minimize the blank values and to decrease the consumption of reagents and the consequent generation of laboratory residues.

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2. Experimental

2.1. Instrumentation

A microwave oven (Multiwave 3000 microwave sample preparation system, Anton Paar, Graz, Austria) equipped with eight high-pressure quartz vessels was used in the experiments. The internal volume of vessels was 80 mL and the maximum operational temperature and pressure were set at 280 °C and 80 bar, respectively.

Analytes (except Hg) were determined by ICP OES using an axial view configuration spectrometer (Spectro Ciros CCD, Spectro Analytical Instruments, Kleve, Germany) using a cross-flow nebulizer coupled to a Scott double pass type nebulization chamber. Plasma operating conditions and selected wavelengths are listed in Table 1, and they were used as recommended by the instrument manufacturer [25]. Chemical vapor generation (CVG) coupled to ICP-MS (PerkinElmer Sciex, Model Elan DRC II, Thornhill, Canada), equipped with a quartz torch with a quartz injector tube (2 mm i.d.), was used for Hg determination. The CVG system consists of a peristaltic pump (Ismatec, Zurich, Switzerland) and a U-type gas–liquid separator [26]. The mixture was pumped to the gas–liquid separator and Hg measurements were performed by ICP-MS. Plasma operating conditions and selected isotope used for Hg determination are also listed in Table 1. Carbon content in digests (related to 500 mg of sample and dilution to 30 mL) was determined by ICP OES [27,28]. In order to remove the volatile carbon compounds before carbon content determination, digests were previously sonicated with an ultrasonic probe [29] (VCX 130 PB, 130 W, 20 kHz, Sonics and Materials Inc., Newton, CT, USA). This procedure was not applied when determining the analytes in final digests, which were just diluted with water and analyzed by ICP OES and CVG-ICP-MS.

Argon (99.996%, White Martins-Praxair, São Paulo, SP, Brazil) was used for ICP OES and ICP-MS determination for plasma generation, nebulization, auxiliary gas and also for digestion performed under inert atmosphere. Oxygen (99.9991%, White Martins-Praxair) was used as reagent in digestions performed under oxygen pressure.

It is important to mention that all procedures under oxygen pressure were performed employing safety conditions, as recommended by the microwave oven manufacturer [30].

Results for residual acidity were obtained using a titration system (Titrando 836, Metrohm, Herisau, Switzerland) equipped with a magnetic stirrer (module 803 Ti Stand), 20 mL burette (Dosino 800) and pH electrode (LL Electrode plus, model 6.0262.100).

Table 1
Operational parameters for determination of C, Ca, Cd, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Pb and Zn.

Parameter	ICP OES	ICP-MS
Radio-frequency power (W)	1600	1300
Plasma gas flow rate (L min^{-1})	14.0	15.0
Auxiliary gas flow rate (L min^{-1})	1.0	1.2
Nebulizer gas flow rate (L min^{-1})	0.85	1.13
Spray chamber	Double pass, Scott type	*
Nebulizer	Crossflow	*
Observation view	Axial	–
Analytes	Emission line (nm)	Isotope (m/z)
C (I)	193.091	–
Ca (II)	393.366	–
Cd (II)	226.502	–
Cu (I)	324.752	–
Fe (I)	238.204	–
Hg	–	202
K (I)	766.491	–
Mg (II)	285.213	–
Mn (II)	257.610	–
Mo (II)	202.030	–
Na (I)	589.592	–
Pb (II)	220.353	–
Zn (I)	213.856	–

*Direct introduction by CVG, (I) atomic emission and (II) ion emission.

2.2. Samples, reagents and standards

Preliminary experiments were carried out using whole milk powder. After optimization step, the proposed procedure was applied to the digestion of whey, whole and non-fat milk powders, which were purchased in a local market. All samples were dried at 60 °C using an oven (model 400/2ND, Nova Ética, Vargem Grande Paulista, SP, Brazil). Accuracy was evaluated using CRM of skim milk powder (BCR 151, Community Bureau of Reference, Brussels, Belgium) and of non-fat milk powder (SRM NIST 1549, National Institute of Standards and Technology, NIST, Gaithersburg, MD, USA). Samples were weighed using an analytical balance (model AY 220, max. 220 g, 0.1 mg of resolution, Shimadzu, Kyoto, Japan).

Distilled-deionized water (Milli-Q, 18.2 MΩ cm, Millipore, Billerica, MA, USA) and analytical-grade nitric acid (Merck, Darmstadt, Germany) were used to prepare samples and standards. Carbon reference solutions used in external calibration for carbon content determination were prepared by dissolution of citric acid (Merck) in water (25 to 500 mg L^{-1} of C). Yttrium (1.0 mg L^{-1}, Spex CertPrep, Metuchen, NJ, USA) was used as internal standard in all samples, blanks and reference solutions for carbon content determination. Metal determination by ICP OES was performed with external calibration using analytical solutions ranging from 1.0 (Cd, Mn, Zn), or 5.0 (Cu, Fe, Mg, Mo), or 10 µg L^{-1} (Ca, K, Na, Pb) up to 100 µg L^{-1}, prepared in 0.7 mol L^{-1} HNO$_3$ by appropriate dilution of the multi-element stock solution (SCP33MS, SCP Science, Quebec, Canada). Mercury determination by CVG-ICP-MS was performed with external calibration by dilution of inorganic mercury (Hg$_{inorg}$) standard stock solution Titrisol (1000 mg L^{-1}, Merck), which was prepared just before use. Sodium tetrahydroborate (0.2% m/v) used for CVG was obtained from Vetec (Duque de Caxias, RJ, Brazil). A 0.1 mol L^{-1} KOH (Merck) solution was used for residual acidity determination. Glass and quartz material were soaked in 1.4 mol L^{-1} HNO$_3$ for 24 h and further washed with water before use.

2.3. Microwave-assisted acid digestion

Samples (up to 500 mg) were transferred to the quartz vessels. Digestion efficiency using nitric acid solutions (6 mL) was evaluated in the following concentrations: 1, 2, 3, 7, and 14 mol L^{-1} HNO$_3$. After closing and capping the rotor, vessels were pressurized with 2.5, 5, 7.5, 10, 15 and 20 bar of oxygen. The gases were introduced into the vessels using the valve originally designed for pressure release after conventional acid sample digestion. Then, the rotor was placed inside the oven, and microwave-heating program was started by applying (i) 1000 W with a ramp of 5 min, (ii) 1000 W for 10 min, and (iii) 0 W for 20 min (cooling step) [30]. After digestion, the pressure of each vessel was carefully released. In this work, each run was performed using a minimum of four vessels. The resulting solutions were transferred to 30 mL polypropylene vials and diluted to the mark with water. Cleaning of digestion vessels was carried out with 6 mL of concentrated HNO$_3$ in the microwave oven at 1000 W for 10 min and 0 W for 20 min for cooling. All statistical comparisons were performed using Student's t test, (GraphPad InStat Software Inc., Version 3.00, 1997). A significance level of $P < 0.05$ was chosen for all comparisons.

3. Results and discussion

3.1. Preliminary evaluation of digestion efficiency

Preliminary tests were carried out with air at atmospheric pressure in order to evaluate the effect of oxygen pressure on whole milk powder digestion. Nitric acid concentration was varied in order to achieve a condition of efficient organic matter digestion using as low as possible concentrated acid solution, which was evaluated by the carbon content in digests and residual acidity determination. The same procedure was repeated using oxygen for pressurization of