



ELSEVIER

Contents lists available at ScienceDirect

Talanta

journal homepage: [www.elsevier.com/locate/talanta](http://www.elsevier.com/locate/talanta)

# Microwave-assisted wet digestion with H<sub>2</sub>O<sub>2</sub> at high temperature and pressure using single reaction chamber for elemental determination in milk powder by ICP-OES and ICP-MS



Edson I. Muller<sup>\*</sup>, Juliana P. Souza, Cristiano C. Muller, Aline L.H. Muller, Paola A. Mello, Cezar A. Bizzi

Departamento de Química, Universidade Federal de Santa Maria, 97105-900 Santa Maria, RS, Brazil

## ARTICLE INFO

### Article history:

Received 8 February 2016

Received in revised form

2 May 2016

Accepted 3 May 2016

Available online 10 May 2016

### Keywords:

Hydrogen peroxide

Milk powder

Single reaction chamber

Microwave-assisted wet digestion

Elemental analysis

Trace elements

## ABSTRACT

In this work a green digestion method which only used H<sub>2</sub>O<sub>2</sub> as an oxidant and high temperature and pressure in the single reaction chamber system (SRC-UltraWave™) was applied for subsequent elemental determination by inductively coupled plasma-based techniques. Milk powder was chosen to demonstrate the feasibility and advantages of the proposed method. Samples masses up to 500 mg were efficiently digested, and the determination of Ca, Fe, K, Mg and Na was performed by inductively coupled plasma optical emission spectrometry (ICP-OES), while trace elements (B, Ba, Cd, Cu, Mn, Mo, Pb, Sr and Zn) were determined by inductively coupled plasma mass spectrometry (ICP-MS). Residual carbon (RC) lower than 918 mg L<sup>-1</sup> of C was obtained for digests which contributed to minimizing interferences in determination by ICP-OES and ICP-MS. Accuracy was evaluated using certified reference materials NIST 1549 (non-fat milk powder certified reference material) and NIST 8435 (whole milk powder reference material). The results obtained by the proposed method were in agreement with the certified reference values (*t*-test, 95% confidence level). In addition, no significant difference was observed between results obtained by the proposed method and conventional wet digestion using concentrated HNO<sub>3</sub>. As digestion was performed without using any kind of acid, the characteristics of final digests were in agreement with green chemistry principles when compared to digests obtained using conventional wet digestion method with concentrated HNO<sub>3</sub>. Additionally, H<sub>2</sub>O<sub>2</sub> digests were more suitable for subsequent analysis by ICP-based techniques due to of water being the main product of organic matrix oxidation. The proposed method was suitable for quality control of major components and trace elements present in milk powder in consonance with green sample preparation.

© 2016 Elsevier B.V. All rights reserved.

## 1. Introduction

Milk powder is a complex matrix composed of several organic molecules, such as proteins, carbohydrates and fat. It is a kind of food that is normally a source of essential minerals, but it can also contain toxic elements. In order to ensure health properties, quality control of milk powder should be carefully performed [1]. To avoid mistakes in this step, mainly those related to matrix effects during elemental determination, samples should be previously digested to bring the analytes into solution for subsequent analysis by using suitable analytical techniques. In this sense, a lot of methods are available in the literature to oxidize refractory matrices and the most common are dry ashing, combustion in closed systems and wet digestion methods in high pressure systems [2–9].

<sup>\*</sup> Corresponding author.

E-mail address: [edson\\_muller@yahoo.com.br](mailto:edson_muller@yahoo.com.br) (E.I. Muller).

The dry ashing method is usually performed by calcination at atmospheric pressure in a muffle furnace at temperatures around 450 °C. The main advantage of dry ashing for milk powder is the possible oxidation of a large sample mass and subsequent dissolution of ash in a small volume of acids that allow better limits of detection (LODs) when compared with other digestion methods. However, this method is prone to several drawbacks, such as losses of analyte and contamination during sample preparation [3,10,11].

Nowadays, access to commercial microwave-induced combustion (MIC) systems has allowed the efficient digestion of several refractory organic matrices. Diluted acid or alkaline solutions are used to absorb analytes during combustion and final digest solutions are suitable for determination of trace elements using ICP-based techniques. In this sense, MIC has been used for milk and related matrices, thus allowing for digests with very low residual carbon content (RCC) [12,13].

Undoubtedly, wet digestion methods are widely used for the digestion of organic matrices [14]. Among the different oxidants

used in wet digestion methods,  $\text{HNO}_3$  is preferred because it allows for oxidation of almost all organic compounds and can be obtained in high purity [14]. However, for efficient digestion of refractory organic matrices high temperature and pressure systems are required to improve the oxidation capacity of  $\text{HNO}_3$  [15,16].

By contrast, hydrogen peroxide presents weak acidity (a solution of 50%  $\text{H}_2\text{O}_2$  presents a pH of 4.3) and can oxidize organic compounds with the generation of water as the only theoretical byproduct [14]. In this sense, digests containing only  $\text{H}_2\text{O}_2$  were less prone to signal suppression using plasma-based techniques compared to digests prepared with mineral acids. The explanation of signal suppression of analytes in acid digests with respect to water is complex due to the combination of several possible effects, such as a decrease in the sample aspiration rate as a result of increased viscosity, a change in nebulizer efficiency and droplet size distribution, a variation in the aerosol transport efficiency and a change in plasma conditions [17–19]. For ICP-OES, when analyses were carried out using robust conditions (MgII/MgI ratio higher than 8) the changes in thermal characteristics of plasma were minimized and signal suppression could be attributed to the reduction of the analyte transport to the plasma [17]. In regard to ICP-MS analysis, changes in aerosol properties or plasma temperature caused by mineral acids affect the distribution of analyte ions close to the sampling orifice, and suppression of the signal can be observed. According to the literature, and depending on the operating conditions employed, signal suppression is more pronounced for elements with high ionization potential. In order to minimize drawbacks related to acid effect on ICP-MS, robust plasma conditions should also be used [18,19]. Additionally, the use of  $\text{H}_2\text{O}_2$  for oxidation of organic compounds is compatible with green chemistry principles because it is relatively cheap and environmental friendly [20]. According to the literature  $\text{H}_2\text{O}_2$  is also useful for the extraction of trace elements from refractory matrices, but the digests can contain solid residues that cause interference in determination with plasma based techniques [21,22]. However, applications that only use  $\text{H}_2\text{O}_2$  for digestion of organic matrices are scarce in the literature and it is usually used in combination with other concentrated acids [14].

