JAAS

TECHNICAL NOTE

View Article Online
View Journal

Cite this: DOI: 10.1039/c5ja00388a

The synergic effect of microwave and ultraviolet radiation for chocolate digestion and further determination of As, Cd, Ni and Pb by ICP-MS

Carla A. Hartwig,[a] Rodrigo M. Pereira,[a] Filipe S. Rondan,[a] Sandra M. Cruz,[b] Fabio A. Duarte,[b] Erico M. M. Flores[b] and Marcia F. Mesko*[a]

Received 14th September 2015
Accepted 28th October 2015

DOI: 10.1039/c5ja00388a

www.rsc.org/jaas

The synergic effect between microwave and ultraviolet radiation was proposed for chocolate digestion and further toxic element determination by inductively coupled plasma mass spectrometry (ICP-MS). As, Cd, Ni and Pb were chosen to show the applicability of the proposed method. Ultraviolet radiation was generated *in situ* using a low-pressure Cd discharge microwave lamp inside each digestion vessel. HNO_3 solutions of several concentrations (2, 4, 7 and 14.4 mol L^{-1}) were evaluated as digestion media. Parameters such as sample mass and microwave irradiation time were also evaluated in order to provide the best conditions for chocolate digestion. A relatively high sample mass, up to 600 mg of chocolate (white and milk), was digested using a diluted acid solution (10 mL of 4 mol L^{-1} HNO_3), allowing for a final solution with dissolved carbon content lower than 100 mg L^{-1}, which was suitable for ICP-MS measurements. The accuracy of the proposed method was evaluated by the digestion of a certified reference material (CRM, BCR 414), and the agreement with the certified values for all analytes was between 95 and 98%. Recovery tests were also performed and results between 95 and 104% for all analytes were obtained. The limits of detection for As, Cd, Ni and Pb by the proposed method were 0.87, 0.98, 29.7 and 7.85 ng g^{-1}, respectively. Thus, for the first time, chocolate was efficiently digested using only a diluted acid solution (digestion efficiency was higher than 90%). Moreover, the digests were suitable for subsequent ICP-MS analysis without any filtration and/or extra-dilution step, as generally reported in the literature.

1. Introduction

Chocolate is a food product widely consumed around the world, in all segments of society and by people of all ages. This type of food is produced from cocoa beans, the fruit of the cocoa tree (*Theobroma cacao*), to be consumed in bars or as powder for the preparation of several foods (cakes, candies, breads, *etc.*).[1,2] Although there are relatively high levels of sugar and fat in these products, the consumption of chocolate and other cocoa products is frequently associated with beneficial health effects. The presence of cocoa, milk and sugar in chocolate composition can help to increase the ingestion of proteins, carbohydrates, fats, minerals and vitamins.[2,3] However, many studies have shown that potentially toxic elements can be found in chocolates.[3–18] This contamination has been attributed to several factors, such as the raw materials, process of manufacturing, wrappers where they are stored, environmental pollution, among others.[2,3,6,10,13,14,16,17]

Arsenic, cadmium, nickel and lead are examples of elements that need to be investigated in chocolate, due to their potential toxicity to the human body. The presence of As in chocolate has been studied by other authors considering that the inorganic species of this element are carcinogenic to humans even at low concentration.[9,10,16,19] In the same way, studies about Cd and Pb concentration in chocolate necessarily take into account the toxic effects on the human body, such as damage in hepatic and reproductive systems, kidney failure, problems in brain functions, among others.[2,3,6,7,9,10,13,16,17,19] Nickel is considered one of the main contaminants in chocolate, and a potentially carcinogenic element to humans. Additionally, this element may cause, among other damages, dermal symptoms of allergy in susceptible individuals.[2,6,14,15,19,20]

In general, these contaminants are present at trace levels in chocolate products,[2] requiring the use of sensitive analytical techniques for element determination, associated with a suitable sample preparation method. The sensitivity and multielement capability of inductively coupled plasma mass spectrometry (ICP-MS)[21] make this technique attractive for the determination of trace elements in chocolate,[7,14,16,22] although most of the publications on this topic involve the use of analytical techniques based on atomic absorption spectrometry (AAS).[3,4,6,8–13,15–18] Despite the advantages related to the use of

[a]*Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas, 96160-000, Pelotas, RS, Brazil. E-mail: marcia.mesko@pq.cnpq.br; Tel: +55 53 3275 7387*

[b]*Departamento de Química, Universidade Federal de Santa Maria, 97105-900, Santa Maria, RS, Brazil*

ICP-MS for trace element determination,[23,24] this technique usually requires a complete digestion of matrix in order to obtain solutions with lower dissolved carbon content, which could interfere in the determination of some analytes.[21,25] Therefore, the complexity of the chocolate matrix, due to its high sugar and fat content,[1,3,9,22] still makes the preparation step of this sample a challenge.

