Accepted Manuscript

Assessment of arsenic bioaccessibility in raw and cooked edible mushrooms by a PBET method

Toni Llorente-Mirandes, Mariona Llorens-Muñoz, Virginia Funes-Collado, Àngels Sahuquillo, José Fermín López-Sánchez

Assessment of arsenic bioaccessibility in raw and cooked edible mushrooms by a

PBET method

Toni Llorente-Mirandes, Mariona Llorens-Muñoz, Virginia Funes-Collado, Àngels

Sahuquillo and José Fermín López-Sánchez[*]

*Department of Analytical Chemistry, University of Barcelona, Martí i Franquès 1-11,

Barcelona E-08028, Spain*

[*]Corresponding author: J.F López-Sánchez, Department of Analytical Chemistry,

University of Barcelona, Martí i Franquès 1-11, Barcelona E-08028, Spain. Tel.: +34

934034873. Email address: fermin.lopez@ub.edu

Abstract

The present study reports arsenic analysis in *Lentinula edodes*, *Agaricus
bisporus* and *Pleurotus ostreatus* before and after being cooked. Furthermore, arsenic in
raw and cooked mushroom was determined in the gastric and gastrointestinal
bioaccessible fractions obtained after simulating human digestion by means of an *in
vitro* physiologically based extraction test (PBET). Several certified reference materials
(SRM 1568a, SRM 1570a, CRM 7503-a, BC211 and IPE-120) were analysed to
evaluate the proposed methods. Total arsenic content was 1393, 181 and 335 µg As kg^{-1}
for *L. edodes*, *A. bisporus* and *P. ostreatus*, respectively, and decreased by between 53%
and 71% in boiled mushroom and less than 11% in griddled mushroom. High
bioaccessibility was observed in raw, boiled and griddled mushroom, ranging from 74%
to 89% and from 80% to 100% for gastric and gastrointestinal extracts, respectively,

suggesting the need to consider the potential health risk of consumption of the mushrooms analysed.

Keywords: Arsenic; Edible mushrooms; Arsenic bioaccessibility; *in vitro* PBET; Cooking; ICPMS.

1. Introduction

Food and drinking water are the principal routes of exposure to arsenic (As) for humans (IARC, International Agency for Cancer Research, 2012; WHO, World Health Organization, 2011). 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 non-threshold, class 1 human carcinogen) (ATSDR Toxicological profile for arsenic, 2007). On the other hand, organic arsenic forms are mainly considered to be non-toxic (i.e. arsenobetaine) or potentially toxic (e.g. arsenosugars or arsenolipids) (Feldmann & Krupp, 2011).

The European Food Safety Authority (European Food Safety Authority, 2009 and 2014) and the Joint FAO/WHO Expert Committee on Food Additives (JEFCA) (FAO/WHO, Evaluation of certain contaminants in food, 2011) have recently shown an interest in the content of arsenic in food, especially inorganic arsenic, and have evaluated dietary exposure to arsenic. Mushrooms as well as other foods were included among the foodstuffs that contribute to arsenic exposure in the general European population (European Food Safety Authority, 2009). Among the regulations proposing maximum levels of arsenic tolerated in food, few establish specific levels for iAs. Very

2

recently, the European Union published Regulation (EU) 2015/1006 (European Commission 2015) amending Annex to Regulation (EC) No 1881/2006 (European Commission 2006) regarding the maximum levels of iAs in rice and rice-based products but not for other foodstuffs.

The capacity of some mushroom species to accumulate arsenic may represent a serious risk to consumer health (Falandysz & Borovicka, 2013; Kalač, 2010; Vetter, 2004); nonetheless, the consumption of edible mushrooms has increased considerably worldwide in recent years due to their nutritional properties. The most widely cultivated edible mushrooms in the world are *Agaricus bisporus* (also known as the button mushroom, white mushroom, brown mushroom or portobello mushroom), *Lentinula edodes* (often called by its Japanese name of shiitake) and *Pleurotus spp.* (particularly *P. ostreatus*, known as the oyster mushroom or hiratake mushroom) (Kalač, 2013), and they are particularly popular in China, Japan and other Asian countries. The Directorate General for Health and Consumers (DG SANCO) of the European Commission requested the European Union Reference Laboratory for Heavy Metals in Feed and Food (EURL-HM) to test the analytical capabilities of National Reference Laboratories (NRLs) to determine heavy metals in mushrooms. Two proficiency tests were organised via the International Measurement Evaluation Programme (IMEP) on behalf of the EURL-HM using the same test item (shiitake mushroom): IMEP-116 and IMEP-39 (Cordeiro et al., 2015), highlighting the fact that arsenic content in mushrooms is currently a priority issue for the DG SANCO of the European Commission.