The first application using only  $\text{H}_2\text{O}_2$  reported in the literature was proposed for the digestion of sample masses of up to 15 g of more than thirty organic matrices (such as milk, honey, margarine and meat) mixed with 150 mL of 30% ( $\text{w w}^{-1}$ )  $\text{H}_2\text{O}_2$ . According to the author, the reaction rate of organic substances with  $\text{H}_2\text{O}_2$  is increased by high temperature and pressure, and especially constructed high-pressure vessel was necessary to ensure the security. The maximum temperature achieved during the heating program was 370 °C (using a saltpetre bath or electric furnace), and the resulting digests with low acidity were suitable for subsequent analysis using different analytical techniques. Accuracy was evaluated by determining of spike of radiotracers ( $^{85}\text{Sr}$ ,  $^{106}\text{Ru}$ ,  $^{131}\text{I}$  and  $^{137}\text{Cs}$ ) using gamma spectrometry, and recoveries higher than 96% were observed for these elements [23]. Later, the same author proposed the wet digestion with  $\text{H}_2\text{O}_2$  as oxidant for different fatty matrices (such as cocoa butter, olive oil and sunflower oil) and temperature up to 450 °C were necessary to ensure the efficiency of the digestion [24].

More recently, digestion of dried wood samples was also carried out using only  $\text{H}_2\text{O}_2$  for subsequent analysis of digests using electrothermal vaporization inductively coupled plasma optical emission spectrometry (ETV-ICP-OES). A sample mass of up to 120 mg was digested with 1.5 mL of 50% ( $\text{w w}^{-1}$ )  $\text{H}_2\text{O}_2$  in a polytetrafluoroethylene (PTFE) bomb heated up to 125 °C (conventional heating) during 4 h. Final digests did not present wood residue in the solution and determination of 21 elements was carried out using ETV-ICP-OES. The authors of this manuscript

recommended that such procedure must be carefully evaluated for larger sample masses and higher concentration of  $\text{H}_2\text{O}_2$  to obtain a safe method. Additionally, the authors recommended avoiding temperatures higher than 125 °C to prevent the leakage from PTFE bomb vessels [25].

For safety reasons digestion of organic matrices that only use  $\text{H}_2\text{O}_2$  should be performed in systems especially designed for working under high temperature and pressure [23,25]. Recently, the single reaction chamber system (SRC-UltraWave™) with microwave-assisted heating has become commercially available, and it allows maximum temperature and pressure up to 300 °C and 199 bar, respectively [15]. This new system has been used to digest large sample masses even of refractory samples such as active pharmaceutical ingredients and nuts. The combination of high pressure and high temperature allows for the efficient digestion of these samples. Final digests with low RCC were obtained that are suitable for analysis using ICP-based techniques [26,27].

In the present study, a green method using a single reaction chamber (SRC-UltraWave™ system) was evaluated for milk powder digestion using only  $\text{H}_2\text{O}_2$  as an oxidant reagent for subsequent elemental determination by ICP-based techniques. To evaluate the digestion efficiency of the proposed method, sample masses of up to 1 g were digested, and the digestion efficiency was evaluated by the determination of residual carbon content (RCC) and residual carbon (RC) in final digests. The accuracy of the proposed method, was evaluated using reference materials (RMs) and also by comparison with results obtained using digestion with concentrated  $\text{HNO}_3$ .

## 2. Experimental section

### 2.1. Instrumentation

In this study sample digestion was performed using SRC system (UltraWave™, Milestone, Sorisole, Italy) equipped with a five quartz vessel (internal volume of 40 mL). This system has a microwave cavity (1 L) made of stainless steel and covered with PTFE vessel which was sealed and pressurized with 40 bar of argon 99.996% (White Martins, São Paulo, Brazil) before heating. All experiments using SRC system were carried out using 1500 W of microwave irradiation and a maximum temperature and pressure of 250 °C and 199 bar, respectively.

Determination of C, Ca, Fe, K, Mg and Na was carried out in an inductively coupled plasma optical emission spectrometer (Ciros CCD model, Spectro, Kleve, Germany) equipped with a Scott-type double pass spray chamber, a cross-flow nebulizer, a torch and a quartz injector. Additionally, an inductively coupled plasma mass spectrometer (Nexion model, Perkin Elmer, Shelton, USA) equipped with concentric nebulizer and cyclonic spray chamber was used for determination of B, Ba, Cd, Cu, Mn, Mo, Pb, Sr and Zn. For plasma generation, nebulization and auxiliary gas, argon with a purity of 99.996% was used. The conditions used for element determination by ICP-OES and ICP-MS are summarized in Table 1.

### 2.2. Reagents, solutions and samples

Water was purified in Milli-Q system (Millipore Corp. Bedford, USA). Purified water was used to prepare all reference solutions and reagents used in this study. Stabilized 30% ( $\text{w w}^{-1}$ ) and 50% ( $\text{w w}^{-1}$ )  $\text{H}_2\text{O}_2$  (Sigma Aldrich, USA, product numbers H3410 and 516,813, respectively) were used as an oxidant agent. Digestion of milk powder samples was also performed using  $\text{HNO}_3$  (65%,  $\text{w w}^{-1}$ , Merck, Germany) doubly distilled in a quartz sub-boiling device (model duoPUR 2.01E, Milestone, Sorisole, Italy).

