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Arsenic removal from rice by washing and cooking with water

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Abstract

Two Hungarian and one Chinese rice samples were selected in order to establish the extractable arsenic content by washing and cooking in water in a ratio of 6:1, water:rice (cm^3:g) by inductively coupled plasma sector field mass spectrometry (ICP-SF-MS). Total arsenic concentration of the Zhenshan 97, Risabell and Kőröstáj-333 samples were 171.3 ± 7.1 ng g^{-1}, 116.0 ± 3.7 ng g^{-1} and 139.0 ± 6.1 ng g^{-1}, respectively, which did not exceed the toxic limits established for As in Hungary (0.3 μg g^{-1}) or in China (0.7 μg g^{-1}). The predominant chemical form of As in the raw rice samples determined by on-line high performance liquid chromatography and ICP-MS was arsenite. Moreover, enzymatic hydrolysis with α-amylase + protease and microprobe focused sonication proved that arsenite could be removed in the highest extent by washing and cooking, meanwhile the main As form remaining in the cooked rice was As(V). Thus, it is recommended to prepare rice-containing dishes in abundant water, which should be discarded after washing and cooking. The results were validated with a NIST SRM 1568a.

Keywords: Arsenic; Rice; HPLC; ICP-MS; Washing; Cooking

1. Introduction

Great attention is to be paid for the chemical composition levels in rice, which is a staple foodstuff most consumed world-wide. According to the Food and Agriculture Organisation (FAO), the People's Republic of China was the biggest rice producer in the world in 2003, followed by India, Indonesia, Bangladesh and Vietnam. Lately, from these countries, groundwater has been reported to be highly contaminated with arsenic (Abedin, Feldmann, & Meharg, 2002). Hungary also has arsenic contaminated groundwater of geological origin in its south-eastern part, used intensively for agricultural purposes (Varsányi, Fodre, & Bartha, 1991). Inorganic arsenic is predominant in soils and ground waters, As(V) in aerobic and As(III) in anoxic conditions (Abedin et al., 2002). Arsenic methylation can occur in soil systems due to microbial activity (Takamatsu, Aoki, & Yoshida, 1982). The main route for arsenic bioavailability for humans is the chain food (via dietary intake and drinking water) (Gallagher, Wei, Shoemaker, Brockhoff, & Creed, 1999; Tao & Bolger, 1999). The average As concentration in terrestrial vegetables can reach 20 ng g^{-1} (Schoof et al., 1999); however, arsenic concentration in rice can reach even 280 ng g^{-1} (D'Amato, Forte, & Caroli, 2004), which proves relatively high uptake and translocation of arsenic in rice.

The predominant arsenic species found in rice are: arsenite [As(III)], arsenate [As(V)], dimethyl arsinic acid (DMA) and monomethyl arsonic acid (MMA) (D'Amato et al., 2004; Heitkemper et al., 2001; Pizarro et al., 2003a;

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Pizarro et al., 2003b; Sanz et al., 2005). It is well-known that the inorganic forms are more toxic than organoarsenicals like arsenobetaine (AsB) and arsenocholine (AsC); however, it has been reported that DMA and MMA can also act as cancer promoters (Brown, Kitchin, & George, 1997). In the past, MMA and DMA were extensively used as pesticides in cotton lands (Odanaka, Tsuchiya, Matano, & Goto, 1985).

Arsenic speciation can be performed in rice seedlings (Abedin, Cresser, Meharg, Feldmann, & Cotter-Howells, 2002) as well as in grains (D'Amato et al., 2004; Heitkemper et al., 2001; Pizarro et al., 2003a, 2003b; Sanz et al., 2005a; Sanz, Muñoz-Olivas, & Cámara, 2005b); however, extraction procedures have to be applied prior to chromatographic separation, usually solvent or enzymatic extractions. Extraction with methanol:water mixture 1:1 v/v followed by sonication resulted in a recovery of 76% of the certified value for the SRM 1568a; however, the extraction efficiency for the rice samples ranged from 31% to 72% for different white and brown grain rice samples (Heitkemper et al., 2001). The authors suspected that the particle size of the rice samples might be responsible, but the samples were ground and prepared in the same manner. By extracting arsenic species from freeze-dried rice prepared at the Institute for Reference Materials and Measurements (IRMM) of Geel (Belgium), with water or different methanol:water mixtures (1:1 v/v, 9:1 v/v, and 1:1 v/v and subsequently with 9:1 v/v mixture), Pizarro et al. (2003b) established that about 80% of the total arsenic could be extracted with methanol:water 1:1 v/v in the first run.

Accelerated solvent extraction (ASE) is similar to the conventional solid–liquid extraction, but it is generally performed at elevated temperatures and pressures, and thus, it has shorter extraction demand time. However, depending on the sample size, type of solvent, temperature and pressure values used, ASE causes swelling of the rice samples (Heitkemper et al., 2001). Nevertheless, it has been successfully applied for arsenic extraction from marine samples like fish (McKiernan, Creed, Brockhoff, Caruso, & Lorenzana, 1999) and ribbon kelp (Gallagher et al., 1999).

