

Analysis of Main Arsenic Species in Canned Fish Marketed in the Czech Republic and Austria

Antonín Kaňa¹, Richard Koplík², Simone Braeuer³, Walter Goessler³ and Oto Mestek¹

¹Department of Analytical Chemistry, University of Chemistry and Technology Prague, Technická 5, 166 28 Prague 6, Czech Republic

²Department of Food Analysis and Nutrition, University of Chemistry and Technology Prague, Technická 5, 166 28 Prague 6, Czech Republic

³Institute of Chemistry, University of Graz, Universitaetsplatz 1, 8010 Graz, Austria

Correspondence to:

Antonín Kaňa
Department of Analytical Chemistry
University of Chemistry and Technology Prague
Technická 5, 166 28 Prague 6, Czech Republic
E-mail: kanaa@vscht.cz

Received: November 23, 2017

Accepted: January 25, 2018

Published: January 29, 2018

Citation: Kaňa A, Koplík R, Braeuer S, Goessler W, Mestek O. 2018. Analysis of Main Arsenic Species in Canned Fish Marketed in the Czech Republic and Austria. *J Food Chem Nanotechnol* 4(1): 10-17.

Copyright: © 2018 Kaňa et al. This is an Open Access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC-BY) (<http://creativecommons.org/licenses/by/4.0/>) which permits commercial use, including reproduction, adaptation, and distribution of the article provided the original author and source are credited.

Published by United Scientific Group

Abstract

Total arsenic and water-soluble arsenic species were determined in canned fish bought in Graz ($n = 6$) and Prague ($n = 8$) with inductively-coupled plasma mass spectrometry and anion-exchange liquid chromatography. A difference in total arsenic content between samples from Prague (mean 3.4; standard deviation (s.d.) 1.2 mg kg⁻¹) and Graz (mean 2.5; s.d. 0.7 mg kg⁻¹) was not significant, however Prague samples differed in abundance of arsenobetaine (mean 83% of extractable arsenic; s.d. 16%) vs. Graz samples (mean 66%; s.d. 10%). No differences in total arsenic were caused by fish species (mackerel, sardine, herring, tuna) and origin (Northeast and Eastern-central Atlantic).

Keywords

Arsenic, Speciation, Fish, ICP-MS, Ion-exchange chromatography

Introduction

Arsenic has belonged to the category of probable carcinogens for more than a hundred years, but only in the seventies and eighties was it shown that arsenic can cause skin cancer and was therefore moved into the category of proven carcinogens. Over time, it was shown that various arsenic species have different toxicities. Organic arsenic compounds, in particular dimethylarsinic acid (DMA), methylarsonic acid (MA) and arsenobetaine (AB), are less toxic than inorganic compounds containing trivalent (As (III)) or pentavalent arsenic (As(V)) [1-3]. Nowadays, more than 100 different organic arsenic compounds are known. In addition to those above, relatively harmless arsenocholine (AsC) and various arsenosugars and arsenolipids can be mentioned as examples of organic arsenic species. Plant hyperaccumulators, such as some ferns, can bind arsenic in complexes usually with phytochelatin [4, 5]. More information about arsenic species can be found elsewhere, e.g., in [6].

Arsenic enters the human diet via both plant and animal sources. Rice is considered the most important plant source of arsenic. Depending on location, rice contains up to hundreds of ng As g⁻¹ and contaminated rice can cause chronic poisoning in large populations [7-10]. Rice contains arsenic mainly in the inorganic form, followed by DMA [9-11].

Another important source of arsenic in the human diet is represented by fish and seafood. Fish meat contains the largest proportion of arsenic in the form of AB, which is generally regarded as non-toxic. Minor amounts of arsenic may also be present in the form of As(V), MA and DMA. Since in aquatic biota arsenic is a cumulative element, its content in such foods can achieve

considerable levels. Thus, for example, the fish *Urophycis cirrus* caught in the Atlantic Ocean on the shores of Brazil contained 34.0 mg As kg⁻¹ (on dry mass basis); the dominant species was AB (33.5 mg As kg⁻¹) [12]. Recently, extensive consideration was given to the arsenic content in fish meat, with scientific databases containing more than 300 scientific publications. Basic information can be drawn e.g. from reviews [13-15].

Although the number of known arsenic species has reached more than 100, for a basic assessment of food or food raw materials, the analysis of only a few is sufficient. Usually, only polar arsenic compounds are analysed, and consequently aqueous extraction agents are often applied during the sample preparation step [16]. The efficiency and rate of extraction can be increased by the application of microwaves or ultrasound. The analysis is then performed by combining liquid chromatography (LC) with a sensitive element selective detection, typically by inductively-coupled plasma mass spectrometry (ICP-MS). The main separation technique used in this case is anionic ion-exchange chromatography (A-IEC). Using this technique, all cationic species (AsC, tetramethylarsonium salts) and zwitterionic species (AB) are eluted near the dead volume of the column. However, since the dominant component in this group is AB, the entire content of arsenic corresponding to this peak is usually assigned to AB. The elution of anionic species (As(III), DMA, MA, As(V)) normally follows the peak of AB. As(III) is not very well separated from AB when the pH of the mobile phase is below 8. This problem is frequently solved by analysis of the sum of As(III) and As(V) instead of individual species. Therefore, in some procedures, As(III) is converted to As(V) by oxidation with hydrogen peroxide prior to chromatographic analysis (see [17, 18] for fish tissue analysis, [19] for analysis of urine or [20] for analysis of blood). The other species of arsenic MA, DMA, and As(V) are separated with very good resolution. Only in special cases, an analysis by A-IEC is complemented by a parallel analysis using cationic IEC for the determination of cationic species. Of the many articles published recently, the reviews [21-23] can be recommended.