A multi-element stock solution (PlasmaCal SCP33MS, SCP Science, Quebec, Canada) containing 10 mg  $\text{L}^{-1}$  of all the evaluated

**Table 1**  
Operational parameters used for determination by ICP-OES and ICP-MS.

Parameter	ICP-OES	ICP-MS
RF power wavelength (W)	1400	1300
Plasma gas flow rate (L min <sup>-1</sup> )	14	18
Auxiliary gas flow rate (L min <sup>-1</sup> )	1.00	1.20
Nebulizer gas flow rate (L min <sup>-1</sup> )	0.80	0.95
Spray chamber	Scott-type double pass	Cyclonic
Nebulizer	Cross-flow	Concentric
Wavelength (nm)		–
	Ca - 396.847	
	Cd - 228.802	
	Cu - 327.393	
	Fe - 238.204	
	K - 766.490	
	Mg - 280.270	
	Mn - 257.610	
	Mo - 202.031	
	Na - 589.592	
	Pb - 220.353	
	Sr - 407.771	
	C - 193.030	
	Y - 371.029	
Data collection mode	–	Peak hopping
Sampler and skimmer cones	–	Pt
Isotopes (m/z)	–	<sup>11</sup> B
		<sup>138</sup> Ba
		<sup>114</sup> Cd
		<sup>65</sup> Cu
		<sup>55</sup> Mn
		<sup>95</sup> Mo
		<sup>208</sup> Pb
		<sup>88</sup> Sr
		<sup>64</sup> Zn

elements was used to prepare the calibration curve for ICP-OES and ICP-MS determination. As C and Y were not present in the multi-element stock solution, special solutions for these elements were prepared. Reference solutions used for RC determination (in mg L<sup>-1</sup> of C) were prepared by sequential dilution of a carbon stock solution prepared after the dissolution of citric acid (Merck) in boiled ultrapure water. Yttrium (1 mg L<sup>-1</sup>, SpexCertPrep, Metuchen, USA) was used as an internal standard for RCC determination.

Five samples of milk powder (identified as samples 1, 2, 3, 4 and 5) with different content of carbohydrate (ranging from 37 to 58%), protein (ranging from 9.5 to 26%) and fat (ranging to 19–28%), according to package labels, were purchased in local markets. Sample 5 (with the highest fat content) was analyzed for C content using elemental analysis and presented 50 ± 4% of C. This value was used to calculate RCC (in % of C) of final digests. Whole milk powder reference material (NIST 8435) and non-fat milk powder certified reference material (NIST 1549) were used to evaluate the accuracy of the proposed method. Digestions of samples were also carried out using concentrated HNO<sub>3</sub>, using the same heating program used for digestions with H<sub>2</sub>O<sub>2</sub>, as an additional procedure for evaluating the accuracy.

### 2.3. Procedure

Sample masses ranging from 0.1 to 1 g were directly weighed inside the quartz vessels and four different volumes of H<sub>2</sub>O<sub>2</sub> (5, 6, 7 and 8 mL) were added to the sample. The rotor containing the quartz vessels was positioned inside the SRC system, which was previously filled with 120 mL of water and 5 mL of H<sub>2</sub>SO<sub>4</sub>. The SRC system was pressurized up to 40 bar with argon to cover the cap

and keep it in the upper part of the quartz vessel. For safety reasons and to minimize material fatigue the maximum system temperature was arbitrarily fixed at 250 °C. To evaluate the influence of pressure on digestion efficiency, experiments were carried out using the pressure of the SRC system set at 40, 80 and 199 bar. It is important to mention that preliminary experiments showed that 20 min was the minimum hold digestion time necessary to provide digests without insoluble materials. In this sense, the microwave heating program was as follows: 10 min of ramp and hold for 20 min at 250 °C. After cooling down (65 °C) the chamber was depressurized and the resultant solution was diluted in volumetric flasks up to 25 mL. Final digests were analyzed by ICP-OES and ICP-MS. Additionally, carbon content (mg L<sup>-1</sup> of C) was determined in the final digests to evaluate the digestion efficiency. Residual carbon content (RCC) was calculated based on the carbon content in final digest and total carbon originally present in sample 5.

## 3. Results and discussion

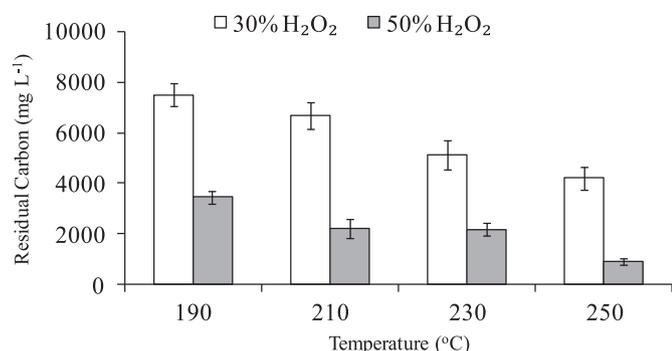
### 3.1. Preliminary results

Initially, experiments were carried out using 0.5 g of milk powder (sample 5 with highest amount of fat), 5 mL of 30% H<sub>2</sub>O<sub>2</sub> at a maximum temperature and pressure of 250 °C and 199 bar, respectively. Under these conditions, the sample was not completely digested and suspended solids remained in the final digest solution. In order to achieve complete digestion of the milk powder sample, different volumes of 30% H<sub>2</sub>O<sub>2</sub> (6, 7 and 8 mL) were also evaluated. Digests without insoluble residues present were only obtained using at least 8 mL of 30%, and in this condition a final system pressure of about 110 bar was observed. Additional experiments were carried out for evaluation of influence of temperature (190–250 °C), concentration of H<sub>2</sub>O<sub>2</sub> (30% and 50%), sample mass (0.1–1 g) and the maximum pressure of microwave system (40, 80 and 199 bar) on digestion efficiency. The efficiency of digestion that only used H<sub>2</sub>O<sub>2</sub> was evaluated based on values of RC and RCC in the final digests. For comparison, the digestion of milk powder was also carried out using 8 mL of concentrated HNO<sub>3</sub>.

Finally, it is important to point out that the concentration of the majority of trace elements in 30% and 50% H<sub>2</sub>O<sub>2</sub> were below the limit of detection (LOD) for ICP-OES with the exception of Sn (used as stabilizer for H<sub>2</sub>O<sub>2</sub>), Ca, Na, Fe and Zn. For these elements, the blank values were minute compared with the concentration present in milk powder, but were discounted from the digest concentration.