Up to the present, the main disadvantage of the enzymatic extraction procedures has been that they are relatively slow processes, which can give place to internal species conversion (Caruso, Heitkemper, & B'Hymer, 2001). Due to its capability to hydrolyse starch, α-amylase was also used by Heitkemper et al. (2001), for the extraction of arsenic from rice samples prior to extraction with methanol:water (1:1 v/v) mixture. An extraction efficiency of 59% was achieved for the white rice grain sample and 97% for SRM 1568a. However, the best results were achieved by using trifluoroacetic acid (TFA), but partial reduction of As(V) to As(III) was observed during the extraction process. The reason for the reduction of arsenate to arsenite by TFA may be due to shift in the redox potential by acidification. Moreover, Pizarro et al. (2003b) discarded the use of TFA for the determination of arsenic species with HPLC-ICP-MS from rice, because it provided poor chromatographic resolution for the extracted arsenic species.

Recently, microprobe focused sonication combined with enzymatic hydrolysis has been successfully used for extraction of arsenic in rice grains for speciation studies (Sanz et al., 2005a) as well as for other biological samples (Brown et al., 1997; Capelo, Ximénez-Embún, Madrid-Albarán, & Cámara, 2004; Gallego-Gallegos, Liva, Olivas, & Cámara, 2005). The stress caused by the passing of a sound wave through the liquid generates bubbles, which continuously compress and decompress, enlarge the extraction surface area, enhance mixing and extraction; thus it is a rapid technique.

Up to now, the majority of the reports has been focusing on chemical speciation in rice flour but rice is hardly consumed in this form. Little is known about the leaching of As from rice prior to consumption. Moreover, the food preparation traditions vary with cultures. For example, in Hungary, rice is generally cooked with an excessive amount of water and the water remaining is discarded; on the other hand, in China, rice is generally cooked with aliquots of water in order to absorb it all. However, recently, arsenic speciation in rice cooked with arsenic contaminated drinking and reagent water in ratios ranging from 1:1 to 4:1 has been reported (Ackerman et al., 2005), proving that rice grains are capable of arsenic absorption from water during cooking. Similar results were obtained by Bae et al. (2002) determining As in home cooked rice in Bangladesh, however, in this case arsenic speciation could not be performed.

Considering the literature data, it can be stated that information on arsenic speciation by HPLC-ICP-MS from a practical point of view (i.e., arsenic availability by washing and cooking) is missing. Thus, the objectives of the present study were: (i) determination of total As content in a selected Chinese rice sample by ICP-MS; (ii) determination of the extent of As removal by extraction with cold and hot water simulating washing and cooking of rice in abundant water; (iii) chemical speciation of As in the washing and cooking water extracts by HPLC-ICP-MS.

2. Experimental

2.1. Instrumentation and operating conditions

Prior to the total As determination, rice samples were digested in an Anton Paar microwave-assisted (MW) digestion system (Anton Paar, Austria). Rice samples were ground in a Moulinex AY47R grinder.

Freeze-drying was performed in a ThermoSavant MicroModulyo instrument (Life Sciences, USA). The sonication of the samples subjected to enzyme-assisted extraction before the HPLC analyses was achieved in an UP50H ultrasonic processor (Hielscher, Germany) equipped with a 3 mm Ø Ti microtip sonotrode.

For total As determination as well as for As speciation, an Element2 inductively coupled plasma sector field mass spectrometer (ICP-SF-MS) instrument (Thermo Finnigan, Germany) was used. The operating conditions of the ICP-SF-MS instrument were the following: the RF power was set to 1200 W. The carrier, the auxiliary and the nebuliser argon gas flow rate were 16.0, 0.8 and 1.1 $dm^3\ min^{-1}$, respectively. Meinhard nebuliser was used for nebulisation. The sampler and skimmer cones, with orifice diameter of 1.0 and 0.4 mm, respectively, were made of Ni. High resolution ($R = 10{,}000$) was used for total As determination. Peak area integration mode was applied for the calculations of the results.

For the chromatographic separations, an HPLC system (GBC, Australia) consisting of a solvent delivery unit (model LC 1140) and an HPLC pump (model LC 1150) was coupled to the ICP-SF-MS instrument operating in low resolution mode ($R = 300$). A Hamilton 10 μm PRP-X100 column of 250 × 4.1 mm equipped with a pre-column of 4.6 × 4.1 mm and 20 mmol dm^{-3} $NH_4H_2PO_4$ (pH 5.6 with NH_3) as mobile phase were used for the anion-chromatographic measurements. If necessary, a Supelcosil 10 μm LC SCX-100 250 × 4.1 mm column equipped with a pre-column of 4.6 × 4.1 mm and 25 mmol dm^{-3} pyridine (pH 2.7 with formic acid) as mobile phase were used for the cation-chromatographic measurements. In both cases isocratic elution was used. Flow rate was 1.5 $cm^3\ min^{-1}$. The injection volume was 20 μl. Chromatographic separations were performed at 22 °C.

2.2. Materials, reagents and standards

About 5 kg of a long-grain Chinese rice (*Oryza sativa* L ssp. indica), named Zhenshan 97, supplied by the School of Environmental Studies of the China Univesity of Geosciences of Wuhan, Hubei province, situated in the southeastern part of China, were transported to the ICP-MS laboratory of L. Eötvös University (Hungary) and stored in the dark at room temperature. Hungarian rice samples, namely Risabell (*Oryza sativa* L. ssp. indica), also long-grained type and Kőröstáj-333 (*Oryza sativa* L. ssp. japonica), round-grained rice, were cultivated in the experimental field of the Agricultural High School of Szarvas, situated in the Hungarian Great Plain. In this region, the water of the river Kőrös is used for irrigation, which brings dissolved As from the Western Carpathian Mountains of the neighbouring Romania.

