Occurrence of inorganic arsenic in edible Shiitake (Lentinula edodes) products

Toni Llorente-Mirandes a, Mercedes Barbero b, Roser Rubio a,c and José Fermín López-Sánchez a,c,*

a Department of Analytical Chemistry, University of Barcelona, Martí i Franquès 1-11, Barcelona E-08028, Spain
b Department of Vegetal Biology, University of Barcelona, Av. Diagonal 643, Barcelona E-08028, Spain
c Water Research Institute, University of Barcelona, Av. Diagonal 684, Barcelona E-08034, Spain

*Corresponding author: Department of Analytical Chemistry, University of Barcelona, Martí i Franquès 1-11, Barcelona E-08028, Spain. Tel.: +34 934034873. E-mail address: fermin.lopez@ub.edu (José Fermín López-Sánchez).

Abstract

The present study reports arsenic speciation analysis in edible Shiitake (Lentinula edodes) products. The study focused on the extraction, and accurate quantification of inorganic arsenic (iAs), the most toxic form of arsenic, which was selectively separated and determined using anion exchange LC-ICPMS. A wide variety of edible Shiitake products (fresh mushrooms, food supplements, canned and dehydrated) were purchased and analysed. A cultivated Shiitake grown under controlled conditions was also analysed. The extraction method showed satisfactory extraction efficiencies (>90%) and column recoveries (>85%) for all samples. Arsenic speciation revealed that iAs was the
major As compound up to 1.38 mg As kg\(^{-1}\) dm (with a mean percentage of 84% of the total arsenic) and other organoarsenicals were found as minor species. Shiitake products had high proportions of iAs and therefore should not be ignored as potential contributors to dietary iAs exposure in populations with a high intake of Shiitake products.

Keywords: Inorganic arsenic; Shiitake; *Lentinula edodes*; Mushrooms; Arsenic speciation; LC-ICPMS.

1. Introduction

The consumption of wild edible mushrooms has increased worldwide during recent years. *Lentinula edodes* (Berk.) Pegler (also known by its Japanese name of Shiitake) is one of the five most cultivated edible mushrooms in the world, being particularly popular in China, Japan and other Asian countries (Kalč, 2013; Chang & Miles, 2004). Furthermore, it is a dietary source of protein, vitamin D, B complex vitamins and minerals. It is one of the best-known and best-characterized mushrooms, having been used in medicine for thousands of years. *Lentinula edodes* mycelium extract and its purified fractions have many physiological properties including antitumour, antiviral, antioxidant, antifungal, hypoglycaemic and immunomodulatory activity (Chang & Miles, 2004; Wasser, 2002).

Regarding the toxicological aspects of arsenic in food, inorganic arsenic (iAs, arsenite or As(III) and arsenate or As(V)) is considered to be the most dangerous form due to its biological availability and physiological and toxicological effects (iAs is classified as a nonthreshold, class 1 human carcinogen) (ATSDR Toxicological profile...
for arsenic, 2007). Other arsenic compounds, such as arsenobetaine (AB), are non-toxic and can be consumed without concern, while arsenosugars are potentially toxic (Feldmann & Krupp, 2011). Therefore, toxicological knowledge of the different arsenic species should be considered by legislators and regulators when establishing maximum arsenic levels in food directives.

The ability of some mushroom species to accumulate arsenic may represent a serious risk to consumer health (Dembitsky & Rezanka, 2003; Falandysz & Borovička, 2013; Kalač, 2010; Vetter, 2004). The arsenic content of mushrooms is regulated by genetic factors and natural conditions (type of soil, bedrock, habitat, environmental factors) (Falandysz & Borovička, 2013; Vetter, 2004). More than 50 different naturally occurring As-containing compounds have been identified, comprising both organic and inorganic forms (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2009). Some of these have been found in mushrooms, including methylarsonate (MA), dimethylarsinate (DMA), As(V), As(III), AB, arseno-choline (AC), trimethylarsine oxide (TMAO), tetramethylarsonium cation (TETRA) and arsenosugars (Koch et al., 2013; Koch, Wang, Reimer, & Cullen, 2000; Larsen, Hansen, & Gössler, 1998; Niedzielski, Mleczek, Magdziak, Siwulski, & Kozak, 2013; Šlejkovec, Byrne, Stijve, Goessler, & Irgolic, 1997; Smith, Koch, & Reimer, 2007; Soeroes, Kienzl, Ipolyi, Dernovics, Fodor, & Kuehnelt, 2005).

The arsenic compounds in edible mushrooms are obviously of concern to the consumer and the regulatory authorities, but currently, no limits exist in the European Union (EU) on arsenic, either total or inorganic, in foods (European Union Regulation 1881/2006). On the other hand, China has a maximum allowable concentration of total arsenic in mushrooms of 0.5 and 1.0 mg As kg\(^{-1}\), for fresh and dry mushrooms, respectively (MHC, 2003; MHC, 2005). Given this situation, the European Food Safety
Authority (EFSA) (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2009) and the FAO/WHO Joint Expert Committee on Food Additives (JECFA) (FAO/WHO, Evaluation of certain contaminants in food, 2011) have evaluated dietary exposure to As. Both reported the urgent need for further data on arsenic species, particularly iAs data, in food commodities, in order to improve the background data for future risk assessment analysis. Furthermore, mushrooms were included among the foods that contribute to iAs exposure in the general European population (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2009). The report also highlighted the need for a robust validated analytical method for the determination of iAs in a range of food items. To this end, several proficiency tests (PTs) on iAs in different foodstuffs have been organised (de la Calle et al., 2011; Baer et al., 2011; de la Calle et al., 2012).

Satisfactory performance was generally found for the determination of iAs in rice, wheat and vegetable food; and it was also emphasized that there is no reason to consider the option of introducing possible maximum levels for iAs in rice, wheat, vegetable food and algae, in further discussions on risk management.