Several methods involving dry ashing,[4,9,11] suspension or emulsion,[5,11,26] acid extraction with conventional heating[22] and even wet acid digestion with conventional[3,5,6,9,11,13,16,18] or microwave-assisted heating[3,9,10,14,15,17,22] have been described to prepare chocolate samples for subsequent determination of several analytes. When ICP-MS is used, the choice of the sample preparation method is important in order to obtain digests with low carbon content allowing lower interference, especially for As. Microwave-assisted wet digestion in closed vessels (MW-AD)[14,22] and conventionally heated wet digestion in open vessels[16] using concentrated acids combined with oxidizing reagents have been successfully applied for chocolate digestion and further toxic element determination by ICP-MS. However, in the case of conventional heating in open systems, despite the application of a filtration step to remove undigested lipids and to obtain a clear solution, the authors reported the need to use matrix matching strategies during the determination step.[16] In this sense, it is important to emphasize that these procedures, involving concentrated reagents and/or additional steps such as filtration or dilution after sample digestion, may lead to inconveniences such as contamination or loss of analytes. In addition, the dilution of digests prior to the determination by ICP-MS, which is usually required when using concentrated reagents, may lead to poor limits of detection (LODs), making difficult the determination of elements at trace levels.[27]

The use of diluted acids has also been reported in the literature for sample preparation of chocolate prior to analyte determination by ICP-MS using the hot extraction method, followed by a filtration step for the removal of the sample matrix.[22] However, ICP-MS was only used for the determination of Cu, Mn and Zn, which are present in relatively high concentration, compared to elements such as As, Cd, Ni and Pb. Therefore, it is expected that interference by dissolved carbon in digests is not very pronounced for the determination of these elements (Cu, Mn and Zn) by ICP-MS, because a previous dilution can be used.

An alternative to sample preparation that allows the use of diluted reagents, with minimal dissolved carbon in digests, is the combination of microwave-assisted wet digestion and ultraviolet radiation (MW-UV). In this system, electrodeless discharge lamps used for ultraviolet generation are introduced into the digestion vessels used for MW-AD.[28] Thus, the synergism of the microwave and ultraviolet radiation can provide a good digestion efficiency using the minimal amount of reagents compared to conventional acid digestion methods, even for samples with high carbon content.[28,29] This system was originally developed by Florian and Knapp[28] for the digestion of skimmed milk powder, and has been applied to other matrices.[27,30–32] However, it is important to notice that this system has never been applied for the digestion of samples with a high fat content (around 30%) such as chocolate.

In the present study, the feasibility of MW-UV digestion was evaluated for the first time for the digestion of chocolate samples and for further determination of As, Cd, Ni and Pb by ICP-MS. A systematic study was performed for the evaluation of HNO_3 concentration, microwave irradiation time and mass of sample, aiming to use only diluted HNO_3 as a digestion solution, without a filtration or extra-dilution step prior to the determination. The digestion efficiency was evaluated by the determination of dissolved carbon in the final digests. The accuracy of the proposed method was evaluated by recovery tests and by analysis of a certified reference material (CRM). The proposed method was applied for the digestion of a variety of white and milk chocolates.

2. Experimental

2.1. Instrumentation

In this study, a microwave sample preparation system (Multiwave 3000, Anton Paar, Austria) equipped with eight high-pressure quartz vessels (internal volume of 80 mL, maximum operating temperature and pressure of 280 °C and 80 bar, respectively) was used. Ultraviolet radiation was produced by using a Cd low-pressure discharge microwave lamp inside each vessel (Anton Paar). The emission domain of this lamp in the UV region is mainly located at 228 nm and the radiation intensity emitted during the microwave heating program is dependent on the absorbed microwave energy and ranges from 1 to 10 W.[28] The same microwave oven (Multiwave 3000, Anton Paar) and quartz vessels were used for chocolate digestion by conventional MW-AD, as well as by the proposed MW-UV method.

An inductively coupled plasma optical emission spectrometer (Spectro Ciros CCD, Spectro Analytical Instruments, Germany) was used for dissolved carbon determination in chocolate digests. The determination of As, Cd, Ni and Pb in the digests was carried out using an inductively coupled plasma mass spectrometer (Elan DRC II, PerkinElmer-SCIEX, USA) equipped with a concentric nebulizer (Meinhard Associates, USA), a cyclonic spray chamber (Glass Expansion, Inc., Australia) and a quartz torch with a quartz injector tube (2 mm i.d.). The operational conditions of both instruments were adapted from previous studies[23,24] and are shown in Table 1.

Argon with a purity of 99.996% (White Martins, Brazil) was used for plasma generation, nebulization and as an auxiliary gas, in both plasma-based instruments. Argon was also used to remove dissolved CO_2 from the digests for carbon determination by ICP OES.

The determination of acidity in the digests was performed using an automatic titrator (Titrando 836, Metrohm, Switzerland). This instrument was equipped with an automatic stirring module (model 803, Metrohm) and a combined pH electrode (model 6.0262.100, Metrohm). Acidity was determined in the digests after dilution up to 25 mL.