### 3.2. Influence of temperature and concentration of H<sub>2</sub>O<sub>2</sub>

In order to optimize the method for allowing the digestion of a large sample mass with minimum oxidant amounts, under safe conditions, the digestion temperature and H<sub>2</sub>O<sub>2</sub> concentration were investigated. Fig. 1 presents the influence of temperature and H<sub>2</sub>O<sub>2</sub> concentration on RC values. It was possible to observe that the increase of temperature digestion allowed the improvement of the digestion efficiency with about 40% of decrease of RC in digests comparing the temperature of 190 °C and 250 °C using 30% H<sub>2</sub>O<sub>2</sub>. The most pronounced effect was observed for 50% H<sub>2</sub>O<sub>2</sub>, which led to a decrease of about 70% in RC, comparing the minimum and maximum temperatures used in the proposed method. Additionally, a significant decrease in RC was observed, from 4218 mg L<sup>-1</sup> to 918 mg L<sup>-1</sup> of C (a reduction of about 4 times) when comparing the digests obtained using 30% and 50% of H<sub>2</sub>O<sub>2</sub>, respectively at 250 °C. In

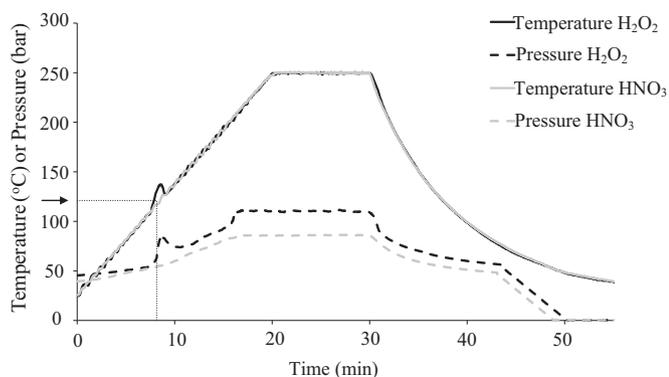


**Fig. 1.** Influence of temperature and H<sub>2</sub>O<sub>2</sub> concentration on residual carbon of milk powder digests (n=3).

this sense, the oxidation efficiency of milk powder matrix by the proposed method is directly dependent on temperature and H<sub>2</sub>O<sub>2</sub> concentration.

According to the literature, digests with carbon concentrations up to 8000 mg L<sup>-1</sup> do not present carbon-related effects on determination by ICP-OES for the majority of elements, with the exception of As, Be and Se [28]. In this sense, all digests obtained using 30% and 50% H<sub>2</sub>O<sub>2</sub> with temperatures ranging from 190 to 250 °C are suitable for ICP-OES analysis. On the other hand, the maximum carbon concentration for accurate analysis by ICP-MS should be as low as possible to minimize spectral interferences and deposits of carbon in the interface [27]. So, only digests obtained using a maximum temperature of 250 °C and 8 mL of 50% H<sub>2</sub>O<sub>2</sub> were suitable for analysis using ICP-OES and ICP-MS. For further experiments, a maximum temperature of 250 °C and 8 mL of 50% H<sub>2</sub>O<sub>2</sub> were selected.

Fig. 2 demonstrates the temperature and pressure of the SRC system during the digestion of milk powder using only 50% H<sub>2</sub>O<sub>2</sub> under optimized conditions and compared with concentrated HNO<sub>3</sub>. Initially, it is important to point out that during the heating program, a significant increase in pressure at a temperature of about 125 °C was only observed for H<sub>2</sub>O<sub>2</sub> digests, which was probably caused by the H<sub>2</sub>O<sub>2</sub> decomposition (Fig. 2, arrow in Y-axis). At the same temperature (125 °C) significant increase in system pressure was not observed for HNO<sub>3</sub> digests. This was probably observed because the increased pressure of HNO<sub>3</sub> digestion is only a result of the gases produced by the oxidation of the organic matrix. Moreover, it was also possible to observe a temperature spike in the heating ramp of the SRC system due to the heat produced by H<sub>2</sub>O<sub>2</sub> decomposition (exothermic reaction) which was not observed in the HNO<sub>3</sub> digestion (Fig. 2).



**Fig. 2.** Profiles of temperature and pressure of digestions carried out using 50% H<sub>2</sub>O<sub>2</sub> and concentrated HNO<sub>3</sub> in SRC system.

### 3.3. Influence of the maximum operation pressure during sample digestion

Pressure can be explored to improve the boiling temperature of the oxidants used in the digestion procedure. In this sense, experiments for the sample digestion were also carried out with the maximum system pressure set at 40, 80 and 199 bar in order to evaluate the influence of pressure on digestion efficiency. In Fig. 2, it is possible to observe that the maximum pressure achieved for milk powder digestion with 50% H<sub>2</sub>O<sub>2</sub> at 250 °C was about 110 bar. Only this combination of high temperature and pressure produced digests without insoluble materials when H<sub>2</sub>O<sub>2</sub> was used as the oxidant. On the other hand, the maximum pressure achieved for digestion using concentrated HNO<sub>3</sub> was about 90 bar ensuring that final digests were free of insoluble materials.

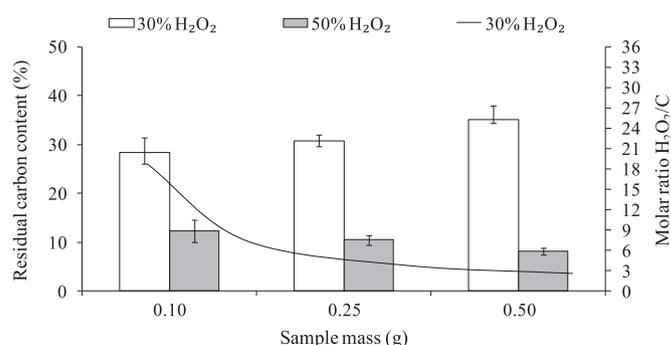
In order to evaluate the digestion efficiency of 50% H<sub>2</sub>O<sub>2</sub> using lower pressures, the maximum pressure of SRC was set at 40 and 80 bar, which are usually the maximum operational pressures allowed by other commercially available microwave systems. Additionally, for experiments carried out using system pressure of 40 and 80 bar, the maximum temperature of 250 °C was not achieved and the final digests presented insoluble materials in both conditions. Based on these results, additional experiments were carried out using maximum pressure of 199 bar to evaluate the sample mass allowed in this system.