Due to the increasing focus on inorganic arsenic in food and given that mushroom consumption had increased considerably in recent years due to their nutritional properties, two PTs, using the same test item, IMEP-116 and IMEP-39, were organised by the Institute for Reference Materials and Measurements (IRMM) (Cordeiro et al., 2013). Thus, the total and inorganic arsenic content in mushrooms is a topic of current priority for the Directorate for Health and Consumers (DG SANCO) of the European Commission. The iAs concentration in the Shiitake test sample was quite high, at around 0.3 mg As kg$^{-1}$, accounting for 50% of the total As. Therefore, arsenic speciation data, particularly iAs data, for Shiitake samples are needed to estimate the health risk associated with dietary As exposure.
Although Shiitake has medicinal properties and is one of the most consumed and
cultivated mushrooms, few studies of arsenic speciation appear in the literature
(Wuilloud, Kannamkumarath, & Caruso, 2004). Thus, more studies on Shiitake are
required to provide information about iAs levels, which would be useful in toxicological
risk assessments. Therefore, the main goal of this study was to determine total arsenic
and arsenic species in several edible Shiitake products. The study focused on the
extraction, identification and accurate quantification of the toxic inorganic arsenic
species. In addition, a preliminary study of Shiitake cultivation was performed in a
small-scale mushroom facility in order to estimate the possible health risks of home-
cultivated Shiitake grown on a commercial substrate. Fruiting bodies and substrate
samples were investigated for total arsenic and arsenic species.

2. Materials and methods

2.1. Reagents and standards

All solutions were prepared with doubly deionised water obtained from
Millipore water purification systems (Elix & Rios) (18.2 MΩ cm⁻¹ resistivity and total
organic carbon <30 µg L⁻¹). Nitric acid (69%, Panreac, Hiperpur) and hydrogen
peroxide (31%, Merck, Selectipur) were used for the digestion and extraction
procedures. Ammonium dihydrogen phosphate (Panreac, p.a.), ammonia solution (25%,
Panreac, p.a.), pyridine (Scharlau, p.a.) and formic acid (98%, Panreac, p.a.) were used
to prepare mobile phases.

External calibration standards for total As were prepared daily by dilution of a
standard stock solution traceable to the National Institute of Standards and Technology
(NIST), with a certified concentration of 1000 ± 5 mg As L⁻¹ (Inorganic Ventures
Standards). An arsenate standard solution of 1000 ± 5 mg As L⁻¹ (Merck) was used for external quality control in total arsenic and arsenic speciation measurements.

Stock standard solutions (1000 mg As L⁻¹) for arsenic speciation were prepared as follows: As(III), from As₂O₃ (NIST, USA, Oxidimetric Primary Standard 83d, 99.99%) dissolved in 4 g L⁻¹ NaOH (Merck, Suprapure); As(V), from Na₂HAsO₄·7H₂O (Carlo Erba) dissolved in water; MA, prepared from (CH₃)AsO(ONa)₂·6H₂O (Carlo Erba) dissolved in water; and DMA, prepared from (CH₃)₂AsNaO₂·3H₂O (Fluka) dissolved in water. AC from (CH₃)₃As⁺(CH₂)CH₂OHBr⁻ was supplied by the “Service Central d’Analyse” (CNRS Vernaison, France); and a certified reference material of AB from (CH₃)₃As⁻CH₂COO⁻ was supplied by National Metrology Institute of Japan (NMIJ, Japan) as NMIJ CRM 7901-a, standard solution. TMAO was prepared from (CH₃)₃AsO (Argus Chemicals srl) dissolved in water. Arsenate, arsenite, DMA, MA, AC, TMAO and AB were standardised against As₂O₃ for our internal quality control. All stock solutions were kept at 4 °C, and further diluted solutions for the speciation analysis were prepared daily.

2.2 Samples and certified reference materials

Different types of Shiitake-based food commodities that are representative of all types of edible Shiitake products consumed in Spain, were purchased from markets, local supermarkets and retail stores in Barcelona, Spain, during 2012. A selection of edible Shiitake products was analysed: five fresh, four dehydrated, three canned and two food supplement samples. The three canned Shiitake are commercialised in glass vessels. According to the manufacturer, Shiitake food supplements contain both mycelium and primordia (young fruit body) cultivated into a biomass that is grown on a
sterilised (autoclaved) substrate. Various brands were purchased and all samples were brought to the laboratory on the day of purchase and kept for no more than a day in the refrigerator until sample preparation, which was performed before the recommended time of consumption.

In addition, Shiitake was home-cultivated in a small-scale facility, from which mushrooms were collected as samples for further analysis, to expand the information collected in the study.

Two certified reference materials (CRMs) and a reference material (RM) were analysed during the study. NIST SRM 1570a spinach leaves was obtained from the NIST (Gaithersburg, MD, USA). WEPAL IPE-120 reference material *Agaricus bisporus* mushroom was produced by the Wageningen Evaluating Programs For Analytical Laboratories (WEPAL, Wageningen, The Netherlands). ERM-BC211 rice was obtained from the IRMM of the European Commission’s Joint Research Centre (Geel, Belgium).

### 2.3 Apparatus and instrumentation

Mushroom samples were dried in an oven with natural convection (Digitronic, JPSelecta, Spain). The dried mushrooms were minced using a commercial mincer (Multiquick 5 Hand Processor, Spain) Braun. A microwave digestion system (Ethos Touch Control, Milestone), was used for the digestion and extraction procedures. An Agilent 7500ce inductively coupled plasma mass spectrometer (ICPMS) (Agilent Technologies, Germany) was used to determine total arsenic content. An Agilent 1200 Series LC system (Agilent Technologies, Germany) was used as the chromatographic system for arsenic speciation via coupling LC-ICPMS. The separations were performed
on an anion-exchange column (Hamilton Company, USA) and cation-exchange column (Agilent Technologies, Germany) (Table 1). The outlet of the LC column was connected via polyetheretherketone capillary tubing to the nebuliser (Burgener Research Inc, Mississauga, Canada) of the ICPMS system (Table 1).