The aim of this publication is to at least partially fill the gap in knowledge concerning arsenic species occurring in foods on the Czech market. Fish consumption in the Czech Republic is very limited compared to coastal states, which may be why this seemingly insignificant source of arsenic in our diet has been omitted so far. To date, only a few studies have been published on the total content of arsenic in the meat of wild and farmed fish from the Czech Republic (see the investigation of chub [24], various fish living in Slezska Harta reservoir [25], carp [26, 27], and trout [28]). Information about the arsenic species in food on the Czech market has not yet been published. For our study, canned fish commonly available on the Czech market were chosen. Besides the actual determination of arsenic and its species, the comparison with similar products purchased in Austria was carried out. For this reason, the analyses were conducted at two workplaces, i.e. University of Graz (UG) and University of Chemistry and Technology Prague (UCT).

Material and Methods

Equipment

Speciation analyses were performed in two work places. The laboratory of UG was equipped with an Agilent 8800 (Agilent Technologies, Santa Clara, CA, USA) ICP-MS connected with an Agilent 1100 liquid chromatographic system, while the laboratory of UCT used a NexION 350D (Perkin Elmer, Concord, Canada) ICP-MS and the chromatographic apparatus consisted of a high-pressure pump Series 200 (Perkin Elmer) and a Rheodyne 9010 (Idex Health & Science LLC, Rohnert Park, CA, USA) injector equipped with a PEEK sample loop. Ion-exchange chromatographic columns PRP-X100 (150×4.6 mm, 5 μm, Hamilton) were used for the separation of arsenic species at both workplaces. Sample decomposition for total arsenic determination was performed in UltraClave 3 (Milestone, MLS GmbH, Germany) (UG) or Speed Wave 4 (Berghhof, Eningen, Germany) (UCT). Other equipment used in UG included: freeze-dryer Christ GAMMA 1-16 LSC (Martin Christ, Osterode am Harz, Germany), and centrifuge Rotina 420 R (Hettich, Tuttlingen, Germany). Similar equipment used in UCT was: freeze-dryer Christ Alpha 2-4 LD, ultrasonic baths U300 (Ultrawave, Cardiff, UK), and MPW 211 centrifuge (MPW Med. Instruments, Warsaw, Poland).

Samples and pre-analytical sample preparation

Two series of samples were analysed. The first series was purchased in SPAR and Lidl supermarket chains in Graz (Austria) in 2015 and analysed at UG (samples G1-G6). The next series was purchased from the Kaufland supermarket chain in 2016 in Prague (Czech Republic) and was analysed at UCT (samples P1-P8). Samples comprised both fish meat in brine and in oil; details are given in table 1. After removing the brine or oil, the meat was carefully dried with cellulose tissue and sliced. Pieces of meat were freeze-dried and consequently milled.

Table 1: Overview of analysed samples.

Sample	Description	Origin
G1	Tuna fish in brine	Eastern Central Atlantic
G2	Herring in brine	Northeast Atlantic
G3	Anchovy in olive oil	Southwest Atlantic
G4	Mackerel in sunflower oil	Eastern Central Atlantic
G5	Sardine in sunflower oil	Eastern Central Atlantic
G6	Mackerel in sunflower oil	Northeast Atlantic
P1	Sprats in rapeseed oil	Northeast Atlantic
P2	Tuna in soybean oil	Western Central Pacific
P3	Herring in rapeseed oil	Northeast Atlantic
P4	Sardinella in brine with 3% rapeseed oil	Eastern Central Atlantic
P5	Mackerel in sunflower oil	Eastern Central Atlantic
P6	Sardine in brine	Eastern Central Atlantic
P7	Mackerel in rapeseed oil	Northeast Atlantic
P8	Tuna in brine	Eastern Central Atlantic

Reagents

Sample digestion was performed with sub-boiled (UG) or Suprapur® grade (Merck, Darmstadt, Germany) (UCT) nitric acid.

Standard solutions of $1000 \pm 2 \text{ mg L}^{-1}$ of Ge and As (Certipur®, Merck, Darmstadt, Germany) were used for the preparation of internal standard and calibration solutions in the case of total arsenic content determination.

Calibration standards of arsenic species were (UCT): arsenobetaine (purity $\geq 98\%$), sodium arsenate dibasic heptahydrate ($\geq 98\%$), dimethylarsinic acid (approx. 98%) (all Sigma-Aldrich, St. Louis, USA), and disodium methylarsenate (Supelco, Bellefonte, PA, USA); (UG): in-house prepared arsenobetaine, sodium arsenate dibasic heptahydrate (Merck, Darmstadt, Germany); methylarsenate (in-house prepared from As_2O_3 and CH_3I); and sodium dimethylarsinate (Fluka, Buchs, Switzerland).

The certified reference material DORM-2 “Dogfish Muscle” (NRC, Canada) was used for the method verification.

A solution of 20 mmol L^{-1} ammonium dihydrogen phosphate (99.999%, trace metal basis, Sigma-Aldrich, Steinheim, Germany) served as a mobile phase. The pH was adjusted via the addition of a 25% ammonia solution (Suprapur®, Merck, Darmstadt, Germany) to 6.0. Hydrogen peroxide (Suprapur®, Merck, Darmstadt, Germany) was used for the oxidation of As(III) to As(V). Milli-Q water with a specific resistivity of $18.2 \text{ M}\Omega \text{ cm}$ (Merck-Millipore, Darmstadt, Germany) was used for the preparation of all solutions.

Methods

Both cooperating workplaces used almost identical methods of sample preparation and analysis. Minor differences lie only in the use of chemicals of various origins, but of comparable purity.