A complete food safety assessment should always evaluate the intake of arsenic from food on the basis of the product as ingested by the consumer. In the context of human health risk assessment, bioavailability refers to the fraction of the substance that reaches the systemic circulation (blood) from the gastrointestinal (GI) tract (bioavailable

3

fraction) and which is available to promote its action in the exposed organism (Reeder, Schoonen, & Lanzirotti, 2006). A first step in bioavailability assessment is the study of bioaccessibility, which indicates the maximum fraction of a trace element or other substance in food that is theoretically released from its matrix in the GI tract (bioaccessible fraction), and thus becomes available for intestinal absorption (i.e. enters the blood stream) (Oomen et al., 2002). Both *in vitro* and *in vivo* methods for evaluating bioavailability have been proposed. The *in vitro* methods provide an effective approximation to *in vivo* situations and offer the advantages of good reproducibility, simplicity, rapidity, ease of control, low cost and high precision, as it is possible to control conditions better than with *in vivo* tests (Moreda-Piñeiro et al., 2011). The inclusion of bioaccessibility data when assessing exposure can further refine and improve the risk assessment process. In addition, the fact that food is generally consumed in processed form, after a preservation treatment or cooking, must be taken into account since it has been reported that cooking affects the concentration of arsenic content as well as arsenic species distribution (Devesa, Vélez, & Montoro, 2008; Moreda-Piñeiro et al., 2011).

A limited number of arsenic bioaccessibility studies has been conducted, mostly concerning conventional food items; fish and shellfish (Koch et al., 2007; Moreda-Piñeiro et al., 2012), edible seaweeds (García Sartal, Barciela-Alonso, & Bermejo-Barrera, 2012; García-Sartal et al., 2011; Koch et al., 2007; Laparra, Vélez, Montoro, Barberá, & Farré, 2003), rice (Laparra, Vélez, Barberá, Farré, & Montoro, 2005), vegetables (Calatayud, Bralatei, Feldmann, & Devesa, 2013; Juhasz et al., 2008) and country foods (food obtained by hunting and gathering) from contaminated sites in Canada (Koch et al., 2013). There is thus a lack of data on the bioaccessibility of arsenic in edible mushrooms. Only one recent study has been found which reported high As

bioaccessibility rates in several raw mushrooms (Koch et al., 2013). However, great variability of arsenic bioaccessibility has been reported between different mushrooms samples, suggesting that generalisations about arsenic cannot be made at this point. This highlights the importance of performing more bioaccessibility studies of arsenic in mushrooms to refine and improve the risk assessment process.

To date and to the best of our knowledge, no studies have been published on the bioaccessibility of arsenic in cooked edible mushrooms. Therefore, for the first time, the present preliminary study focused on two objectives to assess the potential health risks involved in the consumption of mushrooms. The first was to assure the reliability of analytical methods by establishing analytical parameters. The second was to determine arsenic content and bioaccessibility by an *in vitro* PBET method in three edible mushrooms, *A. bisporus, L. edodes, P. ostreatus,* before and after being boiled or griddled.

2. Materials and methods

2.1. Reagents, standards and certified reference materials

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 microwave digestion procedure. Pepsin (Panreac), citric acid (Fluka), maleic acid (99%, Aldrich), DL-lactic acid (Sigma-Aldrich), hydrochloric acid (37%, Panreac Hiperpur) and glacial acetic acid (100%, Merck pro-analysis) were used for the gastric solution. Sodium hydrogen

5

carbonate (Merck), porcine bile salts (Sigma-Aldrich), amylase (Sigma-Aldrich) and pancreatin (Sigma-Aldrich) were used for the gastrointestinal solution.