Table 1 Operational parameters for dissolved carbon determination by ICP OES and As, Cd, Ni and Pb by ICP-MS

Parameter	ICP OES	ICP-MS
RF power (W)	1400	1300
Argon flow rate (L min^{-1})		
Plasma	15.0	15.0
Auxiliary	1.0	1.2
Nebulizer	0.7	1.1
Spray chamber	Scott double pass	Cyclonic
Nebulizer	Cross-flow	Concentric
Sampler and skimmer cones	—	Pt
Ion lens	—	Auto lens "on"
Analytes	**Emission line (nm)**	**Isotope (*m*/*z*)**
As	—	75
C	193.094	—
Cd	—	111
Ni	—	58
Pb	—	208
Y	371.029[a]	—

[a] Yttrium was used as the internal standard in the carbon determination.

2.2. Reagents, standards and samples

Nitric acid (Merck, Germany) purified using a sub-boiling system (Duopur, Milestone, Italy) and ultrapure water (resistivity of 18 MΩ cm), obtained from a purification system (Mega Up, Megapurity, South Korea), were used in this study.

A multielement stock standard solution (SCP 33MS, SCP Science, Canada) containing 10 mg L^{-1} of each analyte was used to prepare the calibration curve (in 5% HNO_3, v/v) for ICP-MS determination, ranging from 0.025 to 10 μg L^{-1}. This stock standard solution was also used for the analyte spike for recovery test. Standard solutions used for the dissolved carbon determination by ICP OES were prepared by the dilution of citric acid (Merck) in 5% HNO_3 (v/v), ranging from 10 to 250 mg L^{-1} of carbon. Yttrium (1 mg L^{-1}, Spex CertPrep, USA) was used as the internal standard for carbon determination.

A solution of 0.1 mol L^{-1} KOH (Merck) was used for the determination of residual acidity. This solution was previously standardized using potassium hydrogen phthalate.

All materials used were immersed in 10% HNO_3 solution for 24 h. UV lamps were cleaned for 20 min using concentrated HNO_3 at 120 °C under conventional heating. The quartz vessels and polytetrafluoroethylene (PTFE) devices of UV lamps (spacers and base rings) were cleaned using concentrated HNO_3 under microwave radiation (Multiwave 3000, Anton Paar) for 10 min at 900 W and for 15 min at 0 W (cooling step). Finally, all materials were washed with ultrapure water and dried in a class 100 laminar flow bench (CSLH-12, Veco, Brazil) before use.

Seven chocolate samples from different brands and produced in different regions of Brazil were used. All samples were obtained as bars including milk (MC, numbered from 1 to 4) and white (WC, numbered from 1 to 3) chocolates. The basic composition of white chocolate was sugar, whole and skimmed milk powder, cocoa butter, vegetable fat, emulsifier and flavoring. Milk chocolate was composed of sugar, whole and skimmed milk powder, cocoa butter, cocoa (liquor or powder), emulsifier and flavoring. According to the manufacturers, the fat content in these products ranged from 26 to 34%, which is similar to the fat content reported in the literature for white and milk chocolates.[1,33]

For the optimization of digestion procedures, a milk chocolate sample (MC1) was arbitrarily chosen and other samples were only analyzed under the optimized conditions. A certified reference material of plankton (BCR 414), purchased from the Community Bureau of Reference, was used for accuracy evaluation of the proposed method.

2.3. MW-UV digestion method

For sample preparation by MW-UV, sample masses from 500 to 700 mg were inserted into the quartz vessels along with the UV lamps, PTFE devices and 10 mL of HNO_3 solution. Nitric acid solutions (2, 4, 7 and 14.4 mol L^{-1}) were evaluated as digestion media. Microwave irradiation was carried out using the program shown in Table 2, which was adapted from the microwave oven manufacturer for fat digestion.[34] Variations of microwave irradiation were also performed in view of reducing the digestion time. Thus, microwave irradiation hold times of 20 and 10 min, instead 40 min, were also evaluated. The best heating program was selected taking into account the digestion efficiency, which was monitored by dissolved carbon determination by ICP OES, as well as by recovery tests.

2.4. Conventional microwave-assisted digestion method

For comparison of the digestion efficiency using the proposed method (MW-UV), the digestion of chocolate using MW-AD was performed. In this way, the sample mass, HNO_3 concentration and microwave irradiation program were used according to the best conditions selected for the optimization of the MW-UV method.

2.5. Evaluation of digestion efficiency

The evaluation of the digestion efficiency of each condition was carried out by dissolved carbon determination in the digests

Table 2 Microwave irradiation program used in the MW-UV method for chocolate digestion

	Operational conditions[b]		
Step	Power (W)	Ramp (min)	Hold (min)
1	550	20	40
2[a]	0	0	15

[a] Cooling step. [b] Maximum pressure and temperature were limited to 80 bar and 280 °C, respectively; and a maximum pressure rate of 0.5 bar s^{-1}.

using ICP OES. Prior to this determination, in order to remove dissolved CO_2 from the digests, sample aliquots were purged with argon (0.1 L min^{-1}) for 2 min.[35] The results of the dissolved carbon content in the digests were expressed as mg C/100 mg of the sample. The digestion efficiency was also compared to the final acidity in the digests, in order to know the amount of acid consumed in the oxidation reaction.