Throughout the experiments deionised Milli Q water, Suprapur HNO_3 and H_2O_2 (Merck) were used. For total As measurements, As and Ge (as internal standard) Merck stock solutions were used in concentration of 1 mg cm^{-3} and diluted with Milli Q water and HNO_3 solution to have a 5% acid concentration.

For speciation analyses, the As(III) stock solution in concentration of 0.1 mol dm^{-3} was prepared from arsenic(III) trioxide (Sigma Aldrich) by its dissolution in 1 mol dm^{-3} KOH followed by acidification with 1 mol dm^{-3} HCl similarly to Quaghebeur, Rengel, and Smirk (2003), and then it was kept at 4 °C. The As(V) stock solution was prepared from potassium dihydrogen arsenate (Sigma Aldrich) and acidified with HNO_3 in order to have a 5% acid concentration. DMA was used as cacodylic acid (Fluka), and MMA was prepared by dilution of Strychnotonin injection (Chinoin, Hungary) containing 50 mg disodium salt of MMA dissolved in 1 cm^3 water. The arsenobetaine (AsB) and arsenocholine (AsC) standards (Argus Chemicals, Italy) were dissolved in Milli Q water. Daily dilutions of the stock solutions for the speciation analyses were made with Milli Q water. These individual stock solutions in concentration of 1 mg cm^{-3} were stored at 4 °C.

In order to check the accuracy of the analytical methods, a (National Institute of Standard and Technology) NIST SRM 1568a rice flour sample was used. For the enzyme-assisted extractions, α-amylase from *Bacillus subtilis* and protease Type XIV from *Streptomyceus griseus* were purchased from Sigma Aldrich Ltd. (Hungary).

Ammonium dihydrogen phosphate and ammonia used for preparation of the mobile phase for anion-exchange chromatography, as well as used for total arsenic determination, was of Merck Suprapur quality. HPLC grade pyridine and formic acid for cation-exchange chromatography were purchased from Merck.

A Merck multielement solution of 1 ng cm^{-3} was used for tuning and mass calibration of the ICP-SF-MS instrument. Before the HPLC analysis, the samples were filtered through Millex-GV (Millipore, USA) PVDF filters with a pore size of 0.22 μm.

2.3. Procedures

2.3.1. Analytical procedures applied for determination of total concentration of arsenic

For determination of the total As content of the rice samples, 10 g of the rice grains were ground for four minutes. In case of the NIST SRM 1568a sample, milling was not necessary. From the (ground) samples, 0.25 g were placed into quartz bombs. Then, a mixture containing 1 cm^3 deionised water, 5 cm^3 HNO_3 (purified by subboiling distillation) and 1 cm^3 30% H_2O_2 was added to them. The vessels containing the samples were closed and placed into the microwave oven after the effervescence ceased (~20 min) and digested in two cycles according to the literature data (D'Amato et al., 2004). Six quartz bombs were used simultaneously. After digestion, samples were filled up to 10 cm^3 and diluted 9 times before the ICP-MS analysis. Germanium (in concentration of 10 ng cm^{-3}) was chosen as internal standard in accordance with literature data (Pizarro et al., 2003a). The working range of the Ge containing As standards was between 0.1 and 5 ng cm^{-3}. The final HNO_3 concentration of the digestates as well as of the calibrating solutions was 5%. In order to determine the moisture content of the rice sample, adequate amounts were dried at 95 °C for 48 h in a temperature controlled oven.

2.3.2. Extraction of arsenic species

2.3.2.1. Water extraction. For each water extraction procedure, 50 g of rice were used. Schematic representation of the sample preparation can be seen in Fig. 1. Rice grains were washed three times with 100 cm^3 deionised water at 22 °C, then decantated. About 200 cm^3 of this decantate were freeze-dried. For the total As determination, about 0.25 g of the resulted starch was used, while the rest underwent to speciation analysis.

The wet grains were also boiled with 300 cm^3 deionised water for 15 min. The decantate resulted after boiling was freeze-dried. The resulted starch was used for total as well as for speciation analysis. Adequate amounts of the cooked rice were dried in an oven at 95 °C and subjected to total As determination as well as to speciation analyses after grinding.

2.3.2.2. Enzyme-assisted extraction. Raw and cooked ground rice samples, cold and hot water lyophilizates as well as a NIST SRM 1568a rice flour were enzymatically extracted by microprobe focused sonication with a method described by Sanz et al. (2005a). The procedure was applied in triplicate for each sample. The resulted supernatants were passed through 0.22 μm pore size filters before the HPLC-ICP-SF-MS measurements.

