



ELSEVIER

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.journals.elsevier.com/food-chemistry-x

Arsenic speciation analysis in rice milk using LC-ICP-MS

Francisco Cunha da Rosa^{a,b}, Matheus Augusto Gonçalves Nunes^a, Fábio Andrei Duarte^a,
Érico Marlon de Moraes Flores^a, Flávia Brito Hanzel^a, Agnes Souza Vaz^a, Dirce Pozebon^c,
Valderi Luiz Dressler^{a,*}

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

^b Instituto Federal de Educação, Ciência e Tecnologia do Rio Grande do Sul, 95770-000 Feliz, RS, Brazil

^c Instituto de Química, Universidade Federal do Rio Grande do Sul, 90040-040 Porto Alegre, RS, Brazil

ARTICLE INFO

Keywords:

Arsenic speciation

LC-ICP-MS

Rice milk

ABSTRACT

The consumption of rice milk has increased, mainly by individuals intolerant to lactose or allergic to cow milk. However, rice milk contains As. In this sense, the concentration of As in rice milk should be controlled. In the present study it is proposed a methodology for determination of As(III), dimethylarsenic (DMA), monomethylarsenic (MMA) and As(V) species in rice milk using LC-ICP-MS. The main features of the methodology are fast analysis, easy and simple sample preparation, where the sample is 3-fold diluted in the mobile phase and then filtered. The four arsenic species investigated were detected in the analysed samples, being As(V) the main species. The limit of quantification of the method ranges from 0.25 to 0.43 $\mu\text{g L}^{-1}$ As. The analyte recovery ranged from 81 to 116% for samples spiked to 1.00 $\mu\text{g L}^{-1}$ or 5.00 $\mu\text{g L}^{-1}$ As and the relative standard deviation was better than 5%.

1. Introduction

Cow milk and its derived products are staple food for humans worldwide since they provide many nutrients and can also prevent cardiovascular diseases, some type of cancer, blood pressure, bone and dental pathologies. However, large consumption of cow milk can make children obese (Fernández et al., 2015) and also affect individuals lactose intolerant and/or allergic to milk proteins (Dreborg, 2015).

Given the side effects of cow milk, oat milk, soy milk and rice milk have been increasingly consumed. Rice milk has been considered a good alternative for cow milk because rice milk is cheaper and more easily digested. However, supplementation of protein and fat is needed when rice milk is used for children feeding (Meharg et al., 2008). Besides that, As concentration in rice milk is higher than in cow milk; rice milk usually contains inorganic arsenic (iAs – sum of As(III) + As(V) species) once the plant rice takes up As and accumulates the elements in the grains. Thus, daily consumption of rice milk can lead to the contamination of individuals by As and this is more critical for infants because milk is their basic food.

Rice milk is generally prepared by liquefaction of the whole grain in an aqueous medium containing the enzyme alpha-amylase, followed by a saccharification step in presence of the glucosidase enzyme. Rice milk can also be produced by simply extraction of ground rice in hot water.

Inorganic As (iAs) and organic As (oAs) species such as monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) are the main As species found in rice, comprising 90% of the total As content (Althobiti, Sadiq, & Beauchemin, 2018; Huang, Fecher, Ilgen, Hu, & Yang, 2012). About 60% of As is present in the form of iAs, which is a concern because iAs is very toxic and leads to the development of cancer and cardiovascular diseases (Hughes, Beck, Chen, Lewis, & Thomas, 2011); the iAs toxicity is 100 times higher than that of methylated species (DMA and MMA) (Signes-Pastor, Carey, & Meharg, 2016; Wang, Peng, Tan, Ma, & Rathinasabapathi, 2015). The World Health Organization (WHO, 2010) determines that the safe limit for iAs daily intake is 2 $\mu\text{g kg}^{-1}$ body weight. Once As can be transferred from rice to its derivatives, a maximum concentration of 100 ng g^{-1} iAs in rice destined for the production of food for infants and young children has been established by the Commission Regulation (2015).

Studies about As speciation in rice milk have been conducted and reported. Meharg et al. (2008) determined DMA, MMA and iAs in rice milk produced in European Union countries whereas the main species found was iAs. Similar results were observed by Pedron et al. (2016) for rice milk from Italy; Santos, Pozebon, Cerveira, and Moraes (2017) for rice milk powder from Brazil; and Munera-Picazo, Burló, and Carbonell-Barrachina (2014) for rice milk from Spain. These results demonstrate the importance of the As speciation and that the total concentration of

* Corresponding author.

