Application of GF-AAS Methods for As^{3+} and As^{5+} Determination in Fish Products

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Received 5 February 2004

Methods of As^{3+} and As^{5+} toxic form determination in commerce fish products in Slovakia are described. Conditions of As^{3+} and As^{5+} extraction from sea fish products were optimized. Recovery of As^{3+} and As^{5+} extraction method was determined: at As^{3+} determination in fish it was 80—102 %, at As^{5+} determination in frozen sea fish it was 80—106 %. Portion of As^{3+} from total arsenic content estimated in frozen sea fish was from 3.1 to 15.3 %, and in tuna cans from 3 to 5.7 %. Portion of As^{5+} from total arsenic content was 0—12 % in frozen sea fish and 3.3—6.3 % in tuna cans. GF-AAS method was used for total arsenic content and As^{3+} and As^{5+}species determination. Samples were decomposed in microwave high-pressure system with decomposition mixture 4 cm^3 HNO_3 and 0.5 cm^3 H_2O_2. LOD was determined at the level 0.003 mg kg^{-1} and LOQ at 0.006 mg kg^{-1}. Combined uncertainty of measurement was 7.1 %. The trueness of the method was tested by determination of arsenic concentration in Tuna fish IMEP-20 reference material. The analyzed value was 4.56 mg kg^{-1}, s_w = 0.40 mg kg^{-1}, while certified value was 4.93 mg kg^{-1}, s_w = 0.21 mg kg^{-1}. Estimated contents of toxic arsenic forms in fish products on Slovak market were relatively low and total sum of them never exceeded 20 % portion of total arsenic content. This is in accordance with published data on content of inorganic arsenic in fish products.

Seafood products are not only an important source of nutrients, minerals, and vitamins, but at the same time they are a source of various microelements that, in certain quantities, may be toxic for human beings. One of these elements is arsenic, the toxic potential of which is unquestionable. Approximately 25 chemical forms of As have been detected in seafood [1]. Inorganic arsenic As^{3+} and As^{5+} are the most toxic species, the toxicity of organic arsenical species is lower, and trimethylated species are recognized to be the least toxic [2].

The different chemical forms of arsenic and their different degrees of toxicity make the determination of arsenic species necessary as a basis on which to establish the possible toxicological implications of the arsenic contents of a product. For determination of different As forms only a few analytical methods exist. For analysis of inorganic arsenic in marine food samples hydrochloric acid distillation and flow-injection hydride-generation atomic absorption spectrometry can be used [1]. Different arsenic species in seafood products were separated by means of a switching column system and quantified by the HG-AAS method [3]. For separation of inorganic and organic arsenic compounds from fish matrix some distillation methods can be used [4]. The most common procedures for the total arsenic content determination are atomic emission, fluorescence, and absorption spectrometry. The hydride generation technique has become the most widely used approach, because volatile arsenic hydride is separated from interfering matrix. Determination of arsenic in food by FI-HG-AAS and GF-AAS methods requires complete decomposition of food matrix by acids in the closed system, which enables determination of only total arsenic content. Determination of As^{3+} and As^{5+} is possible by the extraction or ion-exchange chromatographic method. Hydride method enables the direct arsenic As^{3+} form determination in water. *Schaumloeffel* and *Neidhart* [5] optimized this method for As^{3+} and As^{5+} determination in drinking water at LOD of total arsenic of 0.5 μg dm^{-3} and for As^{3+} form at 0.4 μg dm^{-3}. *Chwastowska et al.* [6] used for As^{3+} separation acrylate gum (Bio Beads SM-7) in natural water. *Torralba et al.* [7] compared three multivarietal calibration methods for determination of different arsenic forms in drinking and sea water by the HG-AAS method. For some arsenic forms determination in drinking water *Stummeyer et al.* used coupling of HPLC and hydride method of atomic absorption spectrometry [8]. *Herce-Pagliai et al.* [9] separated organic and inorganic species of arsenic in beer and used for this purposes ion-exchange chromatography. As^{5+} form of arsenic in beer was not found. *Munoz et al.* [10] determined As^{3+} and As^{5+} compounds in sea fish products by inorganic arsenic extraction into the chloroform and re-extraction into 1 mol dm^{-3} HCl solu-

tion. Trueness of determination for As^{3+} was 99 % and for As^{5+} 96 %. *Amran et al.* [11] analyzed six species of arsenic in sea food products by HPLC-ICP-OES and HPLC-HG-QFAAS methods. *Oygard et al.* [1] used for inorganic arsenic determination in sea-food products classical distillation in $AsCl_3$ form.

No common limit values exist for food products within the EU, though the "Joint Expert Committee on Food Additives and Contaminants" suggested a PTWI-value (provisional tolerable weekly intake) of 15 μg inorganic arsenic kg^{-1} body mass. In Slovakia the content of total arsenic in sea fish products is limited to the value of 5.0 mg kg^{-1}, and 1 mg kg^{-1} in the sweet-water fish products [12].