Determination of total arsenic content

Pulverised samples (0.2 g) or sample extracts (2 ml) were decomposed using microwave digestion with 5 mL of conc. nitric acid. After cooling, the samples were spiked with germanium internal standard solution (IS) to make final concentration of $200 \mu\text{g L}^{-1}$ Ge and the volume was filled to 50 mL by water. The ICP-MS measurements were performed under the conditions summarised in table 2. Calibration solutions containing 0, 10 and $50 \mu\text{g As L}^{-1}$ (UCT) and 0, 0.1, 0.5, 1, 5, 10, 50 and $100 \mu\text{g As L}^{-1}$ (UG) were prepared via the dilution of a stock arsenic solution and were also spiked with the IS solution.

Speciation analysis

Portions of freeze-dried samples ($\approx 0.2 \text{ g}$) were weighed into 15 mL polypropylene tubes and extracted with 4 mL of water for 15 min in an ultrasonic bath. The extracts were centrifuged for 10 min at 4500 rpm, and then filtered through a $0.45 \mu\text{m}$ (UCT) or $0.2 \mu\text{m}$ (UG) syringe filter (Whatmann, Buckinghamshire, UK) before analysis. To a 2 mL portion of filtrate, 0.1 mL of hydrogen peroxide was added one hour before

the chromatographic analysis. Oxidation was carried out at approx. $40 \text{ }^\circ\text{C}$. Determination of individual arsenic species was performed by an on-line hyphenation of HPLC and ICP-MS under the conditions given in table 2. Calibration solutions of arsenic species (AB, DMA, MA, As(V)) containing 1, 5, 10, 50 and $100 \mu\text{g As L}^{-1}$ (UCT) and 0.1, 0.5, 1, 5, 10, 50 and $100 \mu\text{g As L}^{-1}$ (UG) were prepared by diluting the stock solutions of arsenic species with water.

Table 2: ICP-MS and chromatographic operating conditions for used MS spectrometers.

	PerkinElmer NexION		Agilent 7500cx	Agilent 8800
	Total arsenic	Speciation analysis	Total arsenic	Speciation analysis
RF power	1100 W		1550 W	
Measurement mode	peak hopping		spectrum	time resolved analysis
Measured nuclides	$^{75}\text{As}, ^{74}\text{Ge}$	^{75}As	$^{75}\text{As}, ^{74}\text{Ge}$	$^{75}\text{As} \rightarrow ^{75}\text{As}^{16}\text{O}$
Acquisition time	500 ms	1000 ms	500 ms	300 ms
Cell gas flow	$0.3 \text{ mL min}^{-1} \text{ NH}_3$		$4.0 \text{ mL min}^{-1} \text{ He}$	$2.5 \text{ mL min}^{-1} \text{ O}_2$
RPq	0.75		---	---
Option gas	---		$15\% \text{ CO}_2$	$16\% \text{ CO}_2$
Total acquisition time	---	540 s	---	600 s
Sample injection volume	100 μL			20 μL
Mobile phase flow	---	1.4 mL min^{-1}	---	1.0 mL min^{-1}
Column temperature	----	$40 \text{ }^\circ\text{C}$	----	$40 \text{ }^\circ\text{C}$

Method validation by analyses of DORM-2 certified reference material

The extracts were prepared from 100 mg samples by extraction into 10-ml portions of water under the conditions described above. Three series of six extracts were prepared. The first series was analysed without any change, whereas the second and third series were spiked by the arsenic species. The concentration of DMA, MA, and As(V) corresponded to $1 \mu\text{g As L}^{-1}$ and $5 \mu\text{g As L}^{-1}$ for each of these species for the second and the third series, respectively. The concentration of added species AB corresponded to $50 \mu\text{g As L}^{-1}$ and $100 \mu\text{g As L}^{-1}$ for the second and the third series, respectively.

Results and Discussion

Optimisation and performance characteristics of speciation analysis

The basic characteristics of the methods of arsenic speciation analysis by coupling of A-IEC and ICP-MS were studied in detail in the workplace of UCT. The determination of arsenic (monoisotopic element ^{75}As) using ICP-MS is disturbed by spectral interference due to the formation of $^{40}\text{Ar}^{35}\text{Cl}^+$ ions. The tests of this interference were performed using the 0.01 mol L^{-1} HCl solution, which corresponds to the expected concentration of chloride ions in the extracts of

fish samples. The spectrometer PE NexIon, which was used for these experiments, is equipped with universal cell technology. This means that the cell is able to work as the collision cell, in which the elimination of polyatomic ions is based on the discrimination of their kinetic energy via collisions with the atoms of collision gas, or the reaction cell, in which the polyatomic ions react with the molecules of reactive gas to form a new product not exhibiting interference. For more details, see e.g. in [29]. When the measurement is carried out in the standard mode (without collision or reaction cell), the background equivalent concentration (BEC) (i.e. the false positive signal caused by the interference) of 0.01 mol L⁻¹ HCl solution was 1.25 µg As L⁻¹. This can cause a considerable distortion of results. The application of collision cell mode (collision gas helium) did not give satisfying results with this instrument (Table 3). In order to operate without a significant loss of sensitivity, it is necessary to work with a low flow rate of He; interferences are removed by the 50% rate only. The increase of He flow allowed the BEC to be reduced to a reasonable level, below 0.05 µg As L⁻¹, but it was accompanied by a substantial decrease in sensitivity to a level of about 10% compared to the standard mode. On the other hand, when reaction cell mode was applied (reaction gas ammonia), the careful optimisation of gas flow and rejection parameter q (RPq, parameter affecting flight of ions through the cell and the removal of reaction product) enabled BEC to be decreased to a negligible level of 0.03 µg As L⁻¹ As without the loss of sensitivity (see Table 3).

Table 3: Spectral interferences due to ArCl⁺ ion (measured on PE NexION spectrometer).