E-mail address: vdressler@gmail.com (V.L. Dressler).

<https://doi.org/10.1016/j.fochx.2019.100028>

Received 25 January 2019; Received in revised form 28 April 2019; Accepted 2 May 2019

Available online 07 May 2019

2590-1575/ © 2019 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license

(<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

the element does not bring the necessary information.

Liquid chromatography (LC) associated with inductively coupled plasma mass spectrometry (ICP-MS) is considered appropriate for As speciation in rice milk. In this case, the good sensitivity of ICP-MS is combined with the good selectivity/resolution of LC. Anion exchange columns are usually employed to separate the As species efficiently.

Munera-Picazo et al. (2014) and Santos et al. (2017) employed liquid chromatography-atomic fluorescence spectrometry (LC-AFS) and hydride generation atomic absorption spectrometry (HG-AAS) for As speciation in rice milk, respectively. These techniques, despite the lower cost and enable the determination of iAs, they are not suitable for determination of DMA and MMA in rice milk due to the lack of sensitivity (in the case of LC-AFS) or inability to quantify them (in the case of HG-AAS).

For reliable speciation analysis using LC-ICP-MS the sample should be submitted to mild treatment conditions; with water or diluted acid combined with short periods of sonication and/or heating in order to avoid species interconversion. Meharg et al., 2008 and Munera-Picazo et al. (2014) used organic solvent for As species extraction from rice milk. In this case several steps were involved, requiring more time for the analysis and are more susceptible to errors. In the present study it is developed a method for As speciation analysis in rice milk by simply diluting the sample in the mobile phase. The As speciation method by means of LC-ICP-MS is proposed by considering (i) the increasing consumption of rice milk, (ii) the presence of As species in such milk and (iii) the usual complexity of speciation analysis. Therefore, it is proposed a more simple method with respect to sample preparation and analysis time.

2. Experimental

2.1. Instrumentation

An inductively coupled plasma mass spectrometer (PerkinElmer Sciex, Model Elan DRC II, Thornhill, Canada) equipped with a concentric nebulizer (Meinhard Associates, Golden, USA), a cyclonic spray chamber (Glass Expansion, Inc., West Melbourne, Australia) and a quartz torch with a quartz injector tube (2 mm i.d.) was employed for As determination. The carrier gas flow rate, ion lens voltage and torch alignment were adjusted according to the spectrometer manufacturer instructions, using conventional pneumatic nebulization. Single ion monitoring at m/z 75 was used to collect the data.

The LC system consisted of a quaternary pump (PerkinElmer, Model Series 200) equipped with a Rheodyne six-port injector valve and an anion exchange separation column (Hamilton, PRP-X100, 250 × 4.1 mm i.d., Bonaduz, Switzerland). The mobile phase was transported from the separation column to the nebulizer by a PEEK-capillary. The LC-ICP-MS operational conditions were optimized according to a previous study (Moreira et al. (2011)). Air in the mobile phase was eliminated by sonication at 37 kHz in ultrasonic bath (Elmasonic, P 60 H, Singen, Germany) for 10 min at room temperature.

For total As (tAs) determination in the rice milk samples they were digested in closed quartz vessels in a microwave system (Milestone Srl., Model Ultrawave, Sorisole, Italy) equipped with a single reaction chamber (SRC). The operational conditions are cited in Table 1 and the heating program was run according to the microwave system manufacturer instructions.

2.2. Reagents and solutions

All chemicals were at least of analytical grade. Nitric acid (HNO_3 65% m m^{-1} , Merck, Darmstadt, Germany) purified by subboiling distillation (in a duoPUR distiller, Milestone) and distilled water further purified to resistivity of 18.2 $\text{M}\Omega\cdot\text{cm}$ (in a Milli-Q system, Millipore Corp., Billerica, USA) were used throughout the study. Stock solutions of As species were prepared in water, containing 1000 mg L^{-1} of As as

Table 1

Operational conditions of the microwave system and ICP-MS equipment for rice milk digestion and As measurement, respectively.

Microwave System	Settings
Pressure, bar	40
Ramp to 230 °C, min	10
Holding at 230 °C, min	20
ICP-MS	Settings
RF power, W	1300
Plasma gas flow rate, L min^{-1}	15
Auxiliary gas flow rate, L min^{-1}	1.2
Nebulizer gas flow rate, L min^{-1}	1.10
Sampler and skimmer cones	Pt
m/z monitored	75
Sweeps	5 (1 for LC-ICP-MS) [*]
Readings per replicate	5 (500 for LC-ICP-MS)
Replicates	3 (1 for LC-ICP-MS)
Dwell time, ms	25 (500 for LC-ICP-MS)

* Additional information about the LC-ICP-MS analysis is given in Table 2.