External calibration standards were prepared daily by dilution of a standard stock solution with a certified concentration of 1000 ± 5 mg As L^{-1} (Inorganic Ventures Standards, arsenic in 2% (v/v) HNO_3) traceable to the National Institute of Standards and Technology (NIST). A standard solution of arsenate with a certified concentration of 1000 ± 5 mg As L^{-1} (Merck, Certipur®, H_3AsO_4 in 2% (v/v) HNO_3) traceable to the NIST was used as internal quality control in arsenic measurements.

Four certified reference materials (CRMs) and a reference material (RM) were analysed during the study. SRM 1570a spinach leaves and SRM 1568a rice flour were obtained from the NIST (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). NMIJ CRM 7503-a white rice flour was purchased from the National Metrology Institute of Japan (NMIJ, Japan). All CRMs were used as provided, without further grinding.

2.2 *Apparatus and instrumentation*

An inductively coupled plasma mass spectrometry (ICPMS) Agilent 7500ce (Agilent Technologies, Germany) was used to determine arsenic content. A microwave digestion system (Ethos Touch Control, Milestone) was used for the digestion procedure. All mushroom samples were minced using a commercial mincer (Multiquick 5 Hand Processor, Braun, Spain). A thermo-agitator Bath Clifton NE5-28D (Fischer

6

Scientific) (37°C ± 0.1) was used for the physiologically based extraction test (PBET) of the samples and CRMs.

2.3 Samples and sample pretreatment

Lentinula edodes, *Agaricus bisporus* and *Pleourotus ostreatus* mushrooms were obtained from a local market in Barcelona (Spain) in 2014. All samples were brought to the laboratory on the day of purchase and kept for no more than one day in the refrigerator until sample pretreatment. Mushrooms were manually cleaned 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. Mushrooms were cut into small pieces before each cooking procedure. Only the edible parts of the mushrooms were used for cooking tests. Each edible mushroom species was manually homogenised and divided into three portions, which were subjected to different cooking treatments. The first one, the raw product, was directly minced until complete homogenisation and the other two subsamples were cooked, i.e. griddled or boiled. After being cooked, mushrooms were minced using a commercial mincer made of stainless steel until complete homogenisation. Care was taken to avoid contamination. Between samples, the mincer was washed once with soap and rinsed several times with deionised water, and then rinsed three times with doubly deionised water, before drying with cleaning wipes. All samples were stored in freezer bags at -4ºC until analysis was performed (up to 24h).

2.4 Cooking procedures of mushroom samples

Around 100 g of mushroom was boiled in approximately 700 mL of doubly deionised water for 10 minutes. Once the mushroom samples had been boiled, the cooking water was separated for further analysis. Furthermore, around 100 g of mushroom was also cooked on a griddle for 10 minutes.

2.5. Moisture determination

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

2.6 Acid digestion for arsenic determination

Microwave acid digestion of raw, griddled and boiled mushroom samples and CRMs was performed as described in detail elsewhere (Llorente-Mirandes, Ruiz-Chancho, Barbero, Rubio, & Lopez-Sanchez, 2010). The digested samples were diluted with water to 25 mL. The digestion blanks were also measured. The digested samples were kept at 4°C until analysis of arsenic content by ICPMS (24-48 h).

2.7 Bioaccessibility extraction using PBET

The physiologically based extraction test (PBET) method was adapted from the previously described method (Funes-Collado, Rubio, & López-Sánchez, 2015). The test was carried out in two stages; gastric (G) and gastro+intestinal (GI). Solution aliquots were separated at each stage for analysis, yielding two solutions per sample; the G solution and the GI solution GI. The reagent blanks were also analysed in each batch of samples.

The gastric step was carried out in triplicate using 5 g of raw, griddled or boiled mushroom sample in a 100 mL stoppered glass flask to which 50 mL of freshly prepared gastric solution was added. The gastric solution contained 1.25 g L^{-1} pepsin, 0.50 g L^{-1} citric acid, 0.50 g L^{-1} maleic acid, 420 µl L^{-1} DL-lactic acid and 500 µl L^{-1} acetic acid dissolved in water, and the pH was adjusted to 1.3 with concentrated hydrochloric acid. After 15 min, the pH value was checked and if necessary readjusted to pH 1.3. Flasks were covered and incubated at 37°C with orbital–horizontal shaking at 150 rpm for 60 min. Then, flasks were placed in an ice-water bath to stop the enzymatic digestion. At the end of the gastric phase mixing, a 5 mL aliquot was collected from the solution for analysis (G sample). Prior to the intestinal digestion step, the pH of the gastric digests was raised to pH 7 by dropwise addition of saturated $NaHCO_3$ solution. Then, 2 mL of the intestinal solution (0.4 g L^{-1} pancreatin, 0.1 g L^{-1} amylase and 1.5 g L^{-1} porcine bile salts) was added and incubation at 37°C continued for an additional 3 h at 37ºC. The enzymatic reaction was stopped by immersing the flasks in an ice-water bath. Following mixing, a 10 mL aliquot (GI sample) was collected.