Condition	Sensitivity (cps per 1 µg As L ⁻¹)	BEC (0.01 mol L ⁻¹ HCl solution, µg As L ⁻¹)
Standard mode	5730	1.25 (equivalent to a signal of 7 160 cps)
Collision mode		
0.6 mL min ⁻¹ He	5300	0.65 (equivalent to a signal of 3 450 cps)
2.6 mL min ⁻¹ He	618	0.04 (equivalent to a signal of 24 cps)
Reaction mode		
0.3 mL min ⁻¹ NH ₃ , RPq = 0.75	6130	0.03 (equivalent to a signal of 158 cps)

The external calibration with multi-species calibration solutions is a very common procedure in speciation analysis and represents a standard approach; its weakness is that some standards could be unavailable and quantification of unidentified species can be questionable. In the ideal case of the same sensitivity for all species of the element, the calibration procedure can be restricted to the use of standard solutions of a single species. Such an approach facilitates the easy quantification of all species, regardless of whether the composition is unknown and/or the standard is unavailable. Therefore, the linearity and slopes of calibration curves of the most common arsenic species were checked. In the first step, stock solutions AB, As(V), MA, and DMA at a concentration corresponding to 1000 mg As L⁻¹ were prepared. The true concentration of arsenic in these solutions was determined

and their uniformity was confirmed by the determination of total arsenic using ICP-MS after decomposition of sample in microwave unit and conversion of all species to As(V). Consequently, five of the mixed calibration solutions containing all four species at levels corresponding to 20, 40, 60, 80, and 100 µg As L⁻¹ were prepared and subjected to speciation analysis. The acquired chromatograms are depicted in figure 1 showing a very good separation of these species, even at high concentrations. The linearity of calibration lines based on the peak area were proven by Mandel's test ($\alpha = 0.05$) [30] for all species. Moreover, the calculated values of slopes of all calibrations (19060 ± 380 for AB, 19300 ± 320 for DMA, 19150 ± 260 for MA, and 19400 ± 270 for As(V)) were identical. The test was performed by the comparison of confidence intervals of individual slope values. However, in the case of a real sample analysis, the sensitivity of individual arsenic species detection could be affected by matrix components, see figure 2. Alkali elements were eluted in a broad tailed peak with a maximum located close to the point of void volume of the column. The signal for chloride ions was also very broad. The figure nicely demonstrates the efficient removal of spectral interferences by using the reaction cell mode of measurement.

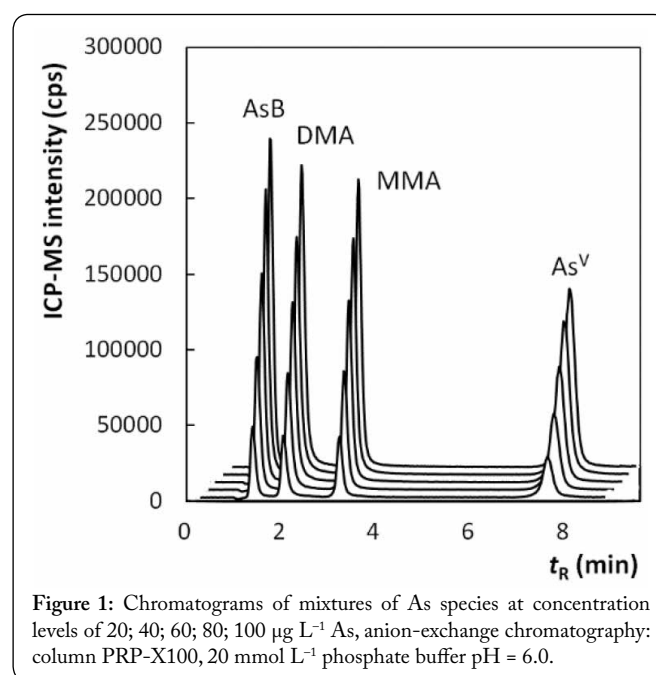


Figure 1: Chromatograms of mixtures of As species at concentration levels of 20; 40; 60; 80; 100 µg L⁻¹ As, anion-exchange chromatography: column PRP-X100, 20 mmol L⁻¹ phosphate buffer pH = 6.0.

The limit of detection (LOD) was estimated by tripling the standard deviation obtained when the solution containing all arsenic species at the very low level of 0.25 µg As L⁻¹ was repeatedly ($n = 6$) analysed. The identified limit of detection for all species was 0.030 µg As L⁻¹. This value is comparable with the BEC value, see above. The chromatogram of AB, DMA, MA and As(V) corresponding to this concentration is shown in figure 3. Corresponding limit of quantification (LOQ) estimated as ten multiple of standard deviation is 0.10 µg As L⁻¹.

The accuracy of the determination was verified using DORM-2 certified reference material of Dog Fish Muscle. Two kinds of test were performed. In the first test, the CRM was utilised only as a sample matrix and the standard additions of individual arsenic species were used for the accuracy tests. The

results are shown in table 4. The accuracy of the determination of all species was proven by the Student t-test ($\alpha = 0.05$). Moreover, the accuracy of arsenic species determination was also proven indirectly: the sum of contents of all species found by speciation analysis corresponds to the value $17.4 \pm 0.7 \text{ mg As kg}^{-1}$, which is in excellent agreement with the certified total content of As in DORM-2 CRM of $18.0 \pm 1.1 \text{ mg As kg}^{-1}$. The second test consists in determination of AB in unspiked samples. Observed value $16.8 \pm 0.6 \text{ mg As kg}^{-1}$ was again in very good agreement with certified value of AB-arsenic content of $16.4 \pm 1.1 \text{ mg As kg}^{-1}$. Although the arrangement of speciation analysis did not enable the application of the internal standard during the ICP-MS measurement, it is obvious that varying the salt elution during the chromatographic separation (see figure 2 and discussion above) did not affect the quality of results.