Table 2

Evaluation of the mobile phase for As speciation analysis in rice milk by LC-ICP-MS.

Parameters	Evaluated conditions			Selected
Mobile phase solution	$(\text{NH}_4)_2\text{HPO}_4$	$(\text{NH}_4)_2\text{CO}_3$	$(\text{NH}_4)_2\text{HPO}_4$	
Mobile phase concentration, mmol L^{-1}	9.0–11.0	9.0–30.0	9.0	
Mobile phase flow rate, mL min^{-1}	0.90–1.10	1.00–1.20	1.00	
Mobile phase pH	4.00–8.00	6.50–10.50	6.00	

dimethylarsenic acid (DMA – Sigma, USA), monomethylarsenic acid (MMA – Sigma), arsenite (As(III) – Riedel-de Haën, Germany), and arsenate (As(V) – Riedel-de Haën). These solutions were stored at 4 °C in the dark. A stock solution containing 10 mg L^{-1} of As (SCP 33MS, SCP Science, PlasmaCAL, Canada) was used in tAs determination by ICP-MS. Calibration solutions were prepared by serial dilution of the stock solutions. The As concentration in the calibration solutions ranged from 0.1 to 10 $\mu\text{g L}^{-1}$ and they were prepared in 5% (v v^{-1}) HNO_3 for tAs and in water or in the mobile phase for As speciation analysis. Monohydrogen phosphate ($(\text{NH}_4)_2\text{HPO}_4$, Merck) and $(\text{NH}_4)_2\text{CO}_3$ were evaluated as mobile phase. Solutions of these salts were prepared in water and then sonicated during 10 min in ultrasonic bath. The pH of the mobile phase and samples were adjusted by adding 1.0 mol L^{-1} ammonium hydroxide (NH_4OH) (Merck) or 1.0 mol L^{-1} HNO_3 .

2.3. Samples

Four samples of rice milk were acquired in local stores. Two of them were produced from polished rice and the other two from brown rice. One of the samples from brown rice contained coconut milk (4%) and natural flavour of coconut. The commercial samples (conditioned in tetra pack packaging) were identified as rice milk I, rice milk II, rice milk with coconut and wholegrain rice milk. They were manually shaken/homogenized before taking aliquots for As speciation analysis or digestion for tAs determination. All As determinations were at least in triplicate ($n = 3$).

2.4. Procedures

2.4.1. Total arsenic determination

Two millilitres of rice milk sample were transferred to quartz vessel to which 4 mL of HNO_3 were added. The vessel was closed, placed into the chamber of the microwave system and irradiated as informed in Table 1. After cooling, the digestate was transferred to a volumetric

