

## Determination of Arsenic in the Rainbow Trout Muscle and Rice Samples

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**Abstract:** The content of arsenic was determined in the rainbow trout muscle (*Oncorhynchus mykiss*) and in rice samples. The method of atomic absorption spectrometry with hydride generation (HG-AAS) in graphite furnace was used for total arsenic determination. Arsenic speciation was done using the high performance liquid chromatography-hydride generation-atomic fluorescence spectrometry (HPLC-HG-AFS). The rainbow trout muscle was extracted by ultra pure deionised water; trifluoroacetic acid was used for the extraction of rice samples. Total arsenic values ranged from 60.90 µg/kg to 310.00 µg/kg in rice samples and from 0.72 mg/kg to 2.23 mg/kg in rainbow trout muscle. An inorganic trivalent arsenic As(III) was determined as the main species in rice and non-toxic arsenobetaine in the samples of rainbow trout.

**Keywords:** arsenic; arsenobetaine; fish muscle; rice; atomic absorption spectrometry; atomic fluorescence spectrometry

### INTRODUCTION

The toxic elements content is one of the main indicators of the hygienic quality of foods. Arsenic is known as a toxic element but its extent of toxicity depends on its chemical form (CORNELIS *et al.* 2005). Determination of the total element content is not sufficient in the case of arsenic.

Arsenic bonded in organic compounds is usually less toxic than arsenic bonded in inorganic compounds. For instance, trivalent inorganic arsenic compounds display a relatively high toxicity whereas the organic compound arsenobetaine is considered to be non-toxic (SOUDEK *et al.* 2006). Arsenobetaine forms a significant part of the overall arsenic sea and freshwater fish species. Little information is available about the arsenic compound species in freshwater and land plants. Based on the limited amount of information available, it seems that as compared to the sea organisms, arsenic

present in freshwater and land organisms namely in the inorganic form (SOUDEK *et al.* 2006).

The highest values of arsenic concentrations are found in sea fish food where arsenobetaine content ranges in the concentrations from 1 to 10 mg/kg. As for the significant sources of arsenic, rice is on the top the same way as mineral water and beer. The concentrations of arsenic in the seashells living at the seabed were more than 100 mg/kg (MANDAL & SUZUKI 2002).

### MATERIAL AND METHODS

**Samples for analysis.** Muscle tissue of 5 rainbow trout available on the market and 12 samples from an intensive fish farming operation was used. Fish from the intensive fish farm operation were kept in vats with potable water. The majority of their feed consisted of a feed mixture

with fish meal content. In market rainbow trout samples, internal organs were analysed – liver and a mixed sample of spleen. Analysis was carried out also in 12 samples of rice available on the Czech market.

**Sample preparation for the total arsenic determination.** Samples were mineralised using wet way (nitric acid 1:1 with hydrogen peroxide), then burned in a muffle furnace and reduced to As(III) by addition of KI in the ascorbic acid (ČELECHOVSKÁ *et al.* 2005).

**Total arsenic determination.** The total content of arsenic was determined using the atomic absorption spectrometry with hydride generation (HG-AAS) in graphite furnace. Total arsenic was established using the ContrAA 700 (Analytik Jena, Germany) device at the wavelength of 193.7 nm. 0.3% NaBH<sub>4</sub> in 1% NaOH and 1.5% HCl was used for analysis. The measurement validity was tested by certified standard material DORM-2 (NRC, dogfish muscle), which contains of  $18.0 \pm 1.1$  mg/kg of arsenic.

**Sample extraction for speciation analysis.** The fish samples were added 10 ml of deionised water, then they were moved into an ultrasonic bath for 2 h at the temperature of 50°C, centrifuged and filtered (NASH & MCSHEEBY 2005). Rice samples were added trifluoroacetic acid and the mixture was extracted over a period of 6 hours in water bath of the temperature of 100°C. After extraction finished, the samples were added deionised water, the samples were centrifuged, filtered and added with deionised water into 10 ml flasks (HEITKEMPER *et al.* 2001).

**Speciation analysis.** Speciation method was done using separation by high performance liquid chromatography and detection by atomic fluorescence spectrometry with hydride generation (HPLC-HG-AFS). An Excalibur atomic fluorescence detector (PS Analytical, UK) equipped with a boosted discharge hollow cathode lamp (Photron, Australia) was used for detection.

The measurements were carried out on the wavelength of As 193.76 nm. As mobile phase, 10 mmol/l phosphate buffer K<sub>2</sub>HPO<sub>4</sub>/KH<sub>2</sub>PO<sub>4</sub>, pH 6.1 was used. For the hydride generation, 1.4% NaBH<sub>4</sub> in 0.1 mol/l NaOH and 6 mol/l HCl was used. An oxidising agent was provided for by the solution of a 1% concentration K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> in 1% NaOH. Oxidation was supported by UV. When analysing samples containing arsenobetaine it was necessary to analyse the sample

twice, once with the UV lamp switched off and once with it switched on. This is due to the fact that arsenobetaine coelutes with arsenite. If the UV lamp is off arsenobetaine do not react with NaBH<sub>4</sub> and do not give a signal.