2.4. Cultivation of Shiitake

Cultivation of Shiitake was performed in a small-scale mushroom facility belonging to the University of Barcelona. Fruiting bodies of Shiitake were produced on a commercial pasteurised substrate inoculated with mycelium intended to be grown at homemade cultivation. The cultivation procedure followed the instructions supplied by the manufacturer. The mushrooms were grown under controlled conditions following the manufacturer’s guidelines, and a large number of fruiting bodies were produced. The original substrate was submerged in tap water in a controlled chamber for 24 hours. Then, the substrate was placed in a cool damp place at a temperature of 17–20 °C, under natural indoor light cycles. After a week the fungi began to fruit and all mushrooms were harvested. After the first harvest, the substrate was air dried for 20 days. After this time, the substrate was submerged in tap water for another 24 hours and the whole process was repeated. This enabled a second Shiitake mushroom harvest. The substrate was randomly sampled in triplicate three times during the cultivation study. Care was taken to collect substrate in which mycelium was not visible to the naked eye. The original substrate was first sampled before submersion and cultivation. A second sample was taken after the first cultivation (medium substrate) and a third sample after the second cultivation (final substrate). The tap water and waste water, and the water remaining after substrate submersion, were also sampled during the
cultivation study. Total arsenic and arsenic species were analysed by ICPMS and LC-
ICPMS, respectively, in the three substrate samples and the tap and waste water
samples.

2.5 Sample pretreatment

Fresh Shiitake mushrooms were cleaned by hand of substrate and foreign matter. The end of the stalk (in contact with the substrate) was removed using a stainless steel knife. Damaged or soiled parts were cut off with a knife and smaller particles were removed using a fine brush. Only the edible parts of the mushrooms were used for the analysis. Mushrooms were cut into small pieces that were then air-dried on filter paper and further dried in an oven at 40ºC for 24–48 hours. The dried mushrooms were minced using a commercial mincer made of stainless steel until complete homogenization. Care was taken to avoid contamination. Between samples, the mincer was washed once with soap and water, rinsed once with HNO₃ (about 10%), rinsed several times with deionised water, and then rinsed three times with doubly deionised water, before drying with cleaning wipes.

Shiitake food supplements, which are commercially available as tablets, were pulverized with an agate mortar, homogenised and stored over silica gel in a desiccator until analysis.

Canned Shiitake samples were drained and then dried in an oven at 40ºC for 24–48 hours and finally minced using a commercial mincer until complete homogenisation.

Powdered samples were stored over silica gel in a desiccator until analysis.

Dehydrated Shiitake samples were cut into small pieces and then minced using a commercial mincer until complete homogenisation and stored over silica gel in a desiccator until analysis.
Cultivated Shiitake were pretreated in the same way as the purchased fresh mushrooms. Substrate samples were pulverized, homogenised, and stored over silica gel in a desiccator until analysis of arsenic and arsenic species. Tap water and waste water were filtered through PET filters (Chromafil® PET, Macherey–Nagel, pore size 0.45 μm) and stored at 4ºC before analysis of total arsenic and arsenic species.

2.6. Moisture determination

Aliquots of 0.5 g samples were dried, in triplicate, at 102 ± 3 ºC to constant weight in an oven. All the results in the study are expressed as dry mass.

2.7 Total arsenic determination

The total arsenic content of the mushroom samples, CRMs, RM and substrate samples was determined by ICPMS measurement after microwave digestion (Llorente-Mirandes, Calderón, López-Sánchez, Centrich, & Rubio, 2012). Helium gas was used in the collision cell to remove interferences in the ICPMS measurements. A solution of $^{9}$Be, $^{103}$Rh and $^{205}$Tl was used as an internal standard. Each sample was digested and analysed in triplicate. The digestion blanks were also measured. Arsenic content in the samples was quantified by means of an external calibration curve for the standards. For quality control purposes, the standards of the calibration curve were run before and after each sample series. The detection (LOD) and quantification limits (LOQ) were estimated and were 0.006 and 0.021 mg As kg$^{-1}$, respectively.

2.8 Arsenic speciation analysis
The extraction of arsenic species was based on our previous studies (Llorente-Mirandes et al., 2012; Llorente-Mirandes, Calderón, Centrich, Rubio, & López-Sánchez, 2014) and was applied here to mushroom samples, CRMs and the RM and substrate samples. Briefly, 0.25 g aliquots of the samples were weighed in PTFE vessels and then extracted by adding 10 mL of 0.2% (w/v) HNO₃ and 1% (w/v) H₂O₂ solution in a microwave system. This extraction method completely oxidises As(III) into As(V), without conversion of the other organoarsenicals into iAs. After extraction, arsenic speciation was carried out in extracts by LC-ICPMS (Llorente-Mirandes et al., 2010 and 2011) using the conditions shown in Table 1. The total arsenic in the extracts was determined by ICPMS (as described above). Arsenic species were quantified by external calibration curves. Extraction blanks were also analysed in each batch of samples. Each sample was extracted and analysed in triplicate. LOD and LOQ were estimated for each As species. The LODs for As(III), DMA, MA, As(V), AB, TMAO and AC were 0.0010, 0.0014, 0.0017, 0.0024, 0.0010, 0.0028 and 0.0018 mg As kg⁻¹, respectively. The LOQs for As(III), DMA, MA, As(V), AB, TMAO and AC were 0.0033, 0.0047, 0.0056, 0.0080, 0.0033, 0.0093 and 0.0060 mg As kg⁻¹, respectively.

3. Results and Discussion

3.1 Quality assessment in the determination of total arsenic and arsenic species

3.1.1 Total arsenic

To evaluate the accuracy of total arsenic measurements a RM and two CRMs were analysed with every batch of samples. The present results in these CRMs showed good agreement with the certified values, as shown in Table 2. The percentage accuracy was 102% and 99% for NIST SRM 1570a and ERM-BC211, respectively.
3.1.2 Extraction efficiency

Extraction efficiencies (calculated as the ratio of total As in the extract to total As in the sample) were calculated. Several extraction solvents have been used for the speciation of arsenic in mushrooms. Extraction efficiencies appear to be highly variable, depending on the mushroom species and extraction solution, ranging from 7% to 129% (Koch et al., 2000; Larsen et al., 1998; Šlejkovec et al., 1997; Slekovč, et al., 1999; Smith et al., 2007; Wuilloud et al., 2004). The present values ranged from 94 to 103% and extracted on average 98% of total arsenic (Table 3). These results indicated full extraction of the arsenic species that may exist in Shiitake mushrooms. The extraction efficiency of ERM-BC211, NIST SRM 1570a and WEPAL-IPE-120 was 98%, 93% and 99%, respectively (Table 2).