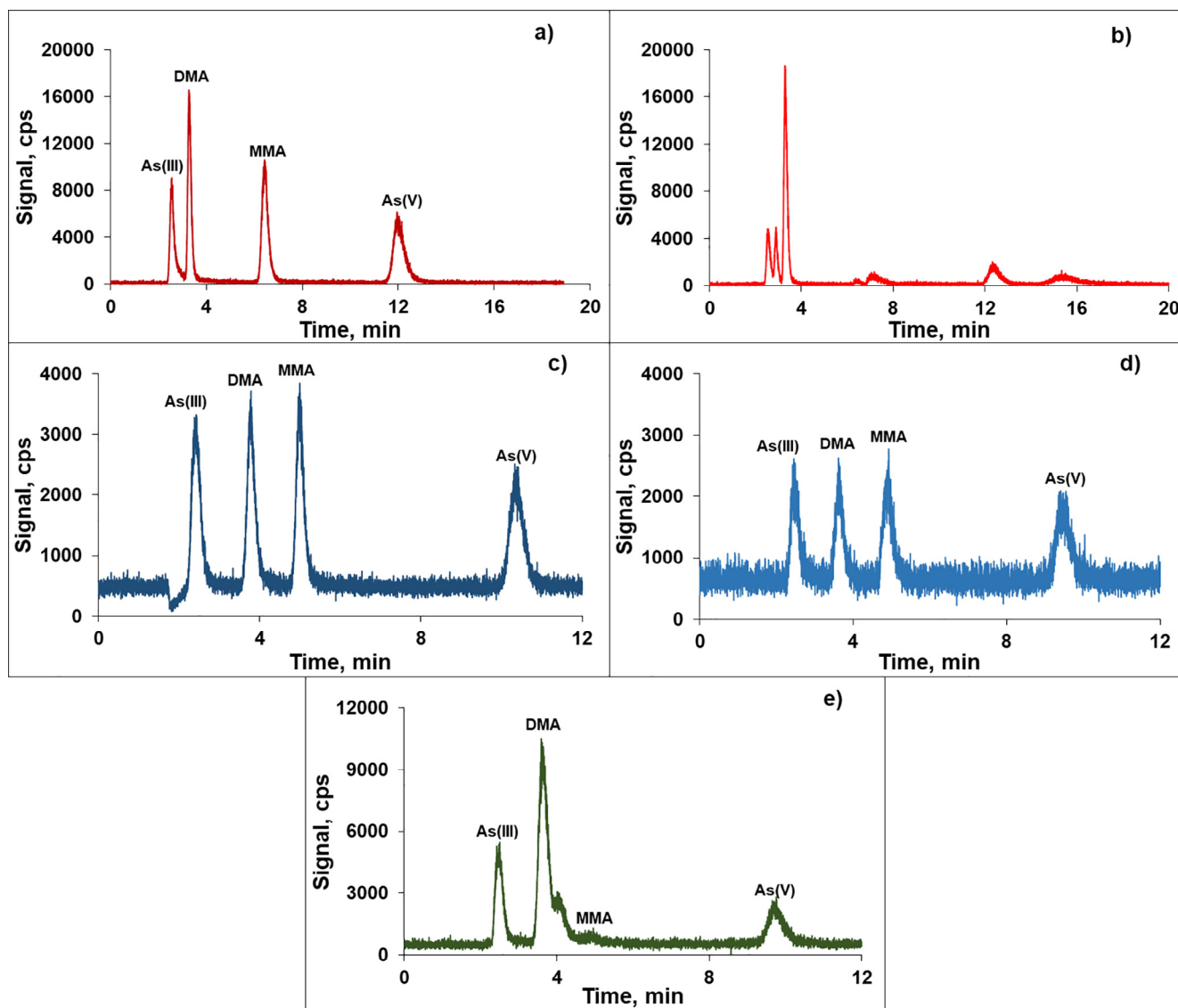


Fig. 1. Chromatograms of As species. (a): solution with $5.0 \mu\text{g L}^{-1}$ of each As species in water; (b) rice milk I diluted 1:2 in $30 \text{ mmol L}^{-1} (\text{NH}_4)_2\text{CO}_3$; (c) and (d): solutions with $1.0 \mu\text{g L}^{-1}$ of each As species prepared in water and $9.0 \text{ mmol L}^{-1} (\text{NH}_4)_2\text{HPO}_4$, respectively; and (e): rice milk I diluted 1:2 in $9.0 \text{ mmol L}^{-1} (\text{NH}_4)_2\text{HPO}_4$. In (a) and (b) the mobile phase was $30 \text{ mmol L}^{-1} (\text{NH}_4)_2\text{CO}_3$ at pH 8.50 while in (c), (d) and (e) the mobile phase was $9.0 \text{ mmol L}^{-1} (\text{NH}_4)_2\text{HPO}_4$ at pH 6.00.

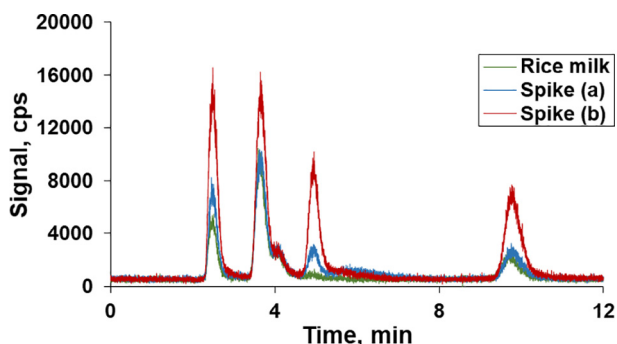


Fig. 2. Chromatograms for As species in rice milk diluted 1:2, without spike and spiked with $1.0 \mu\text{g L}^{-1}$ of As species in (a) and $5.0 \mu\text{g L}^{-1}$ of As species in (b). Chromatographic separation conditions are given in Table 2.

flask and diluted to 25 mL by adding water. Then, tAs concentration was measured by ICP-MS, at the conditions cited in Table 1.