2.6. Accuracy evaluation of the proposed method

The accuracy of the proposed MW-UV method was evaluated by the digestion of CRM BCR 414 under the same conditions applied to samples. This CRM was chosen, considering that there is no CRM of chocolate with certified values for As, Cd, Ni and Pb. Moreover, taking into account that this CRM does not represent the sample matrix (mainly due to the fat content and other major compounds), an additional study was performed in order to evaluate the influence of the chocolate matrix on the analyte recoveries. In this way, a set of solid mixtures containing chocolate (MC1, 450 mg) and BCR 414 (150 mg) was digested. Considering the CRM mass used, the equivalent concentration of As, Cd, Ni and Pb added to the samples was 1705, 57.4, 2820 and 596 ng g^{-1}, respectively. In addition, accuracy was also evaluated using analyte spike (1 μg L^{-1} for As, Cd and Pb, and 10 μg L^{-1} for Ni) of multielement standard solution added to the samples before digestion.

2.7. Analyte determination in chocolate samples

After the optimization of the sample preparation method, the selected conditions of digestion by MW-UV were applied for white and milk chocolates. These conditions were selected taking into account the analyte recoveries and the good digestion efficiency of a high sample mass using a lower acid concentration combined with lower time consumption. The digests were transferred to volumetric vessels using ultrapure water, to carefully wash the digestion vessel walls and UV lamp (including its accessories), and they were diluted to 25 mL for further determination of As, Cd, Ni and Pb by ICP-MS.

All statistical calculations were performed using the software GraphPad InStat Version 3.06 (GraphPad Software, Inc., USA). A significance level of 95% was adopted for all comparisons.

3. Results and discussion

3.1. Optimization of the MW-UV method

The optimization of the MW-UV method for chocolate samples was carried out using a milk chocolate (MC1), which was arbitrarily selected. In these preliminary experiments, parameters such as the concentration of HNO_3 (2, 4, 7 or 14.4 mol L^{-1}) used as digestion solution, sample mass and microwave irradiation time were evaluated.

The evaluation of HNO_3 concentration for chocolate digestion by MW-UV was performed in order to achieve the lowest HNO_3 concentration that ensures an efficient digestion of 500 mg of chocolate. Initially, the heating program was applied as described in Table 2. The results obtained for dissolved carbon content and final acidity in the digests are shown in Fig. 1.

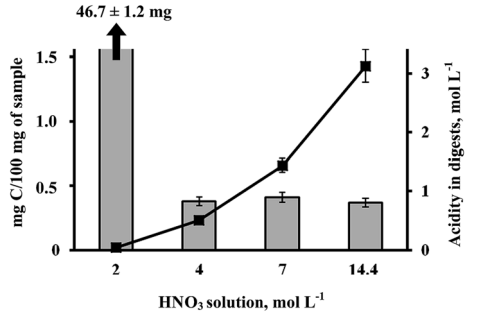

Fig. 1 Dissolved carbon content (▭) and residual acidity (-■-) in the digests of milk chocolate after MW-UV using HNO_3 as a digestion solution ($n = 3$).

Using a 2 mol L^{-1} HNO_3 solution the digestion was not considered effective (about 47 mg C/100 mg of the sample), corresponding to about 7800 mg L^{-1} of dissolved carbon in the digest. However, when 4, 7 or 14.4 mol L^{-1} HNO_3 solutions were used, the dissolved carbon content in the digests was always about 0.4 mg C/100 mg of sample (below 70 mg L^{-1} of dissolved carbon in the digests), which usually did not cause interference in ICP-MS analysis.[25,36] This high digestion efficiency is probably associated with the synergy between the MW and UV radiation, with the formation of highly reactive oxidizing species, which allowed the sample digestion even using diluted acids.[28,31,32]

Regarding the final acidity in the digests, as expected, an increase of values with the use of a higher concentration of HNO_3 was observed (Fig. 1), although the dissolved carbon content was practically the same for HNO_3 concentration equal to or higher than 4 mol L^{-1}. When this HNO_3 concentration was used, a consumption of about 0.025 mol of HNO_3 was observed (about 85% of the acid concentration added) for efficient digestion of 500 mg of the sample, resulting in the final acidity in the digests of around 0.5 mol L^{-1}. However, using 2 mol L^{-1} HNO_3, the final acidity in the digests was about 0.045 mol L^{-1}, corresponding to a consumption of 98% of the acid added. This low residual acidity can explain the low digestion efficiency using this solution.