All G and GI extracts were transferred to polypropylene centrifuge tubes and centrifuged at 3500 rpm for 12 min to separate the soluble fraction. The supernatants were filtered through PET filters (Chromafil PET, Macherey–Nagel, pore size 0.45 µm) prior to performing analysis. The extracts were kept at 4°C until analysis.

2.8 Arsenic determination by ICPMS

Arsenic content was determined in raw, griddled and boiled mushroom samples, in cooking water and in gastric and gastrointestinal fractions by ICPMS. Operating conditions are listed in Table 1. Helium gas was used in the collision cell to remove

9

polyatomic interferences (i.e. $^{40}Ar^{35}Cl$) in the ICPMS measurements. A 20 µg L^{-1} solution of ^{9}Be, ^{103}Rh and ^{205}Tl was used as an internal standard. Arsenic content in the digested samples was quantified by means of an external calibration curve prepared in 2% HNO_3 for the standards. Arsenic content in G and GI fractions was quantified by a standard addition curve in order to minimise matrix effects.

2.9 Quality assurance - Quality control

A rigorous quality control (QC) programme was conducted throughout sample analysis. For ICPMS measurements, acceptance criterion was $R^2 \geq 0.9990$ for every calibration curves (i.e. both for total arsenic and for bioaccessible arsenic methods). The residual errors at each calibration point were checked, accepting a residual error of ≤ 15 % for the lowest calibration level and ≤ 10 % for the others. QC standard solutions, prepared from a different stock standard source at two concentrations levels, were measured after every 5 samples. Data were accepted only when QC samples were 90–110% of the expected value. Additionally, the standards of the calibration curve were run before and after each sample series applying the same criteria. Each sample was digested (section 2.6), extracted (section 2.7) and analysed (section 2.8) in triplicate. Reagent blanks of total arsenic and PBET methods were also analysed in each batch of samples and there were no outliers for blank controls. To assess the accuracy of arsenic measurements, several CRMs were analysed during sample analyses.

2.10 Statistical analysis

A Student's t-test (2 tails) was applied to compare measured total arsenic and certified values in the CRMs. A 95% confidence level was adopted for all comparisons.

The results for analysed samples were analysed statistically by a one-factor analysis of variance (ANOVA). All the assays were performed at least in triplicate. A significance level of p-value < 0.05 was adopted for all comparisons. A p-value of less than 0.05 indicates a statistically significant difference among variances at a 95% confidence level.

3. Results and Discussion

In order to compare the results obtained from the analysis of raw, griddled or boiled mushroom, all results were expressed in the same units, as micrograms per kilogram of mushroom, dry mass (dm). To do this, humidity was calculated (section 2.5) for each cooking process and for each mushroom species. Furthermore, arsenic content in the water used for boiling samples was expressed as micrograms per kilogram of mushroom, dry mass, for comparison purposes. In the following discussion of results, the term "gastric phase (G)" will be used to indicate the bioaccessibility extraction phase representing the stomach, and "gastric+intestinal phase (GI)" will be used for the phase that included both sequential stomach and intestine steps, where results were obtained from the extract produced at the end of the sequence.

3.1 Analytical quality control study

3.1.1 Limits of detection and quantification

Limits of detection (LODs) and limits of quantification (LOQs) were calculated as three times the standard deviation (3σ) and ten times the standard deviation signal (10σ) of ten blanks, respectively, for the total arsenic method and the PBET method (G

11

and GI fractions). The instrumental limits were converted to sample limits by correcting by the sample weight and extraction dilution factor. LODs were 5.2, 9.8 and 11 µg As kg^{-1} dry mass for total arsenic, G and GI fractions, respectively. LOQs were 17, 33 and 36 µg As kg^{-1} dry mass for total arsenic, G and GI fractions, respectively.