2.4.2. Arsenic speciation analysis

Chromatographic conditions were firstly evaluated in order to achieve the best possible resolution at the shortest time, good sensitivity for all As species, low ICP disturbance and reduced carbon deposition on the ICP-MS instrument interface. All experiments were carried out using an anion exchange chromatographic column (PRP-X100, Hamilton) and isocratic elution mode. The chromatographic parameters were optimized using reference solutions containing $5.0 \mu\text{g L}^{-1}$ of As in the form of As(III), DMA, MMA, and As(V). These solutions were prepared in water or in the mobile phase medium. After achieving good separation for the As species in the test solutions, diluted samples were then tested and, if necessary, parameters were re-evaluated.

Before injection in the chromatographic column, samples were 3-fold or 5-fold diluted and filtered through a $0.45 \mu\text{m}$ pore size filter fitted in a syringe. Aliquots of $200 \mu\text{L}$ of diluted sample or As solution were injected in the chromatographic column and the obtained signals

Table 3

Analyte recovery in the spiked rice milk samples 3-fold diluted with the mobile phase. Concentration in $\mu\text{g L}^{-1}$ and chromatographic conditions according to Table 2.

Species	Rice Milk I			Wholegrain Rice Milk		
	Found in the Sample	Found in the Spiked Sample	Recovery, %	Found in the Sample	Found in the Spiked Sample	Recovery, %
As(III)	2.03	3.19 ^a 7.84 ^b	116 116	4.49	5.30 ^a 8.72 ^b	81 84
DMA	5.55	6.40 ^a 9.91 ^b	85 87	7.19	8.04 ^a 11.7 ^b	85 90
MMA	0.21	1.32 ^a 5.92 ^b	111 114	0.41	1.48 ^a 5.45 ^b	107 101
As(V)	1.40	2.30 ^a 6.09 ^b	90 94	2.33	3.29 ^a 7.37 ^b	96 101

^a Spiked to $1.00 \mu\text{g L}^{-1}$ As.

^b Spike to $5.00 \mu\text{g L}^{-1}$ As.

were processed in peak area mode. External calibration was carried out in the determination of As concentration. The accuracy of the As speciation method for rice milk was validated following Food and Drug Administration (FDA) guidelines (FDA, 2018). To this end, aliquots of 3-fold diluted rice milk were spiked with each investigated As species in order to obtain 1.0 or $5.0 \mu\text{g L}^{-1}$ of As. In addition, the sum of As species concentration found by LC-ICP-MS was compared with the tAs concentration determined by ICP-MS, after sample digestion. Standard addition calibration for two rice milk samples was also performed in order to compare the results obtained by external calibration.

The limit of detection (LOD) and limit of quantification (LOQ) were estimated following the 3s and 10s criterion, respectively, where s is the standard deviation of 10 consecutive analyte determinations in the blank. In LC-ICP-MS the mobile phase was considered as blank while HNO_3 treated in the same way as the sample was considered as blank in ICP-MS analysis. The rice milk sample dilution was taken into account to calculate the method LOD and LOQ.

3. Results and discussion

3.1. Arsenic speciation analysis

Tests were initially conducted in order to compare the performance

Table 4

Concentration of tAs determined by ICP-MS and As species by LC-ICP-MS. Concentrations informed are the mean and standard deviation (\pm SD) for three replicates of the sample ($n = 3$).

Sample	As, $\mu\text{g L}^{-1}$					
	As(III)	As(V)	DMA	MMA	Sum ^a	tAs ^b
Rice Milk I	6.03 ± 0.17	4.19 ± 0.04	16.64 ± 0.26	0.63 ± 0.18	27.49 ± 0.36	29.48 ± 1.23
Rice Milk II	2.46 ± 0.06	8.58 ± 0.18	2.37 ± 0.13	< LOD	13.42 ± 0.23	15.33 ± 1.10
Rice Milk with coconut	9.68 ± 0.35	2.87 ± 0.09	< LOD	< LOD	12.55 ± 0.37	14.57 ± 0.96
Wholegrain rice milk	12.48 ± 0.16	6.99 ± 0.02	22.57 ± 0.22	1.24 ± 0.21	43.28 ± 0.33	46.94 ± 1.96
Other works						
Munera-Picazo (2014) ^c	7.3–17.2	< 4	< 4	< 4	7.3–17.2	7.3–19.9
Pedron (2016) ^c	16.8 ± 2.1 – 26.6 ± 9.6 ^d		< LOD – 37.4 ± 1.1 ^e		–	16.4 ± 0.4 – 57.0 ± 3.2
Meharg (2008)	7.1–20.7 ^d		1.1–12.7	< 0.1–0.82	8.0–32.9	10.2–33.2

^a Sum of As(III), As(V), DMA and MMA concentrations.