In addition, in order to ensure the choice of a better digestion solution, analyte spikes were performed for HNO_3 concentrations ranging from 4 to 14.4 mol L^{-1}. For all solutions, recoveries between 95 and 99% were achieved for all analytes (Fig. 2). Thus, 4 mol L^{-1} HNO_3 was considered a suitable solution for chocolate digestion using the MW-UV method. It is important to point out that using this condition the adjustment of solution acidity was not required and it was considered suitable for ICP-MS analysis once calibration curves were obtained using 5% HNO_3 which have practically the same acidity. However, when a more concentrated digestion solution (7 or 14.4 mol L^{-1}) was used, the higher acidity in the digests required an additional dilution step before ICP-MS analysis. In this sense, the selected digested solution in this study (4 mol L^{-1} HNO_3) was the condition that allowed the determination by ICP-MS without an extra-dilution step.

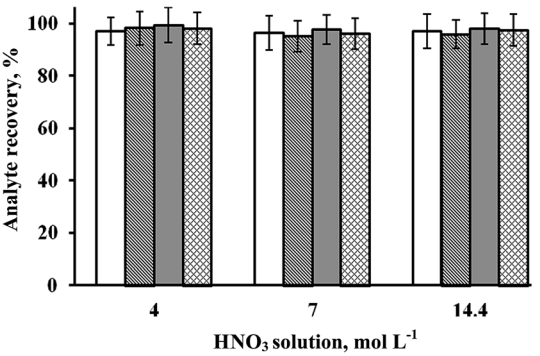

Fig. 2 Recoveries for As □, Cd ▨, Ni ■ and Pb ▩. Determination by ICP-MS after chocolate digestion by MW-UV using 4, 7 or 14.4 mol L^{-1} HNO_3 as a digestion solution ($n = 3$).

Sample mass was another parameter evaluated (up to 700 mg), in order to achieve the digestion of a high mass of chocolate as possible. This evaluation was performed taking into account the residual acidity determined in the final digests (by using 500 mg of chocolate and 10 mL of 4 mol L^{-1} HNO_3), which suggests the availability of acid for digestion of higher sample masses. Thus, when 600 mg of chocolate sample was digested the dissolved carbon content was 0.4 mg C/100 mg of the sample, corresponding to about 80 mg L^{-1} of C in the digests. These digests showed a final acidity of about 0.3 mol L^{-1}. However, when 700 mg of the sample was digested, despite the residual acid observed in the digests (about 0.17 mol L^{-1}), the dissolved carbon was about 1 mg C/100 mg of the sample (233 mg L^{-1} of dissolved carbon). This carbon concentration corresponded to about three times the obtained value for the digests of 600 mg of sample, and it can cause problems such as deposits of carbon in the interface of equipment reducing the sensitivity of analyte determination by ICP-MS.

Based on these findings, for further experiments a mass of 600 mg of chocolate was used, considering the suitable carbon content in the digests, which was similar to those obtained when 500 mg of chocolate was digested. It is important to mention that this is the highest mass of chocolate efficiently digested in closed systems.[3,9,10,14,15,17,22]

In order to reduce the digestion time, the microwave irradiation program was changed, modifying the hold microwave irradiation time from 40 to 10 or 20 min. Although the microwave oven allows the use of a higher power, the maximum irradiation was set at 550 W. This power was used due to the limited pressure (80 bar), which was achieved in the initial minutes of hold time, resulting in a power reduction of about 400 W. For all heating programs evaluated, the ramp time was kept constant in order to avoid sudden spontaneous reactions, considering the high content of organic compounds in the samples.[12]

The maximum temperature observed for all evaluated programs was about 200 °C, which was achieved when maximum pressure was reached. However, the results obtained for dissolved carbon content in the digests of chocolate using 10 min of hold time (about 1.60 mg/100 mg of the sample, corresponding to 267 mg L^{-1} in the solution) and 20 min of hold time (about 0.80 mg/100 mg of the sample, corresponding to 133 mg L^{-1} in the solution) were significantly higher than those obtained using 40 min of hold time (about 0.4 mg/100 mg of the sample). Thus, among the evaluated heating times, 40 min of hold time was chosen as the most satisfactory condition, due to the higher digestion efficiency demonstrated in comparison with others.

3.2. MW-AD method

The digestion of chocolate sample using conventional MW-AD was performed under the selected conditions for the proposed method (MW-UV): 600 mg of the sample, hold time under microwave irradiation of 40 min and using 10 mL of 4 mol L^{-1} HNO_3 as the digestion solution. The only difference in this procedure (MW-AD) was the absence of the UV lamp inside the quartz vessels. The final acidity in the digests under these conditions was 0.24 mol L^{-1} and the solution aspect was clear. However, as expected, digestion by MW-AD was less efficient (dissolved carbon content about 0.8 mg/100 mg of the sample, corresponding to 160 mg L^{-1} in solution) when compared to digestion by MW-UV. This concentration of dissolved carbon in the digests was twice that obtained by the MW-UV method, which confirms the effect of UV radiation on the digestion of organic matrices even using a diluted acid solution.