3.1.3 Column recovery

Column recovery (calculated as the ratio of the sum of the species eluted from the chromatographic columns to the total arsenic in the extract injected into the column) was calculated to guarantee the correctness of the chromatographic separation. This parameter, assessed in replicates with good reproducibility, allowed us to evaluate the quantification of the As species in mushroom samples. Values close to 100% usually indicate that all arsenic extracted was recovered from the analytical column. The present values obtained for column recoveries ranged between 87 and 104% and showed average column recoveries of 97% (Table 3). Satisfactory values were also obtained for the RMs: 102%, 92% and 94% for ERM-BC211, NIST SRM 1570a and WEPAL-IPE-120, respectively (Table 2).
3.1.4 Spiking experiments of inorganic arsenic

To assure the accurate identification and quantification of inorganic As species, three *Shiitake* samples were spiked by adding As(III) and As(V) standards to solid samples and then homogenised. The mixtures were left to stand for 30 min before extraction. Arsenate was the only inorganic species found in the spiked samples, showing the quantitative oxidation of As(III) to As(V) without conversion of the other organoarsenicals into iAs. The concentration of iAs was quantified as As(V) and determined via anion LC-ICPMS. The recovery of iAs from fresh, cultivated and food supplement samples was: 93 ± 6, 97 ± 5 and 94 ± 5, respectively (mean % ± SD, n = 3).

The results show that all of the iAs was recovered successfully (average recoveries of 95% for iAs in Shiitake samples). Furthermore, the ERM-BC211 rice material, which is certified in inorganic arsenic, was also spiked by adding As(III) and As(V) standards. The concentration of iAs was quantified as As(V) and the recovery of iAs was satisfactory: 102 ± 4%, n=3.

3.1.5 Arsenic species in the reference materials

Arsenic speciation was performed on CRMs and the RM and the results are summarised in Table 2. To date, no CRMs are available for arsenic species in mushrooms. Therefore, the ERM-BC211 rice was used throughout the study to assess the accuracy and reliability of the As speciation results. The material was analysed and the results were in agreement with the certified values. The percentage accuracy was 98% and 105% for iAs and DMA in ERM-BC211, respectively (Table 2).

The As speciation results in WEPAL-IPE-120 showed that AB was the major As species (40% of the total As). The inorganic arsenic content was 0.033 ± 0.001 mg As kg\(^{-1}\) (corresponding to 20% of the total As), while DMA accounted for 28% of the total...
As. No arsenic speciation studies on this RM mushroom have been found in the literature. However, studies on *Agaricus* sp. found that AB predominated in this mushroom genus (Koch et al., 2013; Šlejkovec et al., 1997; Smith et al., 2007; Soerões et al., 2005), which is in agreement with the present results. Although WEPAL-IPE-120 (*Agaricus bisporus*) is not certified for arsenic species, the sum of the As species (0.156 ± 0.010 mg As kg\(^{-1}\)) compared well with the indicative total As value of 0.137 ± 0.067 mg As kg\(^{-1}\). An unknown compound was found by the cationic column with a retention time of 380 s and could be attributed to TETRA due to the matching of the retention times when using the same chromatographic conditions (Kirby et al., 2004). However, it was not possible to check this attribution due to the lack of appropriate standards.

Regarding As species in the NIST SRM 1570a, inorganic arsenic was the major compound at 0.059 ± 0.005 mg As kg\(^{-1}\), which was in agreement with the reference value assigned by expert laboratories in the proficiency test IMEP-112: 0.054 ± 0.012 mg As kg\(^{-1}\) (de la Calle et al., 2012).

### 3.1.6 External quality control

This method was tested with participation as an expert laboratory in two recent proficiency tests organised by the European Union Reference Laboratory for Heavy Metals in Feed and Food (EURL-HM) and the International Measurement Evaluation Program (IMEP) from the IRMM, IMEP-116 and IMEP-39, Determination of total Cd, Pb, As, Hg and inorganic As in mushrooms. Satisfactory results were obtained compared with the assigned value for iAs, which demonstrates the validity and reliability of the present method (Cordeiro et al., 2013). Therefore, this method could be recommended for the quantification of inorganic arsenic in edible mushrooms.
3.2 Total arsenic content in purchased Shiitake

The total arsenic content in the purchased edible Shiitake products is shown in Table 3 and ranged from 0.11 to 1.44 mg As kg\(^{-1}\) dry mass (dm). The mean arsenic concentration of 14 samples was 0.51 mg As kg\(^{-1}\) dm. Total arsenic was highest in fresh samples (n=5): 0.90 ± 0.57 mg As kg\(^{-1}\) dm (mean ± SD) with wide variability between the samples. The total arsenic content for dehydrated (n=4) and canned (n=3) samples was 0.26 ± 0.08 and 0.33 ± 0.29 mg As kg\(^{-1}\) dm, respectively. Two food supplements of different brands were analysed and the total arsenic content was 0.45 ± 0.01 and 0.12 ± 0.01 mg As kg\(^{-1}\) dm. Four of the fresh Shiitake samples exceeded the limit of 0.5 mg As kg\(^{-1}\) established by China for fresh mushrooms (MHC, 2003; MHC, 2005). However, none of the dehydrated Shiitake exceeded the limit of 1.0 mg As kg\(^{-1}\) established by China for dry mushrooms (MHC, 2003; MHC, 2005).

The present arsenic results are in the usual range found in mushrooms from unpolluted areas (0.5 to 5 mg As kg\(^{-1}\), Kalač, 2010). However, arsenic content appears to be highly variable, with significant differences according to the soil arsenic concentration as well as the ability of mushroom species to accumulate arsenic (Falandysz & Borovička, 2013; Kalač, 2010). To date, there are few studies of arsenic content in Shiitake in the literature. Several Shiitake purchased in Brazil contained As in concentrations ranging between 0.083 and 0.210 mg As kg\(^{-1}\) dm (Maïhara, Moura, Catharino, Castro, & Figueira, 2008). Another study reported an arsenic content of 1.3 mg As kg\(^{-1}\) dm in a Shiitake sample (Wuilloud et al., 2004). According to Haldimann and co-authors (Haldimann, Bajo, Haller, Venner, & Zimmerli, 1995), the As content in five Shiitake mushrooms varied from 0.04 to 0.07 mg As kg\(^{-1}\) dm. The available results on arsenic in Shiitake-based food are limited and conflicting. Given the number of samples analysed in the present study and the small amount of data available in the
literature, the present values of arsenic content cannot be generalized to indicate the
congJtrations commonly present in Shiitake mushrooms.