^b Arsenic concentration determined by ICP-MS after sample digestion.

^c Arsenic concentration in $\mu\text{g kg}^{-1}$.

^d Sum of As(III) and As(V).

^e Sum of DMA and MMA.

of two common mobile phases used for As speciation by LC-ICP-MS. In this way, $(\text{NH}_4)_2\text{HPO}_4$ and $(\text{NH}_4)_2\text{CO}_3$ solutions at different concentrations, flow rate and pH were evaluated as can be observed in Table 2. Solutions with concentration higher than 11 and 30 mmol L^{-1} of $(\text{NH}_4)_2\text{HPO}_4$ and $(\text{NH}_4)_2\text{CO}_3$ were not tested in order to prevent salt deposition on the interface and ion lens of the ICP-MS instrument, following previous studies (Moreira et al., 2011; Pizarro, Gómez, Palacios, & Cámara, 2003).

It was observed that the separation of As(III) and DMA was not satisfactory when $(\text{NH}_4)_2\text{CO}_3$ was the mobile phase, regardless of its concentration, pH and flow rate (Fig. 1a, b). Better separation of the As species was achieved using $(\text{NH}_4)_2\text{HPO}_4$ as mobile phase at the selected conditions cited in Table 2, as shown in Fig. 1c. These results corroborate those obtained in previous studies about As speciation using LC-ICP-MS, with respect to type of mobile phase, its concentration and pH (Moreira et al., 2011; Pizarro et al., 2003). Through mobile phase optimization, the As species were separated in 10 min, which is an advantage of the proposed method. Good As species separation at pH 6.00 was also observed in a previous study when an anion exchange column PRP-X100 and phosphate solution as mobile phase were used for As speciation by LC-ICP-MS (Wang et al., 2015).

Despite the good separation of the As species, drift of the signal was observed at the beginning of the chromatogram (at 1.8 min in Fig. 1c), making difficult the signal processing and worsening the LOD. This drawback was circumvented by preparing the As species in the mobile phase, as can be observed in Fig. 2d, e.

3.2. Arsenic speciation in rice milk

Arsenic speciation analysis in complex and organic matrices such as rice milk by LC-ICP-MS may require sample preparation in several steps, which is time consuming, prone to contamination and analyte loss. The method proposed by Meharg et al. (2008) using LC-ICP-MS for As speciation in rice milk involved the use of hexane and HNO_3 and clean up of the sample. Despite the satisfactory results obtained, the method is laborious for routine analysis and involves use of organic solvent, which is currently considered not “ecologically friendly”. Pedron et al. (2016) extracted As species in rice milk samples by leaving them in contact with $2\% \text{ v v}^{-1}$ HNO_3 and heating at 95°C for 2.5 h. Munera-Picazo et al. (2014) prepared the samples by adding 2 mol L^{-1} of trifluoroacetic acid (TFA) and keeping the mixture at 100°C for 6 h, followed by centrifugation and filtration. Not using organic solvent is an advantage, but these two procedures had been previously optimized for As speciation in rice and were not evaluated for rice milk, whether there was interconversion of As species in rice milk under heating and

in presence of HNO₃ or TFA. Huang, Hu, Ilgen, and Ilgenet (2012) proposed a method of As speciation in alcoholic rice beverage by simply diluting the sample in the mobile phase (10 mmol L⁻¹ ammonium phosphate at pH 6.00) prior analysis using LC-ICP-MS. However, some signals in the chromatogram could not be identified. In the present study, unidentified signals were not observed at the selected conditions (Table 2) for rice milk 3-fold and 5-fold diluted in the mobile phase. Samples less diluted were not analysed in order to avoid possible interferences by carbon and chloride (Duarte et al., 2007). Considering the low As species concentration and matrix effects the rice milk samples were 3-fold diluted for further analysis.

The four As species investigated were determined in rice milk samples following external calibration; using the chromatographic conditions shown in Table 2. The calibration curves parameters for As(III), DMA, MMA and As(V) were $y = 530.9x + 70.1$ (R²: 0.9994), $y = 562.6x + 88.8$ (R²: 0.9995), $y = 647.0x + 85.3$ (R²: 0.9999), $y = 567.5x + 103.1$ (R²: 0.9993), respectively, where y = counts and x = $\mu\text{g L}^{-1}$ As. As can be seen, the sensitivity differs by about 10% for the As species, which can be considered satisfactory and within the acceptable variation range. The linear correlation coefficient was almost similar for all As species.