3.3. Accuracy evaluation of the proposed method

The accuracy of the proposed MW-UV method was evaluated by digestion of CRM BCR 414 under optimized conditions. The results obtained for As, Cd, Ni and Pb in the CRM showed agreement between 95 and 98% with certified values as can be seen in Table 3. Additionally, recovery tests were carried out by using analyte spike of standard solution prior to sample digestion, and also by using a mixture of the sample with CRM BCR 414. Recoveries in both cases were between 95 and 104% for all analytes, confirming that a 4 mol L^{-1} HNO_3 solution is suitable for chocolate digestion using the MW-UV method.

3.4. Determination of As, Cd, Ni and Pb in chocolate by ICP-MS after digestion by MW-UV

The proposed MW-UV method (600 mg of the sample, 4 mol L^{-1} HNO_3 and hold time under microwave irradiation of 40 min) was applied for digestion of 7 chocolate samples, including white and milk chocolates, for further determination of As, Cd, Ni and Pb by ICP-MS. These types of chocolate were selected because they are the most consumed in Brazil, as well as to evaluate the content of toxic elements between the chocolate types and brands.

The results of the analyte determination in the chocolate samples are shown in Table 3. As can be seen, the Ni concentration was higher than the concentrations of As, Cd and Pb in all samples. These results are in agreement with previous studies, in which Ni concentration was always higher than As, Cd and/or Pb concentration in chocolates.[3,4,6,10,13]

As can be seen in Table 3, Ni concentration was higher in milk chocolate than in white chocolate. The same behaviour

Table 3 Results obtained for As, Cd, Ni and Pb determination in chocolate and the CRM using the proposed MW-UV digestion method and determination by ICP-MS. The results are presented in ng g^{-1} wet weight (values represent the mean and standard deviation of $n = 4$)

		Analyte concentration			
Sample		As	Cd	Ni	Pb
White chocolate	WC 1	15.3 ± 0.7	<0.98[b]	105 ± 5	<7.85[c]
	WC 2	12.4 ± 0.8	<0.98[b]	108 ± 6	15.3 ± 0.9
	WC 3	21.7 ± 1.2	<0.98[b]	97.3 ± 6.7	13.7 ± 0.8
Milk chocolate	MC 1	15.1 ± 1.0	7.41 ± 0.36	559 ± 33	15.1 ± 0.7
	MC 2	11.7 ± 0.8	13.7 ± 0.7	538 ± 29	14.0 ± 0.9
	MC 3	13.0 ± 0.8	16.1 ± 0.8	381 ± 23	13.2 ± 0.6
	MC 4	13.6 ± 0.9	18.2 ± 1.0	652 ± 41	15.2 ± 0.9
BCR 414[a]		6670 ± 379	365 ± 17	18 067 ± 831	3856 ± 201

[a] Certified values for CRM BCR 414 (As: 6820 ± 280 ng g^{-1}; Cd: 383 ± 14 ng g^{-1}; Ni: 18 800 ± 800 ng g^{-1}; Pb: 3970 ± 190 ng g^{-1}). [b] LODs for Cd by the proposed method. [c] LODs for Pb by the proposed method.

was observed for Cd, which was not detected (LOD 0.98 ng g^{-1}) in white chocolate. The source of Cd and Ni is probably the cocoa used as the raw material for milk chocolates, as reported in the literature.[3,6,11,13,15–17] However, it is important to mention that variations in the concentration of toxic elements in chocolates can also be associated with the process of manufacturing, as well as with leaching of metals from the packaging in which they are stored.[3] A different behaviour was observed in the As concentration in that the obtained values for the two types of chocolates varied in a small range (11.7 to 21.7 ng g^{-1}). In the same way, for Pb the obtained values for white and milk chocolates were around 14.0 ng g^{-1}, except for one white chocolate sample, where the Pb concentration was lower than the LOD (7.85 ng g^{-1}) of the method.

The obtained results for As, Cd, Ni and Pb in this study were similar to those reported in the literature on milk chocolate.[4,7,10,17] However, regarding to the determination of these elements in white chocolates, it was not possible to perform a comparison, due to the absence of studies involving the determination of these elements in this type of chocolate.

The obtained values for Cd and Ni were lower than the average of the values reported in 2014 by the U.S. Food and Drug Administration (FDA) Total Diet Study (TDS) for milk chocolates (Cd: 24 ng g^{-1} and Ni: 921 ng g^{-1}).[37] For Pb, the obtained values were similar to the average concentration (13 ng g^{-1}) reported for this element by FDA. It was not possible to compare the results for As concentration with those reported by the FDA, because these were lower than the limit of quantification (40 ng g^{-1}) of the method used by the FDA laboratories. Otherwise, the proposed method allowed the As determination in chocolates even at low concentrations (LOD 0.87 ng g^{-1}).