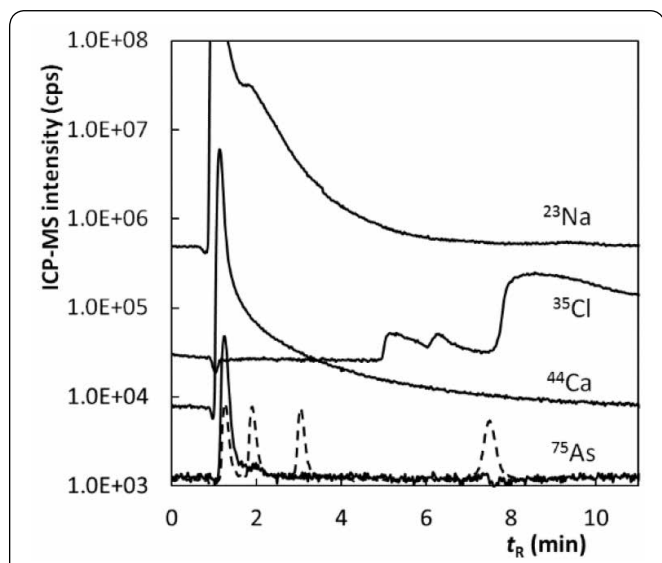


Figure 2: Elution of alkali elements, chloride ions and arsenic species from fish extract, anion-exchange chromatography: column PRP-X100, 20 mmol L⁻¹ phosphate buffer pH = 6.0 Dashed line indicates the hypothetical positions of arsenic species not present in the extract.

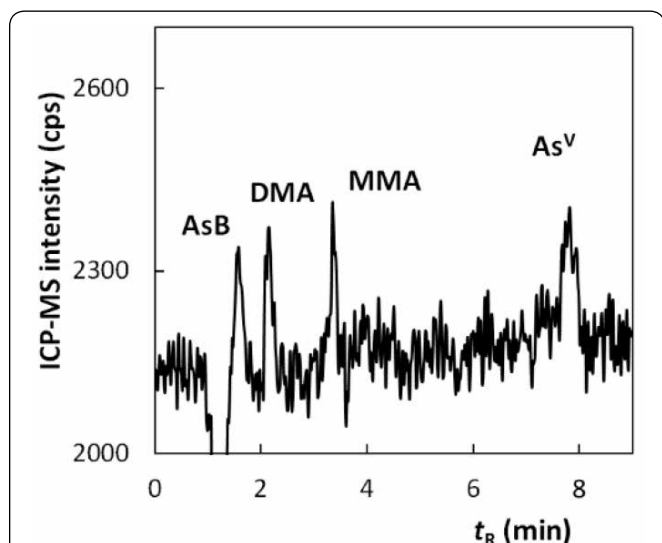


Figure 3: Chromatogram of As species at concentration level of 30 ng L⁻¹ As, anion-exchange chromatography: column PRP-X100, 20 mmol L⁻¹ phosphate buffer pH = 6.0, injection volume 100 μL.

Table 4: Result of determination of arsenic species in original and spiked extracts of DORM-2, results are given as a mean of 6 determinations (RSD%).

Species	1 st series	2 nd series		3 rd series	
	original extract	spiked extract level 1:		spiked extract level 2:	
		AB 50 μg As L ⁻¹		AB 100 μg As L ⁻¹	
		DMA 1 μg As L ⁻¹		DMA 5 μg As L ⁻¹	
		MA 1 μg As L ⁻¹		MA 5 μg As L ⁻¹	
		As(V) 1 μg As L ⁻¹		As(V) 5 μg As L ⁻¹	
	Concentration (μg L ⁻¹ As)	Concentration (μg L ⁻¹ As)	Recovery (%)	Concentration (μg L ⁻¹ As)	Recovery (%)
AB	168 (3)	219 (2)	102 (3)	270 (2)	102 (2)
DMA	5.57 (4)	6.54 (2)	97 (4)	10.3 (2)	95 (4)
MA	0.55 (13)	1.51 (4)	96 (13)	5.50 (2)	99 (13)
As(V)	0.00	1.01 (5)	101 (5)	4.90 (2)	98 (2)

Analysis of canned fish samples

The described methods were used for the determination of total arsenic and major arsenic species in samples of canned fish. Table 1 shows the sample kind, origin and packing media. All samples and sample extracts were analysed for the total arsenic content after microwave decomposition and all sample extracts were subjected to the speciation analysis. The results are shown in table 5; an example of chromatogram is shown in figure 4. Some analyses of total arsenic were performed in duplicates in both UCT and UG laboratories and the results were in a good agreement.

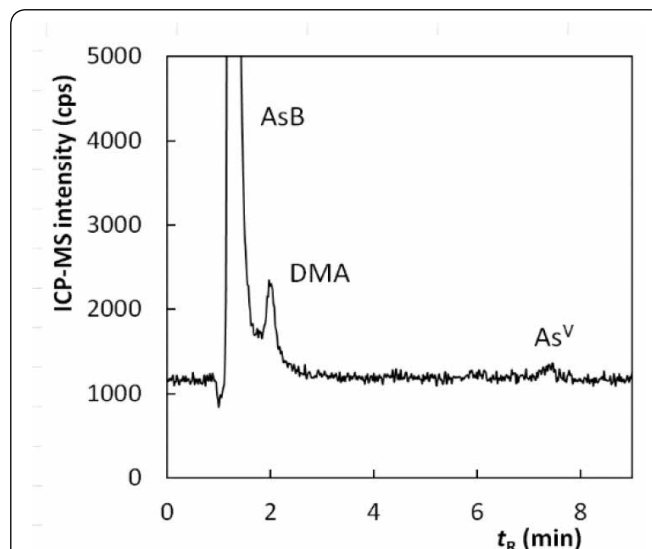


Figure 4: Chromatogram of arsenic species in *Sardinella aurita* extract, anion-exchange chromatography: column PRP-X100, 20 mmol L⁻¹ phosphate buffer pH = 6.0, flow rate 1.4 mL min⁻¹.