3.3 Arsenic species in purchased Shiitake

The arsenic speciation results for the purchased edible Shiitake products are
shown in Table 3. Inorganic arsenic was the predominant As compound in all Shiitake
products and ranged from 0.086 to 1.38 mg As kg\textsuperscript{-1} dm, with a mean value of 0.43 mg
As kg\textsuperscript{-1} dm. Inorganic arsenic accounted for 53 to 99% of the total arsenic with a mean
percentage of 84% of the total arsenic, whereas DMA, MA, AB, and TMAO accounted
for a few percent of the total arsenic. DMA accounted for 2.7 to 28%, MA accounted
for 1.6 to 7.6% and AB accounted for 0.4 to 5.5% of the total arsenic. TMAO was only
quantified in one sample, accounting for 3.1% of total arsenic, and AC was below the
LOQ in all samples. An unknown compound separated by the anionic column was
found in one sample of fresh Shiitake, with a retention time of 255 s. This unknown
anionic arsenic species could be a phosphate arsenosugar. This hypothesis is supported
by the fact that the retention time of phosphate arsenosugar, present in \textit{Fucus serratus}
extract, matches the retention time of the present unknown peak, when using the same
chromatographic conditions (Madsen, Goessler, Pedersen, & Francesconi, 2000).
However, due to the lack of appropriate standards, this identification was not checked.

The finding that almost all the arsenic in the present edible Shiitake products
was present as inorganic As is shown in Figure 1. An example of this behaviour is
illustrated in an anion exchange chromatogram of fresh Shiitake extract in which iAs
was identified as the main arsenic species; DMA was also clearly detected and traces of
MA and cationic species were also present.
To date and to our knowledge, few studies on arsenic speciation in Shiitake are present in the literature. A study on inorganic arsenic content in Hong Kong foods found an iAs value ranging from 0.036 to 0.053 mg As kg\(^{-1}\) dm in dehydrated Shiitake samples (Wong, Chung, Chan, Ho, & Xiao, 2013). Our results on iAs in dehydrated samples (n=4) are consistent with this study, with a mean value of 0.21 mg As kg\(^{-1}\) dm corresponding to 79% of the total arsenic. Wuilloud and colleagues (Wuilloud et al., 2004) analysed Shiitake samples by size-exclusion liquid chromatography (SEC) coupled to UV and ICPMS for detection (SEC-UV-ICPMS). In their study arsenic was found to be associated mainly with a molecular weight (MW) fraction of 4.4–4.9 kDa for all extraction solvents. The authors concluded that the arsenic species are mainly in a form that is not associated with proteins or other high MW compounds, which is consistent with the present results.

Different proportions of arsenic species have been reported in the literature depending on the mushroom species (Dembitsky & Rezanka, 2003; Falandysz & Borovička, 2013; Kalač, 2010). Gonzálvez et al. (Gonzálvez, Llorens, Cervera, Armenta, & de la Guardia, 2009) reported that iAs species were the major compounds in several of the studied mushrooms and that the iAs concentration ranged from 0.14 to 0.89 mg As kg\(^{-1}\), similar to the present results. However, a high iAs content was found in *Lycoperdon* sp. mushroom samples on a gold mine site contaminated with arsenic (Koch et al., 2000). Slekovec and co-authors (Slekovec, Goessler, & Irgolic, 1999) reported that iAs was the predominant As compound, with the sum of arsenite and arsenate up to 35.5 mg As kg\(^{-1}\) dm in *Thelephora terrestris*. A recently study also found high levels of iAs of up to 27.1 and 40.5 mg As kg\(^{-1}\) dm for As(III) and As(V), respectively, for *Xerocomus badius* from different sample collection places (Niedzielski et al., 2013). Arsenic species content could depend on the environment; the site of
sample collection is an important factor that influences both the concentration and form of As present in mushroom fruiting bodies. However, it is not entirely clear whether mushrooms accumulate inorganic arsenic from the soil, or produce it through biotransformations.

The occurrence of inorganic arsenic in food is a complex subject, because foods that are usually high in arsenic, such as seafood and fish (Fontcuberta et al., 2011) or algae (Llorente-Mirandes et al., 2010 and 2011), often have a low iAs content, whereas iAs can be the major arsenic species in other foods with a lower total arsenic content, such as rice (Llorente-Mirandes et al., 2012) and cereal based-food (Llorente-Mirandes et al., 2014). Despite the increased focus in the European Commission (EC) on iAs in food commodities, no maximum levels have been set for iAs to date. However, there are ongoing discussions in the EC and CODEX Alimentarius on the potential future regulation of inorganic arsenic in rice and rice-based products. A maximum level of 0.2 mg As kg\(^{-1}\) has been proposed, but this has not been implemented in the legislation (CODEX, 2012). On the other hand, Australia and New Zealand have established different limits for iAs: 1 mg As kg\(^{-1}\) for seaweed and molluscs and 2 mg As kg\(^{-1}\) for crustaceans and fish (ANFZA 2011). China has maximum limits for inorganic arsenic for different foodstuffs such as rice (MHC, 2005). According to our present results, edible Shiitake products contained in all cases high percentages of toxic inorganic arsenic (accounting for 84% of the total As). These iAs concentrations were higher than those usually found in cereal-based products (Llorente-Mirandes et al., 2014), fish, vegetable foods and meat (Fontcuberta et al., 2011) and similar to those of other widely consumed foods such as rice and rice products (Llorente-Mirandes et al., 2012), and in some cases were even higher (up to 1.38 mg As kg\(^{-1}\) dm, Table 3). Although it is true that the quantity and frequency of Shiitake intake are relatively low compared to that of
rice or cereal-based food in the European population, it should not be ignored as a potential contributor to dietary iAs exposure. Nevertheless, more data on As speciation in edible Shiitake products are needed in order to accurately estimate the dietary exposure to inorganic As in such populations. There is also lack of data on bioaccessibility of iAs species in edible Shiitake products. The consideration of bioaccessibility and arsenic speciation data into the exposure assessment can further refine and improve the risk assessment process. In a recent study, high rates of As bioaccessibility from several mushrooms are reported (Koch et al., 2013).