### 3.4. Influence of sample mass

Digestion efficiency was evaluated for sample mass ranging from 0.1 to 1 g of milk powder and results are presented in Fig. 3. First, it is important to mention that digestion efficiency was evaluated based on residual carbon content (RCC, bars left axis) and calculated using the original C content of the sample. Fig. 3 also shows the molar ratio between H<sub>2</sub>O<sub>2</sub> and C, which is represented in right axis (line) necessary to assure the complete digestion of the milk powder. A sample mass higher than 0.5 g presented incomplete digestion and insoluble residues were observed in the final digests under optimized conditions. These digests were considered unsuitable for elemental analysis using ICP-based techniques. So, only results for masses of 0.1, 0.25 and 0.5 g are shown in Fig. 3. In Fig. 3 (line) it can be observed that the minimum molar ratio between H<sub>2</sub>O<sub>2</sub> and C (only for 30% H<sub>2</sub>O<sub>2</sub>) should be higher than 3 in order to produce milk powder digests without insoluble residues.

### 3.5. Influence of sample composition

According to Fig. 3 it is interesting to observe that for both 30% and 50% H<sub>2</sub>O<sub>2</sub>, the RCC values remained almost constant while



**Fig. 3.** Influence of sample mass and H<sub>2</sub>O<sub>2</sub> concentration on residual carbon content (left axis - bars, n=3). Molar ratio of H<sub>2</sub>O<sub>2</sub>/C in milk powder digests (right axis- line, n=3).

increasing the sample mass. This is probably because some of the major compounds (mainly carbohydrates, protein and fat) in milk are more easily oxidized than others. Under milder oxidation conditions (with 30% H<sub>2</sub>O<sub>2</sub>) about 60% of the original carbon was oxidized, which probably corresponds to the oxidation of the carbohydrates (especially lactose) present in milk powder. On the other hand, under stronger oxidation conditions (with 50% H<sub>2</sub>O<sub>2</sub>) the oxidation of the other compounds in milk (protein and fat) probably occurs, leading to decreased values of RCC. However, even when using 50% H<sub>2</sub>O<sub>2</sub>, about 10% of the original C present in the sample remained in the solution. This result can primarily be attributed to fat content, which is in agreement to results previously reported in the literature where temperatures close to 450 °C were required for the complete oxidation of this class of compounds [24].

In order to better understand the reactivity of the major compounds present in milk (carbohydrates, proteins and fat), the digestion of samples with different contents of these compounds was performed under optimized conditions and the RC in the final digests was determined by ICP-OES. Sample 5, with the highest amount of fat (27%) and the lowest content of carbohydrates (37%), presented an RC in the final digest of 918 mg L<sup>-1</sup>. On the other hand, sample 2 (57% carbohydrates and 27% fat) showed digest with an RC that was 2.5 times lower than that in sample 5. Confirming this data, the RC in the digests obtained for NIST 8435 (whole milk powder, about 43%, 26% and 21% of carbohydrates, proteins and fat, respectively) was about 2 times higher than that obtained for NIST 1549 (non-fat milk powder, about 49% of lactose according to the certificate of analysis). Similar results were obtained by Denbsky, who observed the easy oxidation of food rich in carbohydrates (flour) compared to fatty foods in digestions using only H<sub>2</sub>O<sub>2</sub> and high temperature (up to 370 °C) [23].

According to the literature, lactose (the major disaccharide present in milk) can be completely oxidized to formate in the presence of 30% H<sub>2</sub>O<sub>2</sub> and 25% NH<sub>4</sub>OH during 24 h at room temperature [29,30].

Regarding proteins, according to the literature, peptide bond cleavage is observed and the subsequent oxidation of methionine, cysteine, tryptophan and histidine can also occur with H<sub>2</sub>O<sub>2</sub> at alkaline pH or elevated temperature [31,32].

Alternatively, in this work, the oxidation of the compounds present in milk powder were carried out in the presence of only H<sub>2</sub>O<sub>2</sub>, but in a very high temperature and pressure (250 °C and 199 bar, respectively), which could explain the oxidation of milk carbohydrates and proteins. The formation of organic acids during

the digestion of milk powder with H<sub>2</sub>O<sub>2</sub> (especially formic acid) was demonstrated by the decrease in the pH of digests solution. The initial pH of 50% H<sub>2</sub>O<sub>2</sub>, which was measured before the digestion, was about 4.3 and in the final digests of milk powder the pH decreased to about 3.

Based on published material, saturated fatty acids (SFA) are a majority in the composition of milk fat (about 65%) and the most common way for oxidation reaction is *via* radical chain reaction, requiring the use of a catalyst for the production of these reactive oxygen species from H<sub>2</sub>O<sub>2</sub> [33]. It is also possible to find in the literature, that the presence of small amounts of metals (Fe, Mo, Mn, etc...) can act as a catalyst allowing the decomposition of H<sub>2</sub>O<sub>2</sub> and the resulting OH radicals with nonselective attack of substrate [29,34]. Given this aspect, the main transition metal found in milk powder analyzed in this work was Fe (up to 170 μg g<sup>-1</sup>), but it was not possible to affirm that this concentration was enough to catalyze the H<sub>2</sub>O<sub>2</sub> reactions with fat. Probably, the fats are the more refractory compounds present in milk that correspond to 10% of RCC and were not completely oxidized by the proposed method.