3.1.2 Accuracy of arsenic determination

To assess the accuracy of the total arsenic method, five CRMs were analysed during the study (Table 2). A statistical test was applied to compare determined total arsenic and certified values. The student's t-test indicated that there were no statistically significant differences between the determined and the certified values. Therefore, the accuracy of the total arsenic method was satisfactorily assessed. Moreover, the measured value (170.2 ± 8.2 µg As kg^{-1}) in the WEPAL IPE-120 reference material was in agreement with the indicative value (137 ± 67 µg As kg^{-1}).

3.1.3 Bioaccessibility of arsenic in Reference Materials

Accurate evaluation of the *in vitro* assays for quality control assurance is required prior to application of the approach to specific studies. At present, validation of these approaches is incomplete due to the lack of suitable CRMs. Although several CRMs have been used in bioaccessibility studies (Moreda-Piñeiro et al., 2011), to date, no CRMs are commercially available for bioaccessible arsenic content.

Therefore, to evaluate the PBET method and as our internal quality control, two of the RMs available for total arsenic (WEPAL IPE-120 *A. bisporus* and ERM-BC211 rice) were extracted six times by the same analyst to control the bioaccessibility fractions. The results are shown in Table 3. For our internal QC, arsenic content in G

12

and GI fractions was checked throughout the study and the results for real samples were only accepted when RM values were 85–115% of the established value (Table 3).

The bioaccessibility (BA%) of arsenic was calculated as a percentage using the following equation:

$$BA(\%)=\frac{[\text{As in G or GI extract}]}{[\text{As in sample}]} \times 100$$

where BA (%) is the percentage of bioaccessibility; [As in G or GI extract] is the As concentration in gastric or gastrointestinal phase after PBET extraction; and [As in sample] is the As concentration after the microwave-assisted acid digestion procedure. Gastric and gastrointestinal bioaccessibility values for ERM-BC211 and WEPAL IPE-120 materials are shown in Table 3. In both RMs, there were significant differences ($p < 0.05$) between the bioaccessible concentrations obtained for G and GI fractions, meaning that an increase was observed in bioaccessibility when comparing G fractions versus GI fractions as expected. Data on As bioaccessibility have previously been reported for several CRMs (Leufroy, Noël, Beauchemin, & Guérin, 2012; Moreda-Piñeiro et al., 2011). However, to date, no As bioaccessibility results have been found in the literature on ERM-BC211 and WEPAL IPE-120 materials, and therefore the present results cannot be compared. More results on arsenic bioaccessibility in food CRMs are needed to compare different *in vitro* methods and also to establish the suitability of the same *in vitro* method by different laboratories. The chemical form in which this element is present in the matrix could influence its bioaccessibility. These different As species might influence the greater or lesser bioaccessibility of the arsenic (Leufroy et al., 2012; Moreda-Piñeiro et al., 2011). This assumption obviously requires more research to be confirmed.

13

3.1.4 Repeatability

The repeatability (%) of the methods employed was assessed from the data presented in the accuracy and bioaccessibility sections. In each case, the relative standard deviation (RSD) was calculated of six replicates (n=6) obtained in one day and by the same analyst.

Repeatability values were calculated for the total arsenic method (Table 2) and were below 6% for all CRMs, showing excellent repeatability. For within-day repeatability of the PBET method, six replicates of WEPAL IPE-120 and ERM-BC211 were analysed and the RSD values were below 6% and 9% for the G and GI fractions, respectively (Table 3). As expected, for both CRMs, higher values were obtained for gastrointestinal extracts than for gastric extracts, probably due to the complexity of the GI matrix components, which produced high variability between replicates.

For real samples, each one was digested and analysed in triplicate and replicates had acceptable repeatability with a RSD (n=3) usually below 6% for the total arsenic method in all analysed samples (Table 4). Each real sample was also extracted by the PBET method and analysed in triplicate. Acceptable repeatability was obtained with a RSD (n=3) usually below 8% in bioaccessibility extracts of the G or GI fraction (except for two bioaccessibility extracts, 10.4% and 12.2%) (Table 5). The repeatability values obtained here for G and GI fractions in all samples were in the range previously reported in a study of bioaccessibility in mushrooms using a PBET method which included G and GI fractions (Koch et al., 2013).