3.4 Cultivated Shiitake

As well as dehydrated, fresh, canned and food supplements, another way to consume Shiitake is through its cultivation in commercial substrate inoculated with mycelium intended to be grown at home. Therefore, to investigate the distribution of arsenic compounds and the potential health risks involved in the consumption of cultivated Shiitake, a preliminary cultivation study was performed. In addition, the relationship between iAs contents in the substrate, tap water and in the mushroom fruiting bodies was investigated to check whether iAs originates from the substrate or is produced through biotransformation.

As mentioned earlier, Shiitake was cultivated according to the instructions supplied by the manufacturer. Tap and waste water solutions and substrate samples were analysed before and after each harvest. The first and second harvest produced a considerable number of mushrooms of different sizes. Differences in the total yield were found between harvests: 319 g and 222 g (wet mass) for the first and second harvest, respectively. The total arsenic concentrations and arsenic species in the substrate, water and mushroom samples over the two harvest periods are summarised in
Table 4. Arsenite content is only reported for tap and waste water samples since in the remaining samples As(III) was quantitatively oxidised to As(V) during the microwave extraction procedure.

The total arsenic in the waste water samples collected after each substrate submersion was 3.5 and 4.6 µg As L\(^{-1}\) for the first and second harvest, respectively. Inorganic arsenic (as the sum of arsenite and arsenate) was the major compound, corresponding to 88% and 78% of the total As in the first and second, respectively. Furthermore, DMA and MA were determined as minor species in both cases, probably extracted from the mycelium and/or substrate.

Substrate samples were collected throughout the cultivation study and the total As content was 0.14, 0.12 and 0.15 mg As kg\(^{-1}\) dm for the initial, medium and final substrate, respectively. The major arsenic compound in the three substrate samples was iAs and DMA was also quantified as a minor species. The results showed that the arsenic content of the substrate, either total or species, remained unchanged during the cultivation study.

In terms of fruiting bodies, the total arsenic content in the first and second harvest was 0.39 and 0.42 mg As kg\(^{-1}\) dm respectively (Table 4), which is consistent with the range obtained in the present study for all commercial edible Shiitake (0.11 to 1.44 mg As kg\(^{-1}\) dm) (Table 3) and also within the range reported in the literature (Maihara et al., 2008; Wuilloud et al., 2004). The arsenic concentrations of the fruiting bodies did not differ significantly between the first and second harvest. The distribution of arsenic species in Shiitake was similar to that of the purchased mushrooms and revealed that iAs was the major As compound with a concentration of 0.33 mg As kg\(^{-1}\) dm (accounting for 85% of the total As) and 0.38 mg As kg\(^{-1}\) dm (accounting for 90%
of the total As) in the first and second harvest, respectively. These results are consistent with the range found in commercial edible samples (0.086 to 1.38 mg As kg\(^{-1}\) of iAs) (Table 3). Other arsenic compounds were found as minor species and similar distributions were found in each harvest: DMA 6.7% and 5.2%, MA 8.7% and 2.9% of the total As for the first and second harvest, respectively. AB and TMAO were below the LOQ and AC was below the LOD. Although MA was not found in the initial substrate, it was detected in both mushroom samples. Furthermore, an unknown compound was found by the cationic column with a retention time of 380 s. This unknown cationic arsenic species could be attributed to TETRA due to the matching of the retention times when using the same chromatographic conditions (Kirby, Maher, Ellwood, & Krikowa, 2004). However, it was not possible to check this attribution due to the lack of appropriate standards. This arsenic species was not found in any of the substrate samples and is shown in Table 4 as ‘Unknown cation’.

Few studies on arsenic species in cultivated mushrooms are available in the literature. Smith and co-authors cultivated Agaricus bisporus (Smith et al., 2007), which was grown in compost amended with either arsenic-contaminated mine waste or an arsenate solution. Surprisingly, AB was found in mushrooms and was absent from compost not inoculated with A. bisporus. The authors hypothesised that the biosynthesis of AB was a product of fungal, not microbial, arsenic metabolism. In another study of cultivated A. bisporus (Soeroes et al., 2005) the results showed that mycelia were capable of taking up As(V) of the contaminated substrate. Arsenic speciation revealed that the majority of the incorporated arsenic in the treated A. bisporus was present as inorganic arsenic, highlighting the potential health risk posed by its consumption.

According to the present results, toxic inorganic arsenic was the main arsenic species found in both the cultivated and purchased Shiitake products. However, it is not
entirely clear whether Shiitake mushrooms accumulate inorganic arsenic from the substrate, or produce it through biotransformations. Therefore, more studies on the cultivation of Shiitake grown on different commercial substrates and under different cultivation conditions are needed to investigate the uptake and distribution of arsenic in mushroom fruiting bodies.

4. Conclusions

Total arsenic and arsenic species were determined in several edible Shiitake products as well as in home–cultivated fruiting bodies. Arsenic speciation analysis showed that inorganic arsenic was the predominant arsenic compound in all samples, accounting for 84% of the total arsenic. Moreover, other arsenic species such as DMA, MA, AB, and TMAO were found as minor compounds. Despite the low intake of Shiitake products in the European population, the found inorganic arsenic contents could contribute to iAs exposure and therefore Shiitake products should not be ignored as possible source of iAs.

The analytical method used may contribute to increase the availability of reliable results on inorganic arsenic in edible mushrooms. Furthermore, the present results may be useful in ongoing discussions in the European Commission and the CODEX Alimentarius for establishing and implementing future maximum levels of inorganic arsenic in food commodities.

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Wong, W. W. K., Chung, S. W. C., Chan, B. T. P., Ho, Y. Y., & Xiao, Y. (2013). Dietary exposure to inorganic arsenic of the Hong Kong population: Results of the first Hong Kong Total Diet Study. *Food and Chemical Toxicology, 51*(0), 379–385.