In order to compare the digestion efficiency, 0.5 g of milk powder was digested using 8 mL of concentrated HNO<sub>3</sub>. Final digest solutions presented carbon content up to 300 mg L<sup>-1</sup>, which is 3 times lower than the C concentration obtained in 50% H<sub>2</sub>O<sub>2</sub> digests. This can be explained by the higher oxidation potential of nitric acid under high temperatures and pressure compared to the oxidation potential of H<sub>2</sub>O<sub>2</sub>. However, the final pH of the HNO<sub>3</sub> digest solutions was about 0. It is important to mention that the acidity of the final digests was lower than that observed for other works reported in the literature, which proposed wet digestion methods with concentrated acids for milk powder [3,4]. Additionally, the final acidity of the digest obtained using H<sub>2</sub>O<sub>2</sub> was comparable with acidity provided by MIC methods [5,35]. In this sense, milk powder digests obtained using only H<sub>2</sub>O<sub>2</sub> were more suitable for ICP-based technique analysis in comparison with HNO<sub>3</sub> digests, because they presented low acidity and additional dilution was not required for sample introduction in spectrometer nebulizer.

### 3.6. Elemental determination in milk powder samples and certified reference materials

Certified reference materials and five milk powder samples were digested using the optimized method. The results for fourteen elements are shown in Tables 2 and 3, respectively. According

**Table 2**  
Elemental concentration (mg kg<sup>-1</sup>) in certified materials using the proposed digestion method and determination by ICP-OES and ICP-MS (mean ± standard deviation, n=3).

Element	Whole milk powder (NIST 8435)		Non-fat milk powder (NIST 1549)	
	Found	Certified	Found	Certified
B	1.1 ± 0.1	1.1 ± 0.1	nd.	–
Ba	0.61 ± 0.08	0.58 ± 0.23	nd.	–
Ca	9570 ± 860	9220 ± 490	12,610 ± 740	13,000 ± 500
Cd	0.0003 ± 0.0001	0.0002 <sup>a</sup>	0.0006 ± 0.0002	0.0005 ± 0.0002
Cu	0.47 ± 0.05	0.46 ± 0.08	0.68 ± 0.15	0.7 ± 0.1
Fe	2.2 ± 0.8	1.8 ± 1.1	1.8 ± 0.1	1.78 ± 0.10
K	13,850 ± 510	13,630 ± 470	17,520 ± 600	16,900 ± 300
Mg	790 ± 80	814 ± 76	1130 ± 85	1200 ± 30
Mn	0.20 ± 0.06	0.17 ± 0.05	0.28 ± 0.10	0.26 ± 0.06
Mo	0.23 ± 0.10	0.29 ± 0.13	0.26 ± 0.10	0.34 <sup>a</sup>
Na	3780 ± 420	3560 ± 400	4870 ± 120	4970 ± 100
Pb	0.11 ± 0.03	0.11 ± 0.05	0.022 ± 0.005	0.019 ± 0.003
Sr	4.6 ± 0.4	4.35 ± 0.50	nd.	–
Zn	28.4 ± 3.5	28.0 ± 3.1	47.0 ± 3.0	46.1 ± 2.2

nd.= not determined.

<sup>a</sup> Informed value.

**Table 3**Elemental concentration (mg kg<sup>-1</sup>) in milk powder samples using the proposed method and determination by ICP-OES and ICP-MS (mean ± standard deviation, n=3).

Element	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5	
	HNO <sub>3</sub>	H <sub>2</sub> O <sub>2</sub>								
B	1.1 ± 0.1	0.93 ± 0.12	3.1 ± 0.2	2.9 ± 0.2	2.7 ± 0.3	2.3 ± 0.2	0.51 ± 0.04	0.51 ± 0.03	1.2 ± 0.1	1.2 ± 0.1
Ba	0.67 ± 0.07	0.73 ± 0.08	0.44 ± 0.05	0.55 ± 0.04	0.86 ± 0.07	0.92 ± 0.06	1.15 ± 0.10	1.24 ± 0.12	0.91 ± 0.08	1.03 ± 0.09
Ca	5890 ± 490	5860 ± 420	5390 ± 540	5500 ± 490	5430 ± 510	5640 ± 500	9470 ± 860	9160 ± 810	7560 ± 720	8000 ± 740
Cd	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Cu	5.4 ± 0.5	6.1 ± 0.4	3.1 ± 0.2	2.8 ± 0.2	2.9 ± 0.3	2.7 ± 0.2	0.26 ± 0.02	0.35 ± 0.02	0.29 ± 0.01	0.36 ± 0.01
Fe	86 ± 9	80 ± 8	81 ± 7	89 ± 6	71 ± 6	73 ± 7	168 ± 11	158 ± 10	86 ± 7	82 ± 5
K	6870 ± 840	6820 ± 800	6580 ± 460	6550 ± 490	7690 ± 690	7350 ± 620	10,070 ± 900	11,080 ± 810	8960 ± 760	8680 ± 690
Mg	452 ± 36	473 ± 45	396 ± 33	405 ± 32	593 ± 44	591 ± 45	793 ± 47	813 ± 55	842 ± 47	878 ± 39
Mn	0.39 ± 0.04	0.45 ± 0.05	0.56 ± 0.05	0.63 ± 0.05	0.57 ± 0.04	0.61 ± 0.05	0.57 ± 0.04	0.64 ± 0.04	0.43 ± 0.03	0.47 ± 0.03
Mo	0.50 ± 0.07	0.50 ± 0.06	0.63 ± 0.06	0.68 ± 0.05	0.59 ± 0.05	0.70 ± 0.06	0.68 ± 0.05	0.76 ± 0.06	0.81 ± 0.05	0.81 ± 0.04
Na	1990 ± 190	2000 ± 180	2440 ± 270	2600 ± 245	2180 ± 200	2570 ± 220	3220 ± 290	3410 ± 320	3520 ± 290	3880 ± 280
Pb	0.12 ± 0.01	0.13 ± 0.01	0.15 ± 0.01	0.13 ± 0.01	0.10 ± 0.01	0.12 ± 0.01	0.21 ± 0.01	0.23 ± 0.01	0.16 ± 0.01	0.14 ± 0.01
Sr	3.3 ± 0.34	3.8 ± 0.39	4.1 ± 0.3	4.9 ± 0.4	5.2 ± 0.4	5.5 ± 0.3	4.4 ± 0.2	5.0 ± 0.3	5.1 ± 0.3	5.8 ± 0.4
Zn	58 ± 6	62 ± 5	46 ± 4	44 ± 3	71 ± 6	72 ± 7	34 ± 2	35 ± 3	38 ± 3	37 ± 2

**Table 4**

Limit of detection obtained using plasma-based techniques.