3.2 Arsenic contents in edible mushrooms

3.2.1 Arsenic in raw edible mushrooms

Total arsenic content in raw mushroom is shown in Table 4. The present results are in the usual range found in mushrooms from unpolluted areas, from 500 to 5000 µg As kg^{-1} (Kalač, 2010). However, significant differences in arsenic concentration (p-value < 0.05) were observed depending on the type of mushroom species analysed. Arsenic concentration in raw *A. bisporus* and *P. ostreatus* was below the maximum allowable concentration of 500 µg As kg^{-1} established by China for edible mushrooms (MHC, 2012). In contrast, arsenic content in raw *L. edodes* exceeded this maximum limit. Furthermore, it has recently been reported that toxic inorganic arsenic was the predominant arsenic species in *L. edodes* (Cordeiro et al., 2015; Llorente-Mirandes, Barbero, Rubio, & López-Sánchez, 2014), suggesting that this mushroom could be a potential contributor to dietary iAs exposure in populations with a high intake of shiitake products.

The arsenic content of mushrooms is regulated by different factors, both environmental, i.e. sampling zone and arsenic content in soil, and genetic, i.e. the ability of mushroom species to accumulate arsenic (Vetter, 2004). High variability in arsenic contents has been reported in the literature (Falandysz & Borovicka, 2013; Kalač, 2010). Some mushroom species can accumulate high amounts of arsenic and this phenomenon seems to be independent of their habitats (Vetter, 2004). For example, for *Laccaria amethysthea*, which is an arsenic accumulator, high arsenic contents have been reported. A mean concentration was above 59000 µg As kg^{-1} dm, with a maximum value of 146900 µg As kg^{-1} dm. Meanwhile, a study analysed 37 common edible mushroom species and the arsenic contents were below 50 µg As kg^{-1} dm in 13 species (Vetter, 2004).

From the present results, *L. edodes* had the highest total arsenic content, which was within the range found in our previous study of this mushroom (range from 110 to 1440 µg As kg^{-1} dm) (Llorente-Mirandes et al., 2014). The few studies on arsenic content in *L. edodes* found in the literature show high variability in As content, e.g. one study reported high As content, at 1300 µg As kg^{-1} dm (Wuilloud, Kannamkumarath, & Caruso, 2004), while other authors have reported low arsenic content in Brazilian shiitake, ranging from 12 to 210 µg As kg^{-1} dm (Maihara, Moura, Catharino, Castro, & Figueira, 2008).

A. bisporus is the most commonly consumed mushroom worldwide and consequently several authors have analysed this mushroom. Furthermore, it has been reported that some species of the genus *Agaricus* have the capacity to accumulate arsenic. For example, in samples gathered from different habitats in Hungary, the maximum concentrations found were about 13000–18000 µg As kg^{-1} dm (Vetter, 2004). In another study of edible mushrooms collected in Italy, high variability in arsenic content was reported for the *Agaricus* genus, ranging from 210 to 5000 µg As kg^{-1} dm (Cocchi, Vescovi, Petrini, & Petrini, 2006). Meanwhile, we observed low As content in our study, which is in agreement with the results obtained in another study on *A. bisporus*, in which total As ranged from 97 to 163 µg As kg^{-1} dm (Maihara et al., 2008).

The As content in *P. ostreatus* was within the range found in cultivated mushrooms, especially in wood-rotting fungi such as *Pleurotus sp*., which are generally in the range of 90 to 500 µg As kg^{-1} dm (Vetter, 2004) or even lower, as in the case of a study of Brazilian mushrooms in which several *Pleurotus sp*. samples were analysed and low As content was found, ranging from 9 to 73 µg As kg^{-1} dm (Maihara et al., 2008).

3.2.2 Arsenic in cooked edible mushrooms

Mushrooms are generally consumed after a cooking treatment, e.g. boiled, griddled, baked or grilled, which may alter the concentration of arsenic (Devesa et al., 2008). Therefore, the effect of griddling or boiling on the arsenic content was evaluated for each of the mushroom species analysed, and the arsenic results are shown in Table 4**.** The effect of cooking, i.e. griddling or boiling, on arsenic content was different for each of the mushroom species analysed.