### Table 1
Operating conditions of the LC-ICPMS system

<table>
<thead>
<tr>
<th>ICPMS parameters</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>RF power</td>
<td>1550  W</td>
</tr>
<tr>
<td>Make up gas flow, Ar</td>
<td>0.32  L min⁻¹</td>
</tr>
<tr>
<td>Carrier gas flow, Ar</td>
<td>0.85  L min⁻¹</td>
</tr>
<tr>
<td>Spray chamber (type and temperature)</td>
<td>Scott-type and 15 ºC</td>
</tr>
<tr>
<td>Sampler and skimmer cones</td>
<td>Nickel</td>
</tr>
<tr>
<td>Nebuliser</td>
<td>BURGENER Ari Mist HP</td>
</tr>
<tr>
<td>Sampling depth</td>
<td>8.0 mm</td>
</tr>
<tr>
<td>Cell exit</td>
<td>-36 V</td>
</tr>
<tr>
<td>Masses</td>
<td>m/z 75 (⁷⁵As), m/z 35 (³⁵Cl) and m/z 77 (⁴⁰Ar³⁷Cl)</td>
</tr>
<tr>
<td>Collision cell</td>
<td>OFF</td>
</tr>
<tr>
<td>Dwell time</td>
<td>2.0 s (m/z 75), 0.1 s (m/z 35 and m/z 77)</td>
</tr>
<tr>
<td>QP/OctP bias difference</td>
<td>3 V</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chromatographic conditions</th>
<th>Anionic exchange</th>
<th>Cationic exchange</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Hamilton PRP-X100 (250 mm x 4.1 mm, 10 µm)</td>
<td>Zorbax 300-SCX. (250 mm x 4.6 mm, 5 µm)</td>
</tr>
<tr>
<td>Pre column</td>
<td>Hamilton PRP-X100. (20 x 2.0 mm i.d., 10 µm)</td>
<td>Zorbax 300-SCX. (12.5 mm x 4.6 i.d., 5 µm)</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>20 mM NH₄H₂PO₄, pH=5.8</td>
<td>20 mM pyridine, pH=2.6</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.5 mL min⁻¹</td>
<td>1.5 mL min⁻¹</td>
</tr>
<tr>
<td>Injection volume</td>
<td>100 µL</td>
<td>50 µL</td>
</tr>
<tr>
<td>Column temperature</td>
<td>Room temperature 24 ºC</td>
<td>Room temperature 24 ºC</td>
</tr>
<tr>
<td>Pressure</td>
<td>145 bar</td>
<td>152 bar</td>
</tr>
<tr>
<td>Arsenic species</td>
<td>As(III), DMA, MA and As(V)</td>
<td>AB, AC and TMAO</td>
</tr>
<tr>
<td>Elution</td>
<td>Isocratic, 8 min</td>
<td>Isocratic, 9 min</td>
</tr>
</tbody>
</table>
### Table 2

Quality assessment of total arsenic and arsenic species in reference materials. Concentrations are expressed as mg As kg\(^{-1}\) dry mass (mean ± SD, n = 3).

<table>
<thead>
<tr>
<th>Reference Material</th>
<th>Total As</th>
<th>Total extracted As</th>
<th>Arsenic species</th>
<th>Sum of As species</th>
<th>Extraction efficiency (%)</th>
<th>Column recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>DMA</td>
<td>MA</td>
<td>iAs</td>
<td>AB</td>
</tr>
<tr>
<td>ERM-BC211 Rice</td>
<td>0.256 ± 0.009</td>
<td>0.252 ± 0.011</td>
<td>0.125 ± 0.005</td>
<td>0.011 ± 0.001</td>
<td>0.122 ± 0.006</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>Certified value</td>
<td>0.260 ± 0.013</td>
<td></td>
<td>0.119 ± 0.013</td>
<td></td>
<td>0.124 ± 0.011</td>
<td></td>
</tr>
<tr>
<td>NIST SRM 1570a Spinach leaves</td>
<td>0.069 ± 0.005</td>
<td>0.064 ± 0.007</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>0.059 ± 0.005</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>Certified value</td>
<td>0.068 ± 0.012</td>
<td></td>
<td>0.054 ± 0.012</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WEPAL-IPE-120 Agaricus bisporus</td>
<td>0.167 ± 0.012</td>
<td>0.166 ± 0.021</td>
<td>0.047 ± 0.004</td>
<td>&lt;LOD</td>
<td>0.033 ± 0.001</td>
<td>0.067 ± 0.004</td>
</tr>
<tr>
<td>Indicative value</td>
<td>0.137 ± 0.067</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(a\) Certified value: mean ± uncertainty.

\(b\) Indicative value: mean ± standard deviation.

\(c\) Reported value for iAs according to expert laboratories in IMEP-112: mean ± expanded uncertainty (k=2) (de la Calle et al., 2012).

\(d\) Unknown cation arsenic species with a retention time of 380 s.
Table 3. Total arsenic and arsenic species in purchased edible Shiitake products. Concentrations are expressed as mg As kg\(^{-1}\) dry mass (mean ± SD, n = 3).