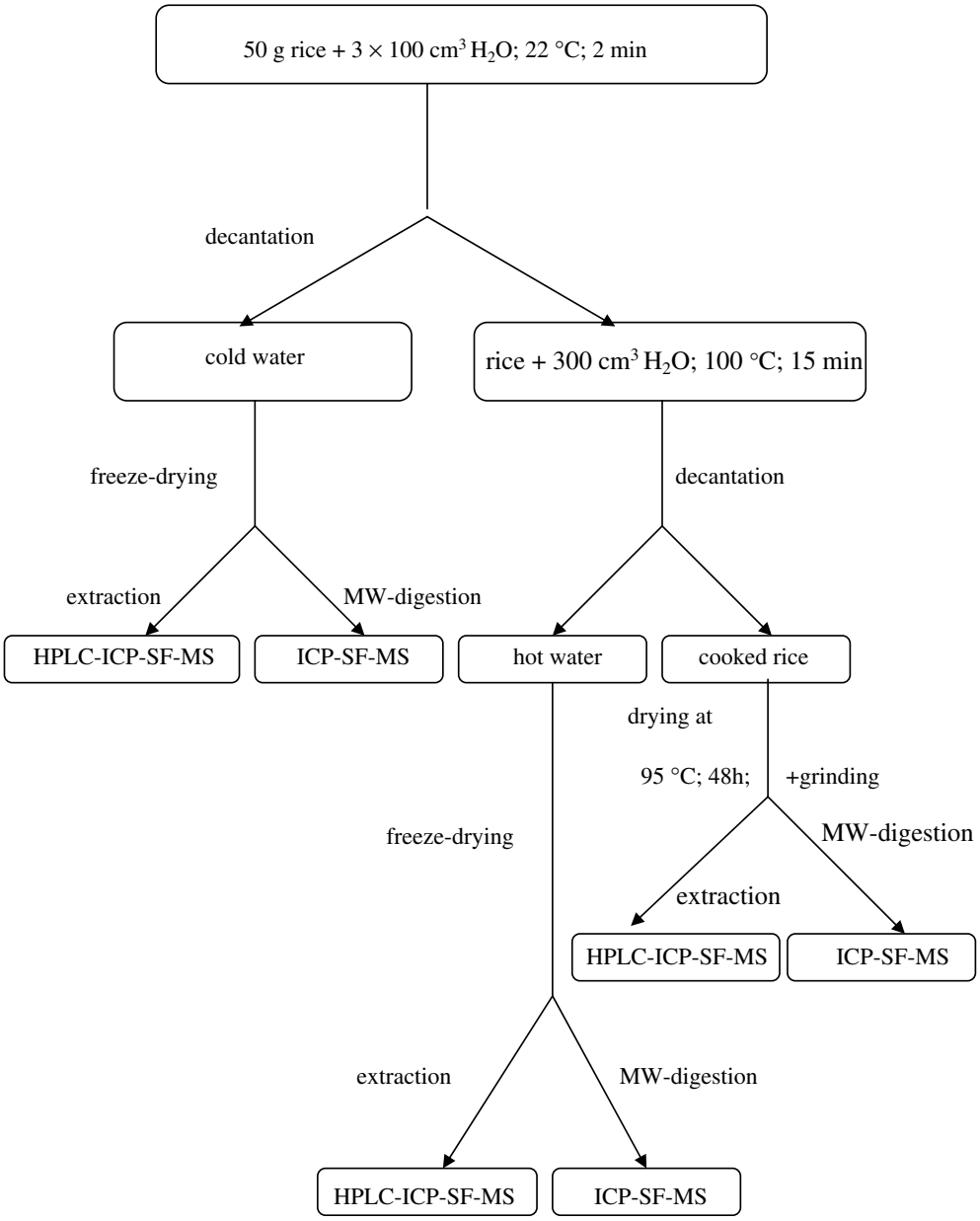

Fig. 1. Schematic representation of the procedures applied for washing and cooking of rice samples with water in a ratio of 6:1 water:rice (cm^3:g). The extraction step corresponds to an enzyme-assisted extraction with α-amylase from *Bacilus subtilis* and protease Type XIV from *Streptomyces griseus* applying microprobe sonication followed by centrifugation (10 min, 4500 rpm). The extraction procedure was repeated two times.

2.3.3. HPLC-ICP-SF-MS measurements

The As species of the investigated samples were separated isocratically on an anion exchange column and measured by on-line coupling of the HPLC device to the ICP-SF-MS instrument according to Quaghebeur et al. (2003), with the only difference that the column temperature was 22 °C. If necessary, the concentrations of AsB were calculated by cation exchange chromatographic measurements with a previously described method (Kuehnelt, Goessler, Schlagenhaufen, & Irgolic, 1997). The working range of the corresponding multispecies calibration standards was between 1 and 15 ng cm^{-3}. The drift in the ICP-MS response was corrected by injection of a multispecies standard after every three chromatographic runs. All extracts were analysed on the day of the extraction.