It is important to emphasize that the As, Cd and Pb concentrations obtained for the evaluated samples were below the maximum levels recommended by the Brazilian Health Surveillance Agency for this type of food (200 ng g^{-1} for all analytes in chocolates with cocoa content lower than 40%).[38] There are no recommendations in Brazilian legislation related to the concentration of Ni in chocolates. However, an ingestion of Ni lower than 100 μg per day is suggested for adults,[19] being 1000 μg per day the maximum level of daily intake that is likely to pose no risk of adverse effects.[39]

3.5. Highlights of the proposed method

Using a diluted HNO_3 solution for chocolate digestion, by the proposed method, it was possible to determine As, Cd, Ni and Pb by ICP-MS with a relative standard deviation (RSD) lower than 7% and LODs of 0.87, 0.98, 29.7 and 7.85 ng g^{-1}, respectively. These LODs were significantly lower than those obtained after MW-UV using 14.4 mol L^{-1} HNO_3 (As: 3.6 ng g^{-1}, Cd: 1.99 ng g^{-1}, Ni: 73.5 ng g^{-1} and Pb: 22.4 ng g^{-1}), showing that the use of diluted acid is suitable for further analyte determination even at a lower concentration. In addition, blank values using diluted HNO_3 (4 mol L^{-1}) for all analytes were always significantly lower than those obtained using concentrated HNO_3.

It is important to emphasize that, in this study, it was possible for the first time to efficiently digest chocolate (efficiency higher than 90% considering the total carbon content in the sample) using only diluted acid, without the addition of other oxidizing reagents. Using diluted HNO_3, an extra-dilution of digests before ICP-MS determination was not necessary (digests can be directly analyzed without interference). Moreover, using the proposed MW-UV method, the presence of the particulate material was not observed in the digests, which eliminates the filtration step after sample digestion as usually performed in other methods applied for chocolate analyses.[16,22]

It is also important to highlight a positive aspect concerning to the low dissolved carbon content in the digests obtained by the proposed method, which reduces the interference with ICP-MS determination. The matrix effects caused by the presence of carbon are explained by changes in the plasma characteristics and the corresponding changes in ion distribution in the plasma. However, for some analytes, such as As, the matrix effects are also related to an increase in analyte ion population, caused by charge transfer reactions involving carbon-containing charged species in the plasma.[25] In this sense, the carbon content in digests is an important parameter to be monitored before the ICP-MS determination, mainly for analytes more susceptible to interference.

4. Conclusions

The proposed MW-UV method was suitable for the digestion of chocolate for further determination of As, Cd, Ni and Pb by ICP-MS. The synergic effect of microwave and ultraviolet radiation enabled the use of diluted HNO_3 4 mol L^{-1} to digest up to 600 mg of the sample. In this way, the MW-UV method avoids the use of concentrated acids or other oxidizing reagents, significantly reducing the interference in the determination step, laboratory waste and blank values. Moreover, using the proposed method, digests with low dissolved carbon content were obtained, which was suitable for analyte determination by ICP-MS, not requiring an extra-dilution or filtration step prior to analysis.

The use of MW-UV and ICP-MS allowed the determination of As, Cd, Ni and Pb content in different types of chocolate, making the procedure suitable for routine analysis. Moreover, it was possible to observe different contents of some analytes in white and milk chocolates, although in all cases the obtained concentration was lower than the limits recommended by legislation.

Acknowledgements

The authors are grateful to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) for supporting this study.