<table>
<thead>
<tr>
<th>Type of Shiitake</th>
<th>Total As</th>
<th>Total extracted As</th>
<th>Arsenic species</th>
<th>Sum of As species</th>
<th>Extraction efficiency (%)</th>
<th>Column recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>DMA</td>
<td>MA</td>
<td>iAs</td>
<td>Unknown anion*</td>
</tr>
<tr>
<td>Fresh-1</td>
<td>1.42 ± 0.06</td>
<td>1.41 ± 0.07</td>
<td>0.070 ± 0.004</td>
<td>0.025 ± 0.002</td>
<td>1.20 ± 0.03</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>Fresh-2</td>
<td>0.58 ± 0.02</td>
<td>0.57 ± 0.03</td>
<td>0.070 ± 0.003</td>
<td>0.009 ± 0.001</td>
<td>0.31 ± 0.01</td>
<td>0.067 ± 0.004</td>
</tr>
<tr>
<td>Fresh-3</td>
<td>0.11 ± 0.02</td>
<td>0.11 ± 0.01</td>
<td>&lt;LOQ</td>
<td>&lt;LOD</td>
<td>0.10 ± 0.01</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>Fresh-4</td>
<td>0.93 ± 0.01</td>
<td>0.93 ± 0.02</td>
<td>0.025 ± 0.001</td>
<td>0.021 ± 0.004</td>
<td>0.90 ± 0.04</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>Fresh-5</td>
<td>1.44 ± 0.04</td>
<td>1.40 ± 0.11</td>
<td>&lt;LOQ</td>
<td>0.041 ± 0.004</td>
<td>1.38 ± 0.08</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>Canned-1</td>
<td>0.15 ± 0.01</td>
<td>0.15 ± 0.01</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>0.15 ± 0.01</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>Canned-2</td>
<td>0.66 ± 0.07</td>
<td>0.62 ± 0.01</td>
<td>&lt;LOQ</td>
<td>0.050 ± 0.004</td>
<td>0.58 ± 0.01</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>Canned-3</td>
<td>0.17 ± 0.02</td>
<td>0.17 ± 0.01</td>
<td>&lt;LOQ</td>
<td>&lt;LOD</td>
<td>0.17 ± 0.01</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>Food supplements-1</td>
<td>0.45 ± 0.01</td>
<td>0.44 ± 0.02</td>
<td>0.012 ± 0.001</td>
<td>0.012 ± 0.001</td>
<td>0.35 ± 0.02</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>Food supplements-2</td>
<td>0.12 ± 0.01</td>
<td>0.12 ± 0.01</td>
<td>0.033 ± 0.001</td>
<td>&lt;LOD</td>
<td>0.086 ± 0.011</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>-------------------</td>
<td>-------------</td>
<td>-------------</td>
<td>----------------</td>
<td>------</td>
<td>----------------</td>
<td>------</td>
</tr>
<tr>
<td>Dehydrated-1</td>
<td>0.14 ± 0.01</td>
<td>0.14 ± 0.01</td>
<td>0.009 ± 0.001</td>
<td>0.006 ± 0.001</td>
<td>0.12 ± 0.01</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>Dehydrated-2</td>
<td>0.27 ± 0.02</td>
<td>0.25 ± 0.02</td>
<td>0.015 ± 0.001</td>
<td>0.010 ± 0.001</td>
<td>0.20 ± 0.01</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>Dehydrated-3</td>
<td>0.29 ± 0.02</td>
<td>0.28 ± 0.01</td>
<td>0.020 ± 0.001</td>
<td>0.014 ± 0.001</td>
<td>0.22 ± 0.01</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>Dehydrated-4</td>
<td>0.34 ± 0.03</td>
<td>0.33 ± 0.05</td>
<td>0.022 ± 0.001</td>
<td>0.014 ± 0.001</td>
<td>0.28 ± 0.02</td>
<td>&lt;LOD</td>
</tr>
</tbody>
</table>

\[a\] Unknown anion arsenic species with a retention time of 255 s.
**Table 4.** Total arsenic and arsenic species in cultivated Shiitake, substrate samples, and tap and waste water. Concentrations are expressed as mg As kg$^{-1}$ dry mass (mean ± SD, n = 3) for Shiitake and substrate samples. Concentrations are expressed as µg As L$^{-1}$ for tap and waste water (mean ± SD, n = 3).

<table>
<thead>
<tr>
<th>Harvest Sample</th>
<th>Total As</th>
<th>Total extracted As</th>
<th>Arsenic species</th>
<th>Sum of As species</th>
<th>Extraction efficiency (%)</th>
<th>Column recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>As (III)</td>
<td>DMA</td>
<td>MA</td>
<td>As (V)</td>
</tr>
<tr>
<td>First Mushroom-1</td>
<td>0.39 ± 0.02</td>
<td>0.38 ± 0.02</td>
<td>-</td>
<td>0.026 ± 0.002</td>
<td>0.034 ± 0.002</td>
<td>0.33 ± 0.01</td>
</tr>
<tr>
<td>Original substrate</td>
<td>0.14 ± 0.01</td>
<td>0.13 ± 0.01</td>
<td>-</td>
<td>0.004 ± 0.001</td>
<td>&lt;LOQ</td>
<td>0.12 ± 0.02</td>
</tr>
<tr>
<td>Medium substrate</td>
<td>0.12 ± 0.02</td>
<td>0.12 ± 0.01</td>
<td>-</td>
<td>0.005 ± 0.001</td>
<td>&lt;LOQ</td>
<td>0.11 ± 0.01</td>
</tr>
<tr>
<td>Tap water-1</td>
<td>0.85 ± 0.04</td>
<td>n.a $^a$</td>
<td>-</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>0.82 ± 0.05</td>
</tr>
<tr>
<td>Waste water-1</td>
<td>3.5 ± 0.30</td>
<td>n.a $^a$</td>
<td>1.06 ± 0.09</td>
<td>0.17 ± 0.01</td>
<td>0.19 ± 0.02</td>
<td>2.03 ± 0.15</td>
</tr>
<tr>
<td>Second Mushroom-2</td>
<td>0.42 ± 0.03</td>
<td>0.42 ± 0.01</td>
<td>-</td>
<td>0.022 ± 0.001</td>
<td>0.012 ± 0.001</td>
<td>0.38 ± 0.02</td>
</tr>
<tr>
<td>Final substrate</td>
<td>0.15 ± 0.01</td>
<td>0.15 ± 0.02</td>
<td>-</td>
<td>0.007 ± 0.001</td>
<td>&lt;LOD</td>
<td>0.13 ± 0.01</td>
</tr>
<tr>
<td>Tap water-2</td>
<td>0.86 ± 0.03</td>
<td>n.a $^a$</td>
<td>0.79 ± 0.04</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>Waste water-2</td>
<td>4.6 ± 0.60</td>
<td>n.a $^a$</td>
<td>0.73 ± 0.04</td>
<td>0.31 ± 0.03</td>
<td>0.24 ± 0.02</td>
<td>2.85 ± 0.20</td>
</tr>
</tbody>
</table>

$^a$ Not analysed, these samples were not extracted.

$^b$ Unknown cation arsenic species with a retention time of 380 s.
Figure captions

Figure 1. Chromatogram from anion exchange by LC–ICPMS of fresh Shiitake extract.