The levels of arsenic and its species in fish can be influenced mainly by the feeding habit of a fish species and the arsenic level in environment, however some changes due to the age of fish [31] or season [32] have also been observed. The significance of variation in the concentrations of arsenic and its species across the various sample origin, fish species, and canned fish origin ($\alpha = 0.05$) was tested by ANOVA and Student t-test. Arsenic extraction efficiency within sample preparation was

different for individual samples. Therefore, in order to assess the variability of arsenic species content, the percentage of arsenic in the corresponding species related to the extractable arsenic content was used as the followed factor instead of the absolute content of individual species. No significant variation in total arsenic content in canned fish marketed in the Czech Republic (3.4 (1.2) mg kg⁻¹) and Austria (2.5 (0.7) mg kg⁻¹) (data are presented as mean and standard deviation in brackets) was found. Prague samples differed from Graz samples only in abundance of arsenobetaine, see Table 6. As to the other parameters, the ANOVA suggests no significant difference in total arsenic, AB, and DMA levels across the fishing areas. Regarding the fish species, differences in total arsenic also were not found, however, differences in AB percentage were noticeable. The AB percentage was found to range from 62 to 96%. The highest AB percentage was found in tuna fish. The differences in DMA percentage and total as content among individual fish species were not significant.

Table 5: Results of determination of total arsenic and arsenic species in canned fish. Results are given in mg As kg⁻¹ and are related to the dry sample. Values of total arsenic are mean of three analyses ± expanded uncertainty ($k = 2$), the other value are mean of two analyses.

Sample	Total As	Extractable As*	AB**	DMA	MA	As(V)
G1	2.60 ± 0.06	2.54 (98%)	1.985	0.005	<0.0006	<0.0006
G2	3.29 ± 0.07	1.70 (52%)	1.227	0.013	<0.0006	<0.0006
G3	1.52 ± 0.21	0.58 (38%)	0.370	0.029	<0.0006	0.011
G4	2.08 ± 0.07	1.45 (70%)	1.021	0.030	<0.0006	0.008
G5	3.09 ± 0.21	1.27 (41%)	0.643	0.129	0.0018	0.0008
G6	2.37 ± 0.10	0.42 (18%)	0.243	0.027	<0.0006	<0.0006
P1	2.84 ± 0.13 2.86 ± 0.12 [§]	0.25 (9%)	0.158	0.018	<0.0006	0.004
P2	3.24 ± 0.21 3.35 ± 0.20 [§]	1.35 (42%)	1.366	0.035	<0.0006	<0.0006
P3	4.37 ± 0.36 4.82 ± 0.78 [§]	0.76 (17%)	0.667	0.014	<0.0006	<0.0006
P4	3.52 ± 0.22 3.93 ± 0.16 [§]	1.09 (31%)	0.868	0.029	<0.0006	<0.0006
P5	2.14 ± 0.19 2.30 ± 0.20 [§]	0.86 (40%)	0.648	0.023	<0.0006	0.003
P6	3.30 ± 0.27 3.95 ± 0.16 [§]	0.84 (25%)	0.704	0.021	<0.0006	<0.0006
P7	5.23 ± 0.31 5.56 ± 0.10 [§]	0.40 (8%)	0.263	0.047	<0.0006	0.003
P8	1.76 ± 0.18 1.95 ± 0.20 [§]	1.06 (60%)	1.135	0.049	<0.0006	<0.0006

* Percentage in brackets is related to the total arsenic content.

** This value represents the sum of AB and other cationic species, however their abundance is less than 3%.

§ Results of parallel analyses performed in UG laboratory.

The extraction yield of arsenic achieved by the applied procedure was low for most canned fish samples. This means that for most samples, the contents of individual arsenic species shown in table 5 (expressed as arsenic) are not related to the whole mass of samples. Consequently, the evaluation of the results of speciation analysis from the point of food toxicology and nutrition is questionable. Nevertheless, all

the analyses showed non-toxic AB as the most abundant arsenic species followed by low-toxic DMA. On the other hand, the more toxic species MA and highly toxic inorganic arsenic (represented by As(V)) were not detected at all in the overwhelming majority of samples. This indicates that the danger of toxic action of arsenic compound originating from canned fish is unlikely. Moreover, the total content of As in canned fish samples is low, especially in view of the fact that EU legislation [33] does not set any limit for arsenic in fish. The total content of arsenic in dry matter of fish samples ranged from 1.52 to 5.23 mg kg⁻¹ corresponding approx to 0.5 to 1.7 mg kg⁻¹ in fresh matter.

Table 6: The average total As content (mg kg⁻¹, dry weight) and its species percentage of extractable As (m/m%, dry weight) in the canned fish. The averages marked by the same letter did not significantly differ at $\alpha < 0.05$ within individual columns; data are presented as mean (standard deviation).