The purpose of this work was modification of extraction and AAS method for total arsenic and its As^{3+} and As^{5+} species determination in commercial fish products in Slovakia.

EXPERIMENTAL

All chemicals, acids, and solvents used were of anal. grade. Redistilled water was used throughout the experiment. Calibration solution for As^{5+} determination was prepared from standard solution of As_2O_5, 1.000 g dm^{-3}, Titrisol (Merck), and for As^{3+} determination from standard solution of $As(NO_3)_3$, 1.000 g dm^{-3}, CertiPUR (Merck).

Total arsenic content in digested and extracted fish samples was determined by an internal accredited and validated GF-AAS method. Atomic absorption spectrometer Perkin—Elmer 4100 (Norwalk, CT, USA) coupled with graphite furnace HGA-700 and autosampler AS-70 was used. Conditions of measurement were: wavelength 193.7 nm, current in EDL 360 mA, split 0.7 nm, background correction on, and argon gas 4.6. The pyrolysis temperature with palladium nitrate—magnesium nitrate modifier was 1300 °C and the atomization temperature was 2400 °C. Parameters of the method were LOD 0.003 mg kg^{-1}, LOQ 0.006 mg kg^{-1}, range of measurement from 0.006 to 2.5 mg kg^{-1}, and extended uncertainty of measurement 14.2 %. The trueness of the method was tested by determination of arsenic concentration in Tuna fish IMEP-20 reference material. The analyzed value was 4.56 mg kg^{-1}, $s_w = 0.40$ mg kg^{-1}, while certified value was 4.93 mg kg^{-1}, $s_w = 0.21$ mg kg^{-1}.

Determination of sample digestion for As^{3+} and As^{5+} was realized as step 1: To 0.2 g dried fish (or 2.0 g frozen fish, 1.0 g tuna in can) 4 cm^3 of redistilled water were added and homogenized until a fine suspension was obtained. Then 3.5 cm^3 of concentrated $HClO_4$ and 25 mg $Fe_2(SO_4)_3$ were added, mixed and digested at 80 °C for 60 min. After cooling to room temperature, the digest was transferred with water to 10 cm^3 volumetric flask and diluted to this volume.

Step 2 was determination of As^{3+} form: 5 cm^3 of digested sample were added into the separation funnel with 10 cm^3 of concentrated HCl and well shaken. Then 10 cm^3 of chloroform were added and shaken for 3 min. Chloroform fraction was decanted to the second separation funnel. Into the first funnel again 10 cm^3 of HCl were added and shaken for 3 min. From the joined chloroform fractions in the second separation funnel As^{3+} was extracted into 10 cm^3 of 1 M-HCl and determined by GF-AAS.

Determination of As^{5+} form was realized as step 3: Into the residual water fraction (step 2), from which As^{3+} was extracted and determined, 1 cm^3 of HBr and 15 mg hydrazine sulfate were added for reduction of As^{5+} to As^{3+} form. After incubation at 80 °C for 30 min As^{3+} was extracted with 10 cm^3 of chloroform for 3 min. Chloroform fraction was decanted and 10 cm^3 of chloroform added to the water phase. After 3 min extraction chloroform phase was decanted and joined with the first one. Then As^{3+} was extracted into 10 cm^3 of 1 mol dm^{-3} HCl and determined by GF-AAS.

We compared the extraction method of determination of inorganic arsenic with the distillation method. Into the distillation flask 1—2.0 g frozen fish was weighed, 15 cm^3 of 3 mol dm^{-3} HCl and 5 cm^3 of 30 % KI solutions added and kept under the refrigerator for 5 min. First 10 cm^3 of distillate was captured and then 15 cm^3 of 3 mol dm^{-3} HCl and 5 cm^3 of 30 % KI were added to distillation flask and kept for 5 min under the refrigerator. 10 cm^3 of distillate was taken, joined with the first phase. To these distillates rinsing refrigerator water was added and volume completed to 25 cm^3 with redistilled water. Inorganic arsenic content was then determined by GF-AAS.

RESULTS AND DISCUSSION

In Tables 1—3 are results of recovery of As^{3+} and As^{5+} content determined by the extraction method. Recovery was assessed in frozen fish – sea pike and tuna in oil by analysis of three fortified samples. Fortification of samples was performed before extraction using solution of As^{3+} and As^{5+}. In all cases recovery of determination is very good at all levels of added arsenic species to the fish product samples.