References

1 E. O. Afoakwa, A. Paterson and M. Fowler, *Trends Food Sci. Technol.*, 2007, **18**, 290–298.
2 R. R. Watson, V. R. Preedy and S. Zibadi, *Chocolate in Health and Nutrition*, Humana Press, London, 2013.
3 N. Jalbani, T. G. Kazi, H. I. Afridi and M. B. Arain, *Pak. J. Anal. Environ. Chem.*, 2009, **10**, 48–52.
4 L. Jorhem and B. Sundström, *J. Food Compos. Anal.*, 1993, **6**, 223–241.
5 I. Karadjova, S. Girousi, E. Iliadou and I. Stratis, *Microchim. Acta*, 2000, **134**, 185–191.
6 S. Dahiya, R. Karpe, A. G. Hedge and R. M. Sharma, *J. Food Compos. Anal.*, 2005, **18**, 517–522.
7 C. W. Rankin, J. O. Nriagu, J. K. Aggarwal, T. A. Arowolo, K. Adebayo and A. R. Flegal, *Environ. Health Perspect.*, 2005, **113**, 1344–1348.
8 N. Jalbani, T. G. Kazi, M. K. Jamali, M. B. Arain, H. I. Afridi, S. T. Sheerazi and R. Ansari, *J. AOAC Int.*, 2007, **90**, 1682–1688.
9 M. Güldas, *J. Food Nutr. Res.*, 2008, **47**, 92–99.
10 M. Güldas, A. F. Dagdelen and G. F. Biricik, *J. Food, Agric. Environ.*, 2008, **6**, 90–94.
11 A. Duran, M. Tuzen and M. Soylac, *Environ. Monit. Assess.*, 2009, **149**, 283–289.
12 C. V. S. Ieggli, D. Bohrer, P. C. Nascimento, L. M. Carvalho and L. A. Gobo, *J. Food Compos. Anal.*, 2011, **24**, 465–468.
13 S. Rehman and M. Husnain, *J. Trace Elem. Anal.*, 2012, **1**, 1–11.
14 J. Scancar, T. Zuliani and R. Milacic, *J. Food Compos. Anal.*, 2013, **32**, 83–89.
15 A. Bartos, I. Majak and J. Leszczynska, *Food Res. Int.*, 2014, **55**, 412–417.
16 R. L. Yanus, H. Sela, E. J. C. Borojovich, Y. Zakon, M. Saphier, A. Nikolski, E. Gutflais, A. Lorber and Z. Karpas, *Talanta*, 2014, **119**, 1–4.
17 J. E. L. Villa, R. R. A. Peixoto and S. Cadore, *J. Agric. Food Chem.*, 2014, **62**, 8759–8763.
18 G. Ramtahal, I. C. Yen, I. Bekele, F. Bekele, L. Wilson, B. Sukha and K. Maharaj, *J. Food Res.*, 2015, **4**, 193–199.
19 *Trace elements in human nutrition and health*, World Health Organization, Geneva, 1996.
20 J. Wittsiepe, K. Schnell, A. Hilbig, P. Schrey, M. Kersting and M. Wilhelm, *J. Trace Elem. Med. Biol.*, 2009, **23**, 183–194.
21 J. S. Becker, *Inorganic Mass Spectrometry: Principles and Applications*, John Wiley & Sons Ltd, Chichester, 2007.
22 J. E. L. Villa, C. D. Pereira and S. Cadore, *Microchem. J.*, 2015, **121**, 199–204.
23 J. V. Maciel, C. L. Knorr, E. M. M. Flores, E. I. Muller, E. G. Primel, M. F. Mesko and F. A. Duarte, *Food Chem.*, 2014, **145**, 927–931.
24 P. A. Mello, L. O. Diehl, J. S. S. Oliveira, E. I. Muller, M. F. Mesko and E. M. M. Flores, *Spectrochim. Acta, Part B*, 2015, **105**, 95–102.
25 G. Grindlay, J. Mora, M. Loos-Vollebregt and F. Vanhaecke, *Spectrochim. Acta, Part B*, 2013, **86**, 42–49.
26 C. V. S. Ieggli, D. Bohrer, P. C. Nascimento and L. M. Carvalho, *Food Chem.*, 2011, **124**, 1189–1193.
27 J. S. S. Oliveira, R. S. Picoloto, C. A. Bizzi, P. A. Mello, J. S. Barin and E. M. M. Flores, *Talanta*, 2015, **144**, 1052–1058.
28 D. Florian and G. Knapp, *Anal. Chem.*, 2001, **73**, 1515–1520.
29 J. S. F. Pereira, E. Wiltsche and G. Knapp, in *Microwave-Assisted Sample Preparation for Trace Element Determination*, ed. E. M. M. Flores, Elsevier, Oxford, 1st edn, 2014, ch. 7, pp. 205–229.
30 A. Limbeck, *Anal. Chim. Acta*, 2006, **575**, 114–119.
31 J. S. F. Pereira, R. S. Picoloto, L. S. F. Pereira, R. C. L. Guimarães, R. A. Guarnieri and E. M. M. Flores, *Anal. Chem.*, 2013, **85**, 11034–11040.
32 M. F. Mesko, R. S. Picoloto, L. R. Ferreira, V. C. Costa, C. M. P. Pereira, P. Colepicolo, E. I. Muller and E. M. M. Flores, *J. Anal. At. Spectrom.*, 2015, **30**, 260–266.
33 M. Torres-Moreno, E. Torrescasana, J. Salas-Salvadó and C. Blanch, *Food Chem.*, 2015, **166**, 125–132.
34 Anton Paar GmbH, *Multiwave 3000 microwave sample preparation system, Software version v1.27-Synt*, Graz, Austria, 2003.
35 C. A. Bizzi, E. L. M. Flores, J. A. Nóbrega, J. S. S. Oliveira, L. Schmidt and S. R. Mortari, *J. Anal. At. Spectrom.*, 2014, **29**, 332–338.
36 H. Vanhoe, J. Goossens, L. Moens and R. Dams, *J. Anal. At. Spectrom.*, 1994, **9**, 177–185.

37 *Total Diet Study – Elements Results Summary Statistics Market Baskets 2006 through 2011*, US Food and Drug Administration (FDA), College Park, 2014.
38 *Technical Regulation MERCOSUL on Maximum Inorganic Contaminants in Foods*, Brazilian Health Surveillance Agency (ANVISA), Brasilia, 2013.
39 *Dietary Reference Intakes: Applications in Dietary Planning. Institute of Medicine (IOM)*, National Academy of Sciences on Dietary Reference Intakes (DRIs), National Academies Press, Washington, 2003.