3. Results and discussion

3.1. Evaluation of arsenic removal by cold and hot water extraction

For the investigations, two white (Zhenshan 97 and Risabell) and one brown (Kőröstáj-333) rice samples were used. The samples belong to the long-grain category, which is very popular in Europe. Moreover, for the selection of the Hungarian rice samples, special attention was paid so that the samples should originate from the south-western region of the country, also affected by natural As contamination. Taking into consideration the water moisture of the investigated rice samples (ranging between 9.34% and 11.25%), the total arsenic concentration of the Zhenshan 97, Risabell and Kőröstáj-333 samples amounted to 171.3 ± 7.1 ng g^{-1}, 116.0 ± 3.7 ng g^{-1} and 139.0 ± 6.1 ng g^{-1}, respectively. These values do not exceed the toxic limits established for As in Hungary (0.3 μg g^{-1}) or China (0.7 μg g^{-1}). The As concentrations obtained for the investigated samples were within the concentration range already reported (D'Amato et al., 2004). Additionally, washing and cooking of the rice grains with deionised water were further investigated in order to determine the removable proportion of As by these everyday processes. Fifteen minutes cooking time was chosen, which was necessary for the grains to be soften. This is in good agreement with the recommendation of the instant and long grain rice producers. Moreover, a mass balance could be established by performing the total As determination of the freeze-dried samples resulted after extraction with cold and hot water as well as of the raw and cooked (dried and then) ground samples. Procedural loss was not observed. Thus, the sum of the arsenic concentrations gave between 96% and 102% of the arsenic content of the raw ground rice samples. The accuracy of the analytical methods was checked with the total As determination of NIST SRM 1568a rice flour standard. The results as well as the percentages corresponding to the arsenic removal are listed in Table 1. According to the water extraction results, 8–17% and 29–42% of As could be removed from rice grains by washing and cooking, respectively. The arsenic removal by water extraction was independent on the fact the investigated samples would be white or brown. These results are in consistence with those obtained by Bae et al. (2002), who reported that 57–81% of As was retained in Bangladesh rice grains cooked in households, however, in that study, rice possibly absorbed arsenic from the water used for cooking.

Table 1
Arsenic distribution among water extracts and cooked rice compared to the total As concentration of the raw Chinese and Hungarian rice samples related to dry weight; SD = standard deviation ($n = 3$); $\sum$ = sum

Sample	$c_{As} \pm$ SD (ng g^{-1})	%
Zhenshan 97 (*Oryza sativa* var. Indica)		
Cold water extract[a]	12.6 ± 0.6	8[c]
Hot water extract[b]	48.1 ± 1.2	29[c]
Cooked rice	103.7 ± 4.7	63[c]
$\sum$ As	164.4	96[d]
Raw rice	171.3 ± 7.1	
Risabell (*Oryza sativa* var. Indica)		
Cold water extract[a]	17.2 ± 0.4	15[c]
Hot water extract[b]	31.3 ± 0.8	26[c]
Cooked rice	70.0 ± 2.6	59[c]
$\sum$ As	118.4	102[d]
Raw rice	116.0 ± 3.7	
Kőröstáj-333 (*Oryza sativa* var. Japonica)		
Cold water extract[a]	23.0 ± 1.5	17[c]
Hot water extract[b]	57.8 ± 4.1	41[c]
Cooked rice	58.9 ± 4.7	42[c]
$\sum$ As	139.7	101[d]
Raw rice	139.0 ± 6.1	
NIST 1568a (measured) in μg g^{-1}	0.294 ± 0.01	
NIST 1568a (certified value) in μg g^{-1}	0.290 ± 0.03	

[a] Water:rice = 6:1 (cm^3:g).
[b] Water:rice = 6:1 (cm^3:g), boiling for 15 min.
[c] Percentage calculated by relating the corresponding values to the sum of their concentration.
[d] Percentage value calculated by dividing the sum ($\sum$) with the digested total As concentration.

3.2. Arsenic speciation in the raw and cooked rice samples as well as in the cold and hot water extracts

The chromatograms of the ground raw rice samples subjected to enzyme-assisted As extraction arsenic and microprobe focused sonication are presented in Fig. 2. The As speciation analysis revealed that the main chemical form of As in the investigated ground raw rice samples was arsenite (Fig. 2), whose percentage in the sample amounted to roughly 50%, independently of the type of rice (white vs. brown). The other main arsenic compound in the samples was As(V), whose percentage varied between 25% and 33% in the samples. The percentage ranges for DMA ad AsC were between 8–10% and 6–10%, respectively. AsB could be determined only in case of the Chinese rice sample in about 5%. These percentages were calculated by relating the obtained values to the sum of species obtained with the chromatographic separation. The chromatographic recov-

ery calculated by dividing the sum of the species separated with the As concentration of the ground sample varied between 76% and 102%.

The main extractable chemical form of As in the freeze-dried samples remaining after washing and cooking with water was As(III), whose percentage ranged roughly between 40% and 70% and between 60% and 75% for the cold water extracts of the white rice samples and the hot water extracts, respectively. However, AsC, DMA and As(V) could also be determined in these samples.