Element	Limit of detection (mg kg <sup>-1</sup> )	
	ICP-OES	ICP-MS
B	0.1	0.02
Ba	0.1	0.01
Ca	3.0	nd.
Cd	0.05	0.0001
Cu	0.1	0.01
Fe	0.3	nd.
K	3.0	nd.
Mg	0.5	nd.
Mn	0.05	0.005
Mo	0.5	0.005
Na	30	nd.
Pb	0.5	0.0002
Sr	0.1	0.01
Zn	0.5	0.1

nd. = not determined.

to Table 2, all the results obtained for the certified reference materials (NIST 8435 and NIST 1549), using the proposed method, did not present a significant difference when compared with certified values (*t* test, 95% confidence level). Accuracy was also evaluated by comparison between values obtained using the proposed method and those obtained by the conventional wet digestion method with concentrated nitric acid and significant difference was not observed for all elements (*t* test, 95% confidence level). Relative standard deviations (RSDs) for the results obtained using digestion with the H<sub>2</sub>O<sub>2</sub> method were lower than 13% and were similar to those reported in the literature [3,4]. Limits of detection were calculated based on three times the standard deviation of the elemental determinations in blanks (*n* = 10). LODs obtained for ICP-OES were suitable for the determination of major and essential elements present in milk powder (Table 4) and were comparable with the values reported in the literature using other wet digestion methods [4,36].

#### 4. Conclusion

The digestion method using H<sub>2</sub>O<sub>2</sub> and high temperature and pressure (SRC -UltraWave™ system) was optimized and a suitable condition for the digestion of milk powder samples was achieved. The use of only H<sub>2</sub>O<sub>2</sub> as an oxidant is in compliance with green chemistry requirements due to the low toxicity and low acidity of

the final digests. In addition, relatively higher sample masses could be digested combining the oxidant action of H<sub>2</sub>O<sub>2</sub>, higher temperature and higher pressure. By the way, up to five vessels could be used simultaneously allowing a better sample throughput. Additionally, the low RCC values and acidity of the digests make the proposed method a promising alternative for sample preparation method for subsequent elemental determination by ICP-based techniques.

#### Acknowledgements

The authors are grateful to CNPq, FAPERGS and CAPES for supporting this study.

#### References

- [1] ([www.searo.who.int/entity/world\\_health\\_day/2015/whd-what-you-should-know/en/#quality](http://www.searo.who.int/entity/world_health_day/2015/whd-what-you-should-know/en/#quality)). Food safety: What you should know. Accessed in January 2016.
- [2] K.E. Levine, J.D. Batchelor, C.B. Rhoades, B.T. Jones, Evaluation of a high-pressure, high-temperature microwave digestion system, *J. Anal. At. Spectrom.* 14 (1999) 49–59.
- [3] A. Krushevska, R.M. Barnes, C.J. Arnanarasiwaradana, H. Foner, L. Martines, Comparison of sample decomposition procedures for the determination of zinc in milk by inductively coupled plasma atomic emission-spectrometry, *J. Anal. At. Spectrom.* 7 (1992) 851–858.
- [4] L. Noël, M. Carl, C. Vastel, T. Guerin, Determination of sodium, potassium, calcium and magnesium content in milk products by flame atomic absorption spectrometry (FAAS): a joint ISO/IDF collaborative study, *Int. Dairy J.* 18 (2008) 899–904.
- [5] E.M.M. Flores, E.I. Muller, F.A. Duarte, P. Grinberg, R.E. Sturgeon, Determination of trace elements in fluoropolymers after microwave-induced combustion, *Anal. Chem.* 85 (2013) 374–380.
- [6] A.L.H. Muller, C.C. Muller, F. Lyra, P.A. Mello, M.F. Mesko, E.I. Muller, E.M. Flores, Determination of toxic elements in nuts by inductively coupled plasma mass spectrometry after microwave-induced combustion, *Food Anal. Methods* 6 (2013) 258–264.
- [7] T.L. Marques, H. Wiltse, H. Motter, J.A. Nobrega, G. Knapp, High pressure microwave-assisted flow digestion system using a large volume reactor-feasibility for further analysis by inductively coupled plasma-based techniques, *J. Anal. At. Spectrom.* 30 (2015) 1898–1905.
- [8] J.S. Barin, J.S.F. Pereira, P.A. Mello, C.L. Knorr, D.P. Moraes, M.F. Mesko, J. A. Nóbrega, M.G.A. Korn, E.M.M. Flores, Focused microwave-induced combustion for digestion of botanical samples and metals determination by ICP OES and ICP-MS, *Talanta* 94 (2012) 308–314.
- [9] M. Murillo, N. Carrion, J. Chirinos, Determination of sulfur in crude oils and related materials with a Parr bomb digestion method and inductively-coupled plasma-atomic emission-spectrometry, *J. Anal. At. Spectrom.* 8 (1993) 493–495.
- [10] M. Hoenig, Dry ashing, in: Z. Mester, R.E. Sturgeon (Eds.), *Sample Preparation for Trace Element Analysis*, Elsevier, Amsterdam, 2003, pp. 235–254.
- [11] J.S. Barin, B. Tischer, R.S. Picoloto, F.G. Antes, F.E.B. da Silva, F.R. Paula, E.M. Flores, Determination of toxic elements in tricyclic active pharmaceutical