By employing the optimized conditions, the LOD for As(III), DMA, MMA and As(V) were 0.10, 0.11, 0.08 and 0.13 $\mu\text{g L}^{-1}$ As, respectively. The LOQ for As(III), DMA, MMA and As(V) were 0.32, 0.37, 0.25 and 0.43 $\mu\text{g L}^{-1}$ As, respectively. The LOQ is sufficiently low for the determination of most of the investigated As species in the analyzed samples.

In order to verify the accuracy of the proposed method, analyte recovery tests were then carried out. As can be seen in Table 3, analyte recoveries ranged from 81 to 116%, which can be considered a good result for the analysed rice milk whose matrix is complex. The analyte recovery is also demonstrated in Fig. 2 that shows the chromatograms for a sample of rice milk and the same sample spiked with the investigated As species. Method accuracy was also evaluated by comparing the tAs concentration with the sum of As species (Table 4). According to Table 4, the sum of As species are slightly lower than the tAs concentration determined by ICP-MS after sample digestion. However, according to the *t*-test at the confidence level of 95% they are not statistically different. It is believed that the small and systematic bias is due to losses that occur during separation in the chromatographic column (Michalke, 2002). The lower results could also be due to retention of As species within the insoluble filtering residue.

In addition, the As species concentrations determined in two rice milk samples by means of external calibration and standard addition calibration were similar according to the *t*-test at a 95% confidence level, revealing that matrix interference was very low or absent for the 3-fold diluted rice milk.

In Brazil there is still no legislation for As species in rice and rice-based products. The Brazilian legislation (ANVISA, 2013) establishes only the maximum tAs concentration allowed in rice based-products, which is 0.3 mg kg⁻¹. Considering that 1.0 kg of rice milk is about 1.0 L of the product, the tAs concentration in the analysed samples satisfies the Brazilian legislation. On the other hand, it might not meet the WHO (2010) recommendations with respect to iAs; the maximum iAs daily intake recommended is 2 $\mu\text{g kg}^{-1}$ body weight. Thus, the maximum daily intake of iAs should be only 10 μg by an infant weighing 5 kg, which could be achieved or even surpassed by consuming 1 L of rice milk (Table 4) per day.

The tAs and As species concentrations in the samples varied (Table 3) in view of the type of rice used for preparing the milk and other components of the formulation. Milk from brown rice (rice milk with coconut and wholegrain rice milk) contains more As than rice milk I and rice milk II that were obtained from polished rice. This can be explained by the higher concentration of As in the bran that remains in the grain of brown rice. Santos et al. (2017) found that iAs is in general the predominant As form in rice milk, following the same pattern

observed for rice. The mean concentration of iAs (As(III) + As(V)) found in the present study ranged from 10.19 to 19.47 $\mu\text{g L}^{-1}$, which is close to that found by Munera-Picazo et al. (2014) Meharg et al. (2008). Pedron et al. (2016) found higher concentration of iAs in rice milk from Italy. The higher concentration in rice milk was attributed to Italian soil that contains more As.

4. Conclusions

The proposed method allows determination of As(III), As(V), DMA and MMA in rice milk by simply diluting the sample in the mobile phase, followed by filtration. In this way, organic solvents and oxidizing/reducing reagents that may cause As species interconversion, are not necessary, making the sample preparation more simple. The four As species investigated in rice milk can be efficiently determined using LC-ICP-MS, following external calibration. Analyte recovery test and standard addition calibration confirmed that the method is free from matrix interferences. The sum of the As concentration species determined by LC-ICP-MS agreed well with the tAs concentration determined by ICP-MS after sample decomposition, that also highlighted the accuracy of the proposed method.

Given the good sample throughput, fast and ease sample preparation, the method could be adopted by regulatory agencies for routine quality control of As in rice milk, mainly iAs that is considered more toxic.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgements

Authors are gratefully to CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico (Processes 401135/2016-0; 480929/2011-4; 458004/2014-6; 308745/2013-2) and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for financial support and scholarships.