Griddling produced significant differences ($p < 0.05$) in *P. ostreatus*, where arsenic decreased by around 11% in griddled mushroom with respect to raw mushroom. However, griddling did not produce significant differences ($p > 0.05$) in *L. edodes* and *A. bisporus* with respect to the arsenic content of the raw product.

Boiling, meanwhile, decreased arsenic content by between 53% and 71% in all mushroom species analysed, producing significant differences ($p < 0.05$) in all mushrooms with respect to the arsenic content of the raw mushroom assayed. Samples of the water used to boil mushrooms were analysed and the arsenic concentrations obtained are shown in Table 4 (water results expressed as dry mass of mushroom weight). The results obtained suggest that a high percentage of arsenic was leached into the boiling water during the cooking treatment. Therefore, for an overall and accurate study of risk assessment, the effect of food processing for each type of mushroom should be considered. Even though boiling *L. edodes* caused a significant reduction ($p < 0.05$) in arsenic with respect to the raw sample, both griddled and boiled shiitake exceeded the limit of 500 µg As kg^{-1} established by China for mushrooms (MHC, 2012).

To date and to the best of our knowledge, no data on arsenic content in these mushrooms subjected to cooking treatments have been reported in the literature, therefore the results obtained in this study cannot be compared. However, our results are in agreement with other arsenic studies on cooking foods. For example, it has been reported that boiling food decreases arsenic content substantially (Devesa et al., 2008), and several studies have been published on foods in which high percentages of arsenic were released from food into the cooking water, e.g. seaweeds (García Sartal et al., 2012; García-Sartal et al., 2011; Laparra et al., 2003), rice (Raab, Baskaran, Feldmann, & Meharg, 2009) and pasta samples with a significant decrease in arsenic (about 60%) after a cooking process (Cubadda, Raggi, Zanasi, & Carcea, 2003).

3.3 Bioaccessible arsenic in mushrooms

3.3.1 Bioaccessible arsenic in raw edible mushrooms

The arsenic content in the G and GI bioaccessible fractions in raw mushroom is shown in Table 5. Significant differences were found in arsenic concentrations in G and also in GI extracts (p-value < 0.05) depending on the type of mushroom species analysed, because of the difference in the contents in the initial raw samples.

The bioaccessibility of arsenic (BA, %) was calculated as a percentage using the equation shown above, and results varied between 74% and 88% for the G fraction (Figure 1a) and 86% and 97% for the GI fraction (Figure 1b). In raw mushroom, an increase in bioaccessibility was observed when comparing G fractions versus GI fractions (Figures 1a and 1b). This finding seems to be quite obvious since these are the consecutive steps of the PBET method. In the G step, part of the arsenic was solubilised and when the extraction time was extended to the intestinal phase, an increased

18

bioaccessibility value was observed. This increase was statistically significant ($p < 0.05$) when BA values for the G and GI fractions from all raw mushrooms were considered together, and also for BA values for *L. edodes*. However, the same was not observed for *A. bisporus* and *P. ostreatus* ($p > 0.05$), in which no significant differences were found between BA values in the G and GI fractions.

To the best of our knowledge, only one study on bioaccessible arsenic content in raw mushrooms exists in the literature (Koch et al., 2013), in which it was found that BA values in several raw mushrooms ranged from 20% to 91% in G extracts and from 22% to 94% in GI extracts. The results obtained in the present study for *A. bisporus* are in agreement with this study, which reported BA values higher than 58% in both G and GI extracts in *Agaricus* sp. The same authors reported an increase in the bioaccessibility of arsenic in the seven mushroom species analysed when comparing G and GI values (Koch et al., 2013).

3.3.2 Bioaccessible arsenic in cooked edible mushrooms

The arsenic content in the G and GI bioaccessible fractions in griddled and boiled mushroom is shown in Table 5**.** The effect of cooking on the arsenic content in the G and GI fractions was different for each of the mushroom species analysed.

For *L. edodes,* no significant differences were observed ($p > 0.05$) between As content in the G fraction of griddled mushroom and the G fraction obtained from raw mushroom, whereas boiling produced significant differences ($p < 0.05$) with respect to raw mushroom. This was to be expected because of the difference in As content in the initial sample (raw, griddled or boiled). However, for GI fractions, both griddling and boiling treatments produced significant differences ($p < 0.05$) in *L. edodes* compared to arsenic content in the GI fraction of raw mushroom.