At As^{3+} and As^{5+} extractions small sample from each fraction was taken for arsenic content determina-

Table 1. Recovery of As^{3+} Extraction from Frozen Fish – Sea Pike

$\rho(As^{3+}$ added)	$\rho(As^{3+}$ extracted)	$\rho(As^{3+}$ found)	Recovery
μg dm^{-3}	μg dm^{-3}	μg dm^{-3}	%
0	1.0	0	–
20	19.7	18.7	93.5
28	29.6	28.6	102.1

Table 2. Recovery of As^{5+} Extraction from Frozen Fish – Sea Pike

$\rho(As^{5+}$ added)	$\rho(As^{5+}$ extracted)	$\rho(As^{5+}$ found)	Recovery
μg dm^{-3}	μg dm^{-3}	μg dm^{-3}	%
0	3.5	0	–
5	8.9	5.4	108.0
10	13.4	9.9	99.0
15	18.2	14.7	98.0

Table 3. Recovery of As^{5+} Extraction from Tuna in Oil

$\rho(As^{5+}$ added)	$\rho(As^{5+}$ extracted)	$\rho(As^{5+}$ found)	Recovery
μg dm^{-3}	μg dm^{-3}	μg dm^{-3}	%
0	1.1	0	–
10	9.9	8.8	88.0
16	14.9	13.8	86.3

tion. Balance of arsenic species was observed in dried fish samples.

Average content of arsenic and standard deviation of measurement were calculated from five repeated extractions (Table 4). Results of arsenic species balance refer to approximately 100 % of inorganic arsenic content. Similar arsenic content should be measured in the 2nd and the 4th fraction, but lower amount in the 4th fraction was observed because of action of hydrazine sulfate and HBr on measured signal sensitivity at reduction of As^{5+} to As^{3+}. In chloroform fraction arsenic was not found. The balance of observed arsenic species is acceptable and corresponds to total content of this element in fish matrix.

Found average values for contents of total arsenic, inorganic arsenic determined by the distillation method, and extracted As^{3+} and As^{5+} forms are presented in Table 5. According to these results we can see a very good commensurability of used methods, but for practice uses distillation method is less laborious than extraction.

Tables 5 and 6 show the results of total arsenic content and content of its forms in sea fish products. Measured values of As^{3+} and As^{5+} inorganic forms of arsenic in fish and fish products are relatively low and their sum did not exceed 20 % of total arsenic content, which is in accordance with published data about inorganic arsenic in fish products [4]. Found values are similar to the contents of arsenic species referred for example by *Holak* and *Specchio* [13], *Munoz* [10], *etc.* Present work confirmed the assumption that the major part of the arsenic in fish exists as non- or low-toxic organic compounds [14]. Found values of total arsenic content in fish products did not exceed the limit value defined by the Slovak Food Codex.

The detection limit of the method defined as the arsenic concentration of the reagent blanks corresponding to the three-fold standard deviation of these blanks ($n = 10$) and the quantitation limit corresponded to the ten-fold standard deviation of the reagent blanks.

CONCLUSION

The procedures described provide some useful detailed information concerning the presence of arsenic species in fish products. Measured values of As^{3+}

Table 4. Balance of As^{3+} and As^{5+} Extraction from Dried Fish Cod ($n = 5$)

Fraction	ρ(As)/(μg dm^{-3})	w_r(As)/(mg kg^{-1})	w / %
1 Total As	60 ± 6	1.98 ± 0.198	100 ± 10
2 After As^{3+} extraction	56 ± 2	1.848 ± 0.066	93.3 ± 3.5
3 Extracted As^{3+}	5 ± 1	0.165 ± 0.033	8.3 ± 1.7
4 Reduced As^{5+} from the 2nd fraction	50 ± 3	1.65 ± 0.099	83.3 ± 5.0
5 Extracted As^{5+}	6 ± 1	0.198 ± 0.033	10.0 ± 1.7
6 As after inorg. As extraction	43 ± 4	1.419 ± 0.132	71.7 ± 6.7

Table 5. Comparison of Distillation and Extraction Methods for Inorganic Arsenic Determined in Fish Products ($n = 3$)

Sample	As total w_r / mg kg^{-1}	Inorganic As Distillation method w_r/(mg kg^{-1})	As^{3+} + As^{5+} Extraction method w_r/(mg kg^{-1})	w(from total As) / %
Frozen fish	0.222 s_w = 0.020	0.041 s_w = 0.006	0.042 s_w = 0.004	18.5—18.9
Dried fish	1.043 s_w = 0.092	0.187 s_w = 0.016	0.192 s_w = 0.020	17.9—18.4

Table 6. Total As Content and Proportion of As^{3+} and As^{5+} in Fish Products

Sample	n	Proportion of As^{3+} from total As w/%	Proportion of As^{5+} from total As w/%	Total As content w_r/(mg kg^{-1})
Salmon	4	9.7—15.3	5.4—10.7	0.109—0.117
Cod frozen	5	3.1—8.0	9.0—12.0	0.102—0.260
Sea pike	4	0—9.2	0—9.3	0.180—0.376
Tuna canned	4	3.0—5.7	3.3—6.3	0.409—0.520

and As^{5+} inorganic forms of arsenic in fish and fish products were relatively low and their sum did not exceed 20 % of total arsenic content. Present work confirms the assumption that the major part of the arsenic in fish exists as non- or low-toxic organic compounds. Modified extraction method for arsenic species determination was in a good relation to the distillation method, but it was more laborious. Found values of total arsenic content in fish products did not exceed the limit values determined by the Slovak Food Codex [12].

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