References

- Althobiti, R. A., Sadiq, N. W., & Beauchemin, B. (2018). Realistic risk assessment of arsenic in rice. *Food Chemistry*, 257, 230–236.
- ANVISA, Agência Nacional de Vigilância Sanitária (2013). *Maximum limits of inorganics contaminants in food. Resolução RDC n° 42. Brazil.*
- Commission Regulation (EU) 2015/1006 of 25 June 2015 amending Regulation (EC) No 1881/2006 as regards maximum levels of inorganic arsenic in foodstuffs. Official Journal of the European Union. L 161/14-16.
- Dreborg, S. (2015). Debates in allergy medicine: Food intolerance does not exist. *World Allergy Organization Journal*, 8, 37–42.
- Duarte, F. A., Pereira, J. S. F., Mesko, M. F., Goldschmidt, F., Flores, E. M. M., & Dressler, V. L. (2007). Evaluation of liquid chromatography inductively coupled plasma mass spectrometry for arsenic speciation in water from industrial treatment of shale. *Spectrochimica Acta Part B*, 62, 978–984.
- FDA (2018). *Elemental analysis manual (EAM) for food and related products*. Updated on 02/27/2018 <https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006954.htm>.
- Fernández, E. F., Hernández, J. A. M., Suárez, V. M., Villares, J. M. M., Yurrita, L. R. C., Cabria, M. H., & Rey, F. J. M. (2015). Consensus document: Nutritional and metabolic importance of cow's milk. *Nutrición Hospitalaria*, 31(1), 92–101.
- Huang, J.-H., Fecher, P., Ilgen, G., Hu, K.-N., & Yang, J. (2012). Speciation of arsenite and arsenate in rice grain – Verification of nitric acid based extraction method and mass sample survey. *Food Chemistry*, 130, 453–459.
- Huang, J.-H., Hu, K.-N., Ilgen, J., & Ilgenet, G. (2012). Occurrence and stability of inorganic and organic arsenic species in wines, rice wines and beers from Central European market. *Food Additives and Contaminants Part A – Chemistry Analysis Control Exposure & Risk Assessment*, 29(1), 85–93.
- Hughes, M. F., Beck, B. D., Chen, Y., Lewis, A. S., & Thomas, D. J. (2011). Arsenic exposure and toxicology: A historical perspective. *Toxicological Sciences*, 123(2), 305–332.
- Meharg, A. A., Deacon, C., Campbell, R. C., Carey, A. M., Williams, P. N., Feldmann, J., & Raab, A. (2008). Inorganic arsenic levels in rice milk exceed EU and US drinking water standards. *Journal of Environmental Monitoring*, 10(4), 428–431.
- Michalke, B. (2002). The coupling of LC to ICP-MS in element speciation: I. General

- aspects. *TrAC Trends in Analytical Chemistry*, 21(2), 142–153.
- Moreira, C. L., Duarte, F. A., Leberher, J., Pozebon, D., Flores, E. M. M., & Dressler, V. L. (2011). Arsenic speciation in white wine by LC-ICP-MS. *Food Chemistry*, 126(3), 1406–1411.
- Munera-Picazo, S., Burló, F., & Carbonell-Barrachina, Á. A. (2014). Arsenic speciation in rice-based food for adults with celiac disease. *Food Additives & Contaminants: Part A: Chemistry, Analysis, Control, Exposure & Risk Assessment*, 31(8), 1358–1366.
- Pedron, T., Segura, F. R., Silva, F. F., Souza, A. L., Malteza, H. F., & Batista, B. L. (2016). Essential and nonessential elements in Brazilian infant food and other ricebased products frequently consumed by children and celiac population. *Journal of Food Composition Analysis*, 49, 78–86.
- Pizarro, I., Gómez, M., Palacios, M. A., & Cámara, C. (2003). Evaluation of stability of arsenic species in rice. *Analytical and Bioanalytical Chemistry*, 376(1), 102–109.
- Santos, G. M., Pozebon, D., Cerveira, C., & Moraes, D. P. (2017). Inorganic arsenic speciation in rice products using selective hydride generation and atomic absorption spectrometry (AAS). *Microchemical Journal*, 133, 265–271.
- Signes-Pastor, A. J., Carey, M., & Meharg, A. A. (2016). Inorganic arsenic in rice-based products for infants and young children. *Food Chemistry*, 191, 128–134.
- Wang, X., Peng, B., Tan, C., Ma, L., & Rathinasabapathi, B. (2015). Recent advances in arsenic bioavailability, transport, and speciation in rice. *Environmental Science and Pollution Research*, 22(8), 5742–5750.
- WHO technical series No. 956. World Health Organization/Joint FAO/WHO Expert Committee on Food Additives, Geneva, Switzerland. JECFA. 2010. Evaluation of certain food additives and contaminants: Seventy first report of the joint FAO/WHO 390 expert committee on food additives.