The measurement validity was tested by certified standard material DORM-2 (NRC, dogfish muscle), which consists of  $16.4 \pm 1.1$  mg/kg arsenobetaine.

## RESULTS AND DISCUSSION

In the muscle tissue of rainbow trout, from 0.72 mg/kg to 2.23 mg/kg of total arsenic was found whereas the content of total arsenic in the intensive fish farm operation trout was slightly higher than in market trout. The difference in values could have been caused by the fact that intensive fish farm trout were kept in vats with potable water and they were fed only with a mixture of sea fish meal. These feed mixtures contain much higher arsenic concentrations, at the limit value most of the time. Total arsenic content in the liver of market trout ranged from 0.34 mg/kg to 0.69 mg/kg. The average value of total arsenic in the mixed spleen sample was 0.027 mg/kg. Total arsenic order in muscle tissues in tissues was established: muscle > liver > spleen. The arsenic order matches published data (ČELECHOVSKÁ *et al.* 2007). Concentration of total arsenic in the certified standard material DORM-2 (NRC, dogfish muscle) was 17.1 mg/kg. Limit of detection (LOD) was found to be 3 S/N (signal/noise ratio) 0.170 µg/l, limit of quantification (LOQ, found at 10S/N) was 0.634 µg/l. The Figure 1 shows the time spectrum of rainbow trout muscle sample. The amount of total arsenic in rice samples ranged from 60.90 µg/kg to 310.00 µg/kg.

Extractable arsenic was present in the form of non-toxic arsenobetaine in all analysed samples of fish. After water extraction, no other species were found. Arsenobetaine amount contained in analysed in trout samples ranged from 0.50 mg/kg to 1.55 mg/kg. As regards speciation, arsenobetaine content in rainbow trout muscle tissue from the intensive fish farm operation was higher than in market samples. Muscle chromatogram of rainbow trout muscle tissue is to be seen on Figure 2. Arsenobetaine content in liver of market trout ranged from 0.05 mg/kg

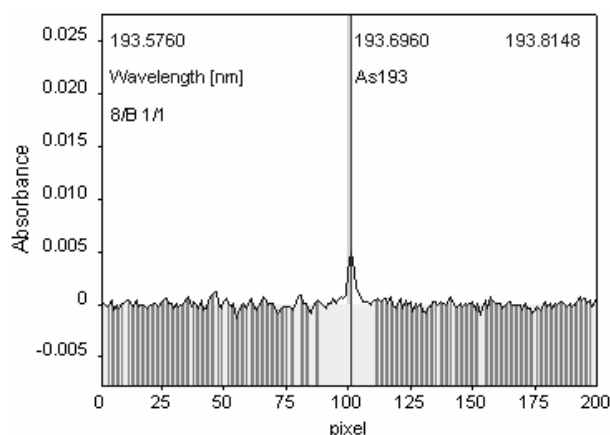


Figure 1. Linear spectrum of a rainbow trout

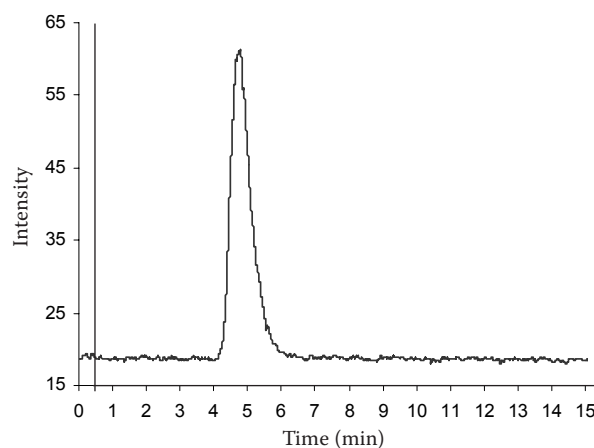


Figure 2. Trout sample chromatogram – arsenobetaine peak

to 0.20 mg/kg, its average content in the mixed spleen sample was 0.013 mg/kg. Concentration of arsenobetaine in the certified standard material DORM-2 (NRC, dogfish muscle) was found to be 17.49 mg/kg. Especially inorganic species As(III) and As(V) were found in rice samples. Their concentration was at low levels but the presence of toxic inorganic species of arsenic in the terrestrial plants was confirmed. From the species present, the most frequent was As(III), being detected in all analysed rice samples but only four samples displayed values above the limit of detection and ranged from 77.04 µg/kg to 122.64 µg/kg.

### CONCLUSIONS

Muscle tissue in rainbow trout was found to contain 0.72–2.23 mg/kg of total arsenic and 0.50 to 1.55 mg/kg of arsenobetaine. The amount of total arsenic in rice samples was 60.90–310.0 µg/kg. The most common arsenic species was As(III).

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