Groups	Total As	AB	DMA
Prague	3.4 (1.2) ^a	83 (16) ^a	4.5 (3.4) ^a
Graz	2.5 (0.7) ^a	66 (10) ^b	4.1 (3.8) ^a
Northeast Atlantic	3.6 (1.2) ^a	69 (12) ^a	5.6 (4.4) ^a
Eastern Central Atlantic	2.6 (0.7) ^a	78 (17) ^a	3.6 (3.2) ^a
Mackerel	3.0 (1.5) ^a	67 (7) ^a	5.7 (4.4) ^a
Sardine	3.2 (0.1) ^a	67 (23) ^{ab}	6.3 (5.4) ^a
Herring	3.8 (0.8) ^a	80 (11) ^{ab}	1.3 (0.8) ^a
Tuna fish	2.5 (0.7) ^a	96 (15) ^b	2.5 (2.2) ^a

The low extraction yield found for most canned fish samples strongly contrasted with the accurate results of DORM reference material analysis (see Table 4). This demonstrates that proving accuracy of a procedure using CRM does not necessarily mean that the procedure works properly in the case of real samples even if the sample matrix is similar. We suppose that the low efficiency of aqueous extraction procedure was caused by insufficient breakage of cellular structures of fish samples and partly also by a fat-coverage of some sample particles originated from sample matrix (fish in oil). Nevertheless for three samples (G1, G4 and P8) the extraction yield was good or satisfactory (60 to 98%). In these samples, the arsenic species distribution was analogous to that mentioned above: AB was the main quantified species followed by DMA, whereas MA was not detected and inorganic arsenic was either not detected or present in only trace amount (0.008 mg kg⁻¹ in G4).

Considering the wider range of circumstances, fish consumption brings important nutritional benefits, such as favourable fatty acids composition, increased intake of lipophilic vitamins and selenium. This partially balances the toxic effects of both mercury and arsenic compounds contained in fish. Seen from this view, the risk associated with toxic arsenic species in canned fish is insignificant.

Conclusion

Our experiments proved a high reproducibility of determination of total arsenic concentration by ICP-MS. The

results of samples analysed in parallel in both laboratories fitted very well. Also, the total concentrations of arsenic in the samples of canned fish purchased in both countries did not differ. Although only a limited set of samples has been analysed, this is an important finding. Recently, differences in the quality of some food between “older” EU countries (like Austria) and “newer” EU countries (like the Czech Republic) were several times noticed. However, canned fish do not seem to be the case.

Conflict of Interest

No potential conflict of interest was reported by the authors.

Acknowledgements

This work was realized within the Operational Programme Prague – Competitiveness (CZ.2.16/3.1.00/24501) and “National Program of Sustainability” (NPU I LO1613) MSMT-43760/2015.