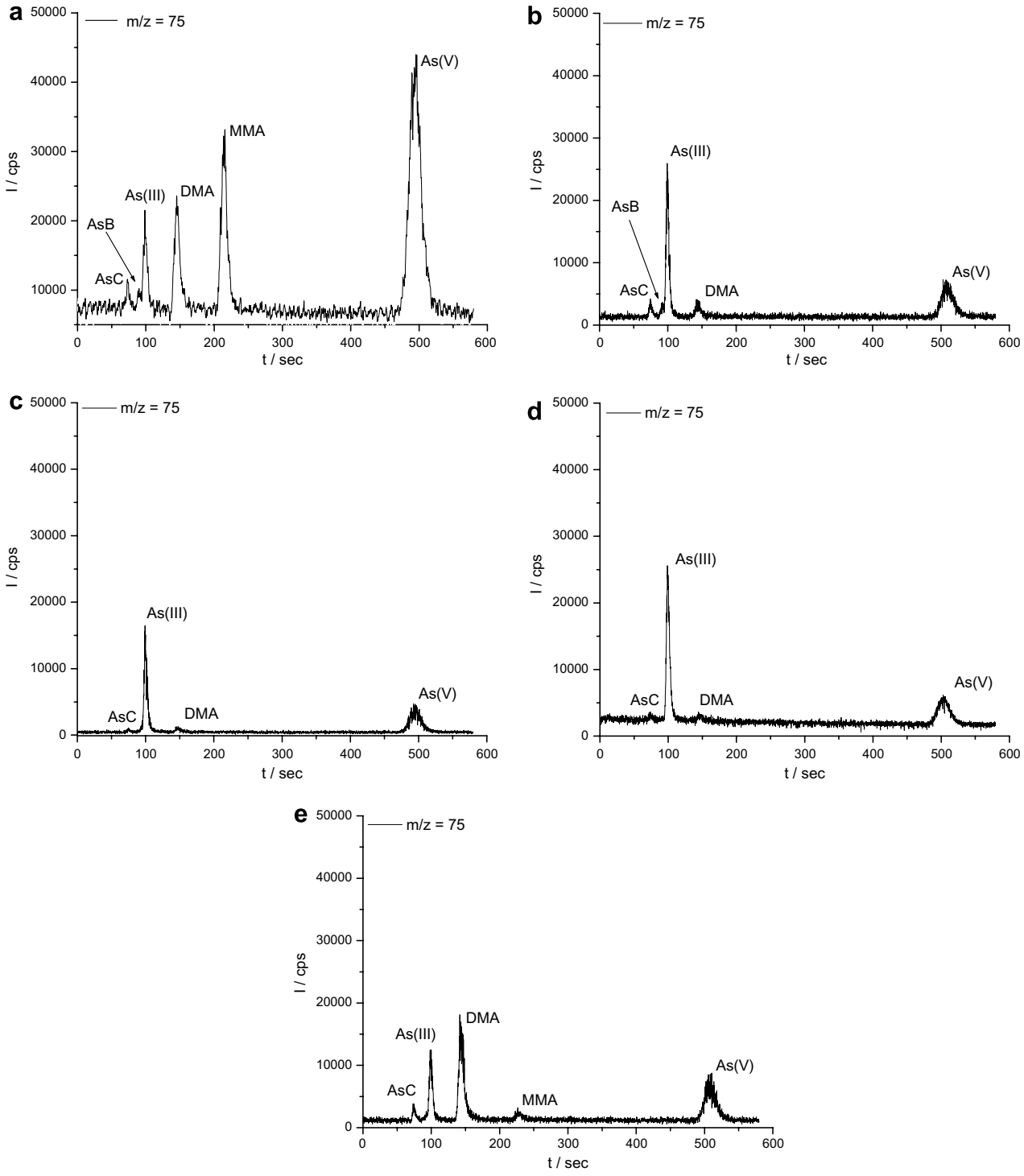

Fig. 2. HPLC-ICP-MS chromatogram of a standard solution containing AsC, AsB, As(III), MMA, DMA and As(V) in concentration of 1, 1, 1, 3, 7 and 15 ng cm^{-3}, respectively (a), of the ground Zhenshan 97 (b), Risabell (c), Kőröstáj-333 (d) and of NIST SRM 1568a (e). Chromatographic conditions: Hamilton PRP X-100 10 μm 250 × 4.1 mm column; mobile phase: 20 mmol dm^{-3} $NH_4H_2PO_4$ (pH 5.6; NH_3); flow rate: 1.5 cm^3 min^{-1}; $t = 22$ °C; injection volume: 20 μl. Detection at $m/z = 75$ in low resolution. Rice samples subjected to enzyme-assisted extraction with α-amylase from *Bacilus subtilis* and protease Type XIV from *Streptomyces griseus* applying microprobe sonication.

Moreover, in case of the Chinese rice sample, AsB could be extracted by hot water. In return, in the freeze-dried cooked samples, generally As(V) is predominant, which may also mean that As(III) oxides during the cooking procedure (Fig. 3). The recovery values by relating the sum of the As species determined to the total arsenic values obtained after microwave-assisted digestion ranged between 73% and 109%.