- ingredients by ICP-MS: a critical study of digestion methods, *J. Anal. At. Spectrom.* 29 (2014) 352–358.
- [12] R.S. Picoloto, M. Doneda, E.L.M. Flores, M.F. Mesko, E.M.M. Flores, P.A. Mello, Simultaneous determination of bromine and iodine in milk powder for adult and infant nutrition by plasma based techniques after digestion using microwave-induced combustion, *Spectrochim. Acta Part B* 107 (2015) 86–92.
- [13] S.V. Silva, R.S. Picoloto, E.M.M. Flores, R. Wagner, N.S.P.S. Richards, J.S. Barin, Evaluation of bromine and iodine content of milk whey proteins combining digestion by microwave-induced combustion and ICP-MS determination, *Food Chem.* 190 (2016) 364–367.
- [14] E.I. Muller, M.F. Mesko, D.P. Moraes, M.G.A. Korn, Wet digestion using microwave heating, in: E.M.M. Flores (Ed.), *Microwave-Assisted Sample Preparation for Trace Element Determination*, Elsevier, Amsterdam, 2014, pp. 99–142.
- [15] J.A. Nobrega, C. Pirola, L.L. Fialho, G. Rota, C.E.K.M.A.C. Jordao, F. Pollo, Microwave-assisted digestion of organic samples: How simple can it become? *Talanta* 98 (2012) 272–276.
- [16] M. Wasilewska, W. Goessler, M. Zischka, B. Maichinc, G. Knapp, Efficiency of oxidation in wet digestion procedures and influence from the residual organic carbon content on selected techniques for determination of trace elements, *J. Anal. At. Spectrom.* 17 (2002) 1121–1125.
- [17] J.L. Todoli, J.M. Mermet, Acid interferences in atomic spectrometry: analyte signal effects and subsequent reduction, *Spectrochim. Acta Part B* 54 (1999) 895–929.
- [18] I.I. Stewart, J.W. Olesik, Steady state acid effects in ICP-MS, *J. Anal. At. Spectrom.* 13 (1998) 1313–1320.
- [19] I.I. Stewart, J.W. Olesik, The effect of nitric acid concentration and nebulizer gas flow rates on aerosol properties and transport rates in inductively coupled plasma sample introduction, *J. Anal. At. Spectrom.* 13 (1998) 1249–1256.
- [20] R. Noyori, M. Aoki, K. Sato, Green oxidation with aqueous hydrogen peroxide, *Chem. Commun.* 16 (2003) 1977–1986.
- [21] N. Mketto, P.N. Nomngongo, J.C. Ngila, An innovative microwave-assisted digestion method with diluted hydrogen peroxide for rapid extraction of trace elements in coal samples followed by inductively coupled plasma-mass spectrometry, *Microchem. J.* 124 (2016) 201–208.
- [22] N. Mketto, P.N. Nomngongo, J.C. Ngila, Development of a novel and green microwave-assisted hydrogen peroxide digestion method for total sulphur quantitative extraction in coal samples prior to inductively coupled plasma optical emission spectroscopy and ion chromatography determination, *RSC Adv.* 5 (2015) 38931–38938.
- [23] G. Denbsky, Rapid wet-ashing of biological-material with hydrogen-peroxide under high-temperature and pressure almost without loss, *Fresenius Z. Anal. Chem.* 267 (1973) 350–355.
- [24] G. Denbsky, Rapid wet-ashing of biogenous fats, oils, waxes and food-stuffs of high lipid-content using hydrogen-peroxide under high-temperature and pressure, *Fresenius. Z. Anal. Chem.* 277 (1977) 375–375.
- [25] H. Matusiewicz, R.M. Barnes, Tree-ring wood analysis after hydrogen-peroxide pressure decomposition with inductively coupled plasma atomic emission-spectrometry and electrothermal vaporization, *Anal. Chem.* 57 (1985) 406–411.
- [26] C.C. Muller, A.L.H. Muller, C. Pirola, F.A. Duarte, E.M.M. Flores, E.I. Muller, Feasibility of nut digestion using single reaction chamber for further trace element determination by ICP-OES, *Microchem. J.* 116 (2014) 255–260.
- [27] A.L.H. Muller, J.S.S. Oliveira, P.A. Mello, E.I. Muller, E.M.M. Flores, Study and determination of elemental impurities by ICP-MS in active pharmaceutical ingredients using single reaction chamber digestion in compliance with USP requirements, *Talanta* 136 (2015) 161–169.
- [28] H. Wilsche, M. Winkler, P. Tirk, Matrix effects of carbon and bromine in inductively coupled plasma optical emission spectrometry, *J. Anal. At. Spectrom.* 30 (2015) 2223–2234.
- [29] S.J.H.F. Arts, E.J.M. Mombarg, H. van Bekkum, R.A. Sheldon, Hydrogen peroxide and oxygen in catalytic oxidation of carbohydrates and related compounds, *Synth.-Stuttg.* 6 (1997) 591–613.
- [30] P. Pullanikat, S.J. Jung, K.S. Yoo, K.W. Jung, Oxidative degradation of reducing carbohydrates to ammonium formate with H<sub>2</sub>O<sub>2</sub> and NH<sub>4</sub>OH, *Tetrahedron Lett.* 51 (2010) 6192–6194.
- [31] R.L. Lundblad, *Chemical Reagents for Protein Modification*, fourth ed., Taylor & Francis Group, Boca Raton, 2014.
- [32] J.L. Cuq, M. Provansal, F. Guilleux, C. Cheftel, Oxidation of methionine residues of casein by hydrogen-peroxide - effects on in-vitro digestibility, *J. Food Sci.* 38 (1973) 11–13.
- [33] D.B. Min, J.M. Boff, Lipid oxidation of edible oil, in: C.C. Akoh, D.B. Min (Eds.), *Food Lipids Chemistry, Nutrition and Biotechnology*, second ed., Marcel Dekker, New York, 2002, pp. 331–363.
- [34] C.P. Shelor, C.A. Campbell, M. Kroll, P.K. Dasgupta, T.L. Smith, A. Abdalla, M. Hamilton, T.W. Muhammad, Fenton digestion of milk for iodinalysis, *Anal. Chem.* 83 (2011) 8300–8307.
- [35] J.S.F. Pereira, L.S.F. Pereira, L. Schmidt, C.M. Moreira, J.S. Barin, E.M.M. Flores, Metals determination in milk powder samples for adult and infant nutrition after focused-microwave induced combustion, *Microchem. J.* 109 (2013) 29–35.
- [36] C.A. Bizzi, J.S. Barin, E.E. Garcia, J.A. Nóbrega, V.L. Dressler, E.M.M. Flores, Improvement of microwave-assisted digestion of milk powder with diluted nitric acid using oxygen as auxiliary reagent, *Spectrochim. Acta Part B* 66 (2011) 394–398.