References

- Bissen M, Frimmel FH. 2003. Arsenic – a review. Part I: occurrence, toxicity, speciation, mobility. *Acta Hydrochim Hydrobiol* 31(1): 9-18. <https://doi.org/10.1002/ahch.200390025>
- Jain CK, Ali I. 2000. Arsenic: occurrence, toxicity and speciation techniques. *Water Research* 34(17): 4304-4312. [https://doi.org/10.1016/S0043-1354\(00\)00182-2](https://doi.org/10.1016/S0043-1354(00)00182-2)
- Roy P, Saha A. 2002. Metabolism and toxicity of arsenic: a human carcinogen. *Current Science* 82(1): 38-45.
- Belogolova GA, Sokolova MG, Gordeeva ON, Vaishlya OB. 2015. Speciation of arsenic and its accumulation by plants from rhizosphere soils under the influence of *Azotobacter* and *Bacillus* bacteria. *J Geochem Explor* 149: 52-58. <https://doi.org/10.1016/j.gexplo.2014.11.017>
- Schmoger MEV, Oven M, Grill E. 2000. Detoxification of arsenic by phytochelatin in plants. *Plant Physiol* 122(3): 793-801. <https://doi.org/10.1104/pp.122.3.793>
- Nearing MM, Koch I, Reimer KJ. 2014. Complementary arsenic speciation methods: a review. *Spectrochim Acta Part B At Spectrosc* 99: 150-162. <https://doi.org/10.1016/j.sab.2014.07.001>
- Chen G, Chen T. 2014. SPE speciation of inorganic arsenic in rice followed by hydride-generation atomic fluorescence spectrometric quantification. *Talanta* 119: 202-206. <https://doi.org/10.1016/j.talanta.2013.11.016>
- Ren JH, Sun H J, Wang SF, Luo J, Ma LQ. 2014. Interactive effects of mercury and arsenic on their uptake, speciation and toxicity in rice seedling. *Chemosphere* 117: 737-744. <https://doi.org/10.1016/j.chemosphere.2014.10.035>
- Rahman MA, Rahman MM, Reichman SM, Lim RP, Naidu R. 2014. Arsenic speciation in Australian-grown and imported rice on sale in Australia: implications for human health risk. *J Agric Food Chem* 62(25): 6016-6024. <https://doi.org/10.1021/jf501077w>
- Shigehiro N, Eri M, Kumiko S, Tsutomu N. 2015. Effects of polishing, cooking, and storing on total arsenic and arsenic species concentrations in rice cultivated in Japan. *Food Chem* 168: 294-301. <https://doi.org/10.1016/j.foodchem.2014.07.060>
- Ma R, Shen J, Wu J, Tang Z, Shen Q, et al. 2014. Impact of agronomic practices on arsenic accumulation and speciation in rice grain. *Environ Pollut* 194: 217-223. <https://doi.org/10.1016/j.envpol.2014.08.004>
- Zmozinski AV, Llorente-Mirandez T, Lopez-Sanchez J, da Silva M. 2015. Establishment of a method for determination of arsenic species in seafood by LC-ICP-MS. *Food Chem* 173: 1073-1082. <https://doi.org/10.1016/j.foodchem.2014.10.102>
- Bosch AC, O'Neill B, Sigge GO, Kerwath SE, Hoffman LC. 2016. Heavy metals in marine fish meat and consumer health: a review. *J Sci Food Agr* 96(1): 32-48. <https://doi.org/10.1002/jsfa.7360>
- Marcovecchio JE, De Marco SG, Buzzi NS, Botte SE, Labudia AC, et al. 2015. Fish and seafood. In: DeLaGuardia M, Garrigues S (eds). *Handbook of mineral elements in food*, Chichester, Wiley, UK, pp 621-643. <https://doi.org/10.1002/9781118654316.ch27>
- Molin M, Ulven SM, Meltzer HM, Alexander J. 2015. Arsenic in the human food chain, biotransformation and toxicology - review focusing on seafood arsenic. *J Trace Elem Med Bio* 31: 249-259. <https://doi.org/10.1016/j.jtemb.2015.01.010>
- Kuehnelt D, Irgolic KJ, Goessler W. 2001. Comparison of three methods for the extraction of arsenic compounds from the NRCC standard reference material DORM-2 and the brown alga *Hijiki fuziforme*. *Appl Organomet Chem* 15(6): 445-456. <https://doi.org/10.1002/aoc.189>
- Krishnakumar PK, Qurban MA, Stiboller M, Nachman KE, Joydas TV, et al. 2016. Arsenic and arsenic species in shellfish and finfish from the western Arabian Gulf and consumer health risk assessment. *Sci Total Environ* 566-567: 1235-1244. <https://doi.org/10.1016/j.scitotenv.2016.05.180>
- Rasmussen RR, Hedegaard RV, Larsen EH, Sloth JJ. 2012. Development and validation of an SPE HG-AAS method for determination of inorganic arsenic in samples of marine origin. *Anal Bioanal Chem* 403(8): 2825-2834. <https://doi.org/10.1007/s00216-012-6006-7>
- Scheer J, Findenig S, Goessler W, Francesconi KA, Howard B, et al. 2012. Arsenic species and selected metals in human urine: validation of HPLC/ICPMS and ICPMS procedures for a long-term population-based epidemiological study. *Anal Methods* 4(2): 406-413. <https://doi.org/10.1039/C2AY05638K>
- Ito K, Goessler W, Gürleyük H, Wels B, Palmer CD, et al. 2011. An interlaboratory study of arsenic speciation analysis of whole blood. *J Anal At Spectrom* 26(9): 1740-1745. <https://doi.org/10.1039/C1JA10040H>
- Dhillon A, Nair M, Kumar D. 2015. Analytical methods for sensing of health-hazardous arsenic from biotic and abiotic natural resources. *Anal Methods* 7(24): 10088-10108. <https://doi.org/10.1039/C5AY02430G>
- Maher WA, Ellwood MJ, Krikowa F, Raber G, Foster S. 2015. Measurement of arsenic species in environmental, biological fluids and food samples by HPLC-ICPMS and HPLC-HG-AFS. *J Anal At Spectrom* 30(10): 2129-2183. <https://doi.org/10.1039/C5JA00155B>
- Wrobel K, Wrobel K. 2015. Methodological aspects of speciation analysis in food products. In: DeLaGuardia M, Garrigues S (eds) *Handbook of mineral elements in food*. Wiley, Chichester, UK, pp 391-453. <https://doi.org/10.1002/9781118654316.ch18>
- Harkabusova V, Celechovska O, Lavickova A, Svobodova Z. 2012. Monitoring of risk metals in chub (*Leuciscus cephalus* L.) from the Svitava and Svratka rivers in the urban area of Brno, Czech Republic. *Acta Vet Brno* 81(1): 69-73. <https://doi.org/10.2754/avb201281010069>
- Rehulka J. 2002. Content of inorganic and organic pollutants in the fish from the Slezska Harta reservoir. *Czech J Anim Sci* 47(1): 30-44.
- Svobodova Z, Zlabek V, Celechovska O, Randak T, Machova J, et al. 2002. Content of metals in tissues of marketable common carp and in bottom sediments of selected ponds of South and West Bohemia. *Czech J Anim Sci* 47(8): 339-350.
- Celechovska O, Svobodova Z, Zlabek V, Macharackova B. 2007. Distribution of metals in tissues of the common carp (*Cyprinus carpio* L.). *Acta Vet Brno* 76(S8): S93-S100. <https://doi.org/10.2754/avb200776S8S093>

28. Svobodova Z, Celechovska O, Machova J, Randak T. 2002. Content of arsenic in market-ready rainbow trout (*Oncorhynchus mykiss*). *Acta Vet Brno* 71(3): 361-367. <https://doi.org/10.2754/avb200271030361>
29. Koyanagi GK, Bohme DK, Bandura DR. 2005. Collision and reaction cells. In: Nelms SM (ed) Inductively coupled plasma mass spectrometry handbook. Blackwell, Oxford, UK, pp 336-384. <https://doi.org/10.1002/9781444305463.ch8>
30. Funk W, Dammann V, Donnevert G, Ianelli S, Ianelli E. 2007. Quality assurance in analytical chemistry: applications in environmental, food, and materials analysis, biotechnology, and medical engineering. (2nd ed) Wiley-VCH, Weinheim, Germany. <https://doi.org/10.1002/9783527609444>
31. Slejkovec Z, Stajnko A, Falnoga I, Lipej L, Mazej D, et al. 2014. Bioaccumulation of arsenic species in rays from the northern Adriatic sea. *Int J Mol Sci* 15(12): 22073-22091. <https://doi.org/10.3390/ijms151222073>
32. Frantzen S, Maage A, Duinker A, Julshamn K, Iversen SA. 2015. A baseline study of metals in herring (*Clupea harengus*) from the Norwegian Sea, with focus on mercury, cadmium, arsenic and lead. *Chemosphere* 127: 164-170. <https://doi.org/10.1016/j.chemosphere.2015.01.037>
33. Commission Regulation (EU) 2015/1006 of 25 June 2015 amending Regulation No 1881/2006 as regards maximum levels of inorganic arsenic in foodstuffs.