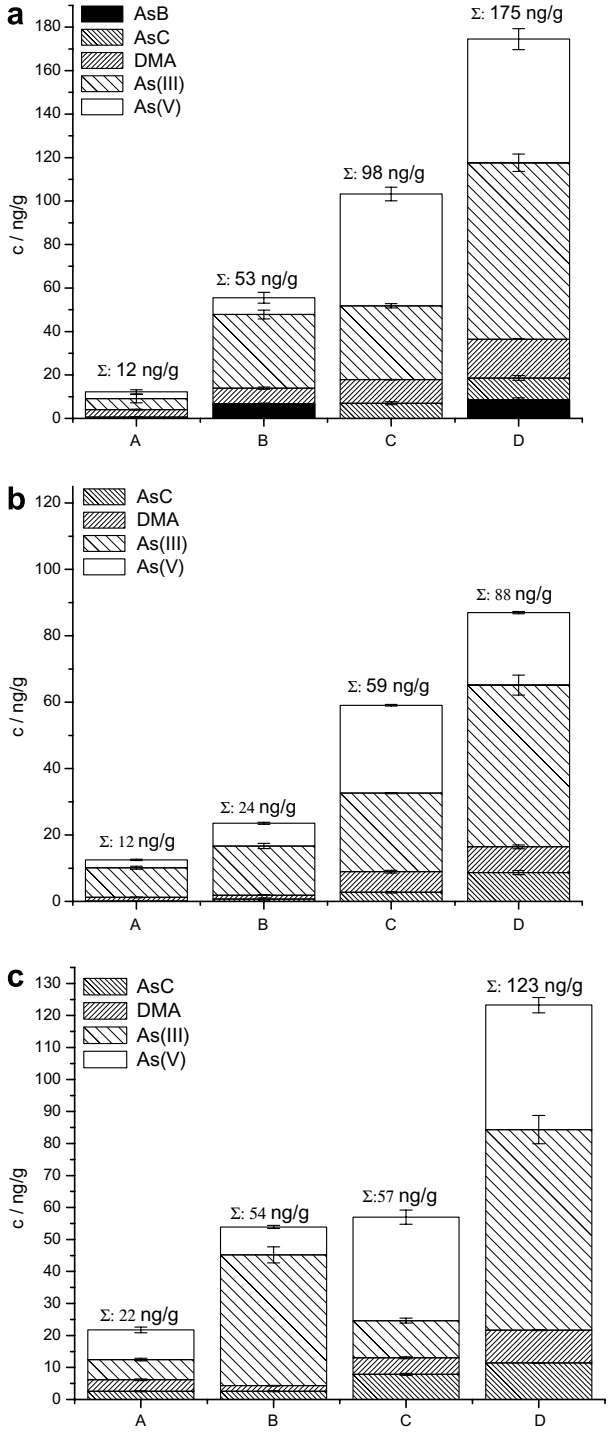

Fig. 3. Distribution of the As species between the cold water (A), hot water (B), cooked (C) and ground (D) of Zhenshan 97 (a), Risabell (b) and Kőröstáj-333 (c) rice samples. $\sum$ = sum.

The accuracy of the results was checked by subjecting the NIST SRM 1568 rice flour standard to the enzyme-assisted extraction and microprobe focused sonication. The elution profile of the NIST SRM 1568a standard can be seen in Fig. 2. In this case, the recovery calculated by relating the chromatographic sum of the species to the value obtained by total As determination was 91%. The species identified in the NIST SRM 1568a rice flour and their percentage order was the following: DMA (42%) > As(V) (28%) > As(III) (20%) > MMA (5%) > AsC (5%). Comparing our results with already reported values for the arsenic speciation of NIST SRM 1568a standard, a good correlation was observed with literature data (D'Amato et al., 2004; Pizarro et al., 2003; Sanz et al., 2005a; Heitkemper et al., 2001) regarding DMA, As(III) and MMA whose percentages vary between 47–60%, 20–30% and 3–5%. However, there is a greater fluctuation in the As(V) percentage: 7% and 18% according to Sanz et al. (2005a) and D'Amato et al. (2004), respectively. Arsenate peak is broader than that of the other eluting species because of being the last eluting species, which conferees a less accurate determination. Moreover, in the cited literatures, the presence of AsC was not reported. The answer to this second phenomenon may be the fact that As speciation was performed, in the cited literatures, with sieved samples: 63 μm (D'Amato et al., 2004), 125 μm (Pizarro et al., 2003a) and 250 μm (Sanz et al., 2005a), and the NIST standard was possibly passed through the above-mentioned mesh size sieve. As our investigations followed the "fit-to-purpose" principle, namely, applying an everyday procedure used for preparation of rice dishes in order to evaluate the leaching of As, the samples were not sieved.

4. Conclusions

Hungarian and Chinese rice samples were subjected to cold and hot water extraction in a ratio of 6:1 water:rice (cm^3:g) in order to investigate the extent of As, which can be removed by these everyday processes. Moreover, enzyme-assisted extraction proved that arsenite could be removed in the highest extent if rice was washed and cooked in abundant water amounts. However, arsenite is also the predominant chemical form in these samples. In return, the predominant As species of the cooked rice samples was arsenate. Thus, it is always recommended to prepare rice dishes in abundant water, which has to be discarded after the elaboration processes.

Further prospects include arsenic speciation in rice boiled in tap water as well as enzymatic hydrolysis studies with human-like gastric fluid in order to assess As bioavailability for humans.

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References

Abedin, M. J., Cresser, M. S., Meharg, A. A., Feldmann, J., & Cotter-Howells, J. (2002). Arsenic accumulation and metabolism in rice (*Oryza Sativa* L.). *Environmental Science & Technology, 36*, 962–968.

Abedin, M. J., Feldmann, J., & Meharg, A. A. (2002). Uptake kinetics of arsenic in rice plants. *Plant Physiology, 128*, 1120–1128.

Ackerman, A. H., Creed, P. A., Parks, A. N., Fricke, M. W., Schwegel, C. A., Creed, J. T., et al. (2005). Comparison of a chemical and enzymatic extraction of arsenic from rice and an assessment of the arsenic absorption from contaminated water by cooked rice. *Environmental Science & Technology, 39*, 5241–5246.

Bae, M., Watanabe, C., Inaoka, T., Sekiyama, M., Sudo, N., Bokul, M. H., et al. (2002). Arsenic in cooked rice in Bangladesh. *Lancet, 360*, 1839–1840.

Brown, J. L., Kitchin, K. T., & George, M. (1997). Dimethylarsinic acid treatment alters six different rat biochemical parameters: Relevance to arsenic carcinogenesis. *Teratogenesis Carcinogenesis and Mutagenesis, 17*, 71–84.

Caruso, J. A., Heitkemper, D. T., & B'Hymer, C. (2001). An evaluation of extraction techniques for arsenic species in freeze-dried apple samples. *Analyst, 126*, 136–140.

Capelo, J. L., Ximénez-Embún, P., Madrid-Albarán, Y., & Cámara, C. (2004). Enzymatic probe sonication: Enhancement of protease catalysed hydrolysis of selenium bound to proteins in yeast. *Analytical Chemistry, 76*, 233–237.

D'Amato, M., Forte, G., & Caroli, S. (2004). Identification and quantification of major species of arsenic in rice. *Journal of AOAC International, 87*, 238–243.

Gallagher, P. A., Wei, X. Y., Shoemaker, J. A., Brockhoff, C. A., & Creed, J. T. (1999). Detection of arsenosugars from kelp extracts via IC-electrospray ionization-MS-MS and IC membrane hydride generation ICP-MS. *Journal of Analytical Atomic Spectrometry, 14*, 1829–1834.

Gallego-Gallegos, M., Liva, M., Olivas, R. M., & Cámara, C. (2005). Focused ultrasound and molecularly imprinted polymers: A new approach to organotin analysis in environmental samples. *Journal of Chromatography A, 1114*, 82–88.

Heitkemper, D. T., Vela, N. P., Stewart, K. R., & Westphal, C. S. (2001). Determination of total and speciated arsenic in rice by ion chromatography and inductively coupled plasma mass spectrometry. *Journal of Analytical Atomic Spectrometry, 16*, 299–306.

Kuehnelt, D., Goessler, W., Schlagenhaufen, C., & Irgolic, K. J. (1997). Comparison of three methods for extraction of arsenic compounds from the NRCC standard reference material DORM-2 and from the brown alga Hijiki fuziforme. *Applied Organometallic Chemistry, 11*, 859–867.

McKiernan, J. W., Creed, J. T., Brockhoff, C. A., Caruso, J. A., & Lorenzana, R. M. (1999). A comparison of automated and traditional methods for the extraction of arsenicals from fish. *Journal of Analytical Atomic Spectrometry, 14*, 607–613.

Odanaka, Y., Tsuchiya, N., Matano, O., & Goto, S. (1985). Characterization of arsenic metabolites in rice plants treated with DSMA (Disodium methanenarsonate). *Journal of Agricultural Food Chemistry, 33*, 757–763.

Pizarro, I., Gómez, M., Palacios, M. A., & Cámara, C. (2003a). Evaluation of stability of arsenic species in rice. *Analytical and Bioanalytical Chemistry, 376*, 102–109.

Pizarro, I., Gómez, M., Cámara, C., & Palacios, M. A. (2003b). Arsenic speciation in environmental and biological samples – Extraction and stability studies. *Analytica Chimica Acta, 495*, 85–98.

Quaghebeur, M., Rengel, Z., & Smirk, M. (2003). Arsenic speciation in terrestrial plant material using microwave-assisted extraction, ion chromatography and inductively coupled plasma mass spectrometry. *Journal of Analytical Atomic Spectrometry, 18*, 128–134.

Sanz, E., Muñoz-Olivas, R., & Cámara, C. (2005a). A rapid and novel alternative to conventional sample treatment for arsenic speciation in rice using enzymatic ultrasonic probe. *Analytica Chimica Acta, 535*, 227–235.

Sanz, E., Muñoz-Olivas, R., & Cámara, C. (2005b). Evaluation of a focused sonication probe for arsenic speciation in environmental and biological samples. *Journal of Chromatography A, 1097*, 1–8.

Schoof, R. A., Yost, L. J., Eickhoff, J., Crecelius, E. A., Cragin, D. W., Meacher, D. M., et al. (1999). A market basket survey of inorganic arsenic in food. *Food and Chemical Toxicology, 37*, 839–846.

Takamatsu, T., Aoki, H., & Yoshida, T. (1982). Determination of arsenate, arsenite, dimethylarsonate, and monomethylarsinate in soil polluted with arsenic. *Soil Science, 133*, 239–246.

Tao, S. S. H., & Bolger, P. M. (1999). Dietary arsenic intake in the United States: FDA total diet study, September 1991–December 1996. *Food Additives and Contaminants, 16*, 465–472.

Varsányi, I., Fodre, Z., & Bartha, A. (1991). Arsenic in drinking-water and mortality in the Southern Great Plain. *Environmental Geochemistry and Health, 13*, 14–22.