Griddling *A. bisporus* did not produce significant differences (p > 0.05) in As content in the G or GI fractions with respect to the As content in G or GI fractions in the raw mushroom. However, the As content in G or GI fractions were significant lower (p < 0.05) in boiled *A. bisporus* than in the G or GI fractions obtained from the raw mushroom.

In the case of *P. ostreatus,* the As content in the G or GI fractions of both griddled and boiled mushroom was significantly lower (p < 0.05) than in the G or GI fractions obtained from raw mushroom**.**

Bioaccessibility (BA, %) of arsenic in both the G and GI fractions was calculated as a percentage using the equation shown above, and the results are shown in Figures 1a and 1b. No significant differences (p > 0.05) were observed in the gastric fraction between values in raw mushroom and after being cooked (griddled or boiled) for any of the mushroom species analysed (Figure 1a). However, bioaccessibility presented a different behaviour in the GI fraction from each of the assayed mushroom species (Figure 1b). A significant decrease (p < 0.05) was observed in *L. edodes* after being griddled but not after being boiled with respect to BA in the GI fraction from raw mushroom. A significant (p < 0.05) increase in BA in the GI fraction was observed after griddling and boiling *A. bisporus.* In *P. ostreatus*, no significant differences (p > 0.05) were observed in BA of the GI fraction between BA in raw mushroom and after being cooked (griddled or boiled).

Bioaccessibility of arsenic in griddled and boiled mushroom varied between 77% and 89% and 80% and 100% for G and GI fractions, respectively. As observed in raw mushroom, an increase was detected when comparing G fractions versus GI fractions in cooked mushroom. This increase was statistically significant (p < 0.05) when BA% values of G and GI fractions from cooked mushroom were considered

together. Considering all mushroom species and all cooking treatments, mean values were 83% and 92% for G and GI fractions, respectively. When all gastric values were compared to gastrointestinal values for each mushroom species and for all types of cooking treatment (raw, griddling and boiling), significant ($p < 0.05$) differences between G and GI fractions were observed. Higher bioaccessibility values of As were found in GI fractions compared to G fractions, indicating that the GI step plays an important role in the solubilisation of arsenic. Therefore, in order not to underestimate the bioaccessibility of arsenic, an intestinal phase should be included in future bioaccessibility studies of mushrooms to ensure an accurate estimation of bioaccessible arsenic.

To date, no previous data are available for bioaccessibility of arsenic in these mushrooms subjected to a cooking treatment and subsequently extracted by means of the PBET method, and therefore the results obtained in this study cannot be compared. The bioaccessibility of an element depends not only on the matrix, but also on the chemical form of the analyte and the model used (Leufroy et al., 2012; Moreda-Piñeiro et al., 2011). Furthermore, it should be borne in mind that cooking not only affects bioaccessible arsenic content but could also modify and transform some arsenic species present in the raw product. Therefore, more studies on arsenic speciation in bioaccessible fractions (G and GI) in raw and cooked mushroom should be performed to improve the risk assessment process.

It might be useful to determine whether the high As bioaccessibility values obtained by the *in vitro* PBET method are in agreement with the high bioavailability As values obtained by *in vivo* assays. Few studies on this subject in some foods have been found in the literature (He & Zheng, 2010; Juhasz et al., 2006 and 2008). In general, a high variability in As bioavailability has been reported, depending on the different types

of food which have been studied. Thus, bioavailability values using an *in vivo* swine model were 33% and 88% for different varieties of rice (Juhasz et al., 2006) and from 50% to 100% in vegetables (Juhasz et al., 2008). Unfortunately, data for arsenic bioavailability in the assayed edible mushrooms have not been reported, therefore the relationship between the present *in vitro* bioaccessibility results and *in vivo* bioavailability cannot be established.

3.4 Mass balance of cooking procedure

To evaluate the accuracy of the cooking procedure, a mass balance approach was performed for each mushroom sample. Arsenic concentrations were determined in raw and boiled mushroom, as well as in the water used to cook each type of mushroom (Table 4). For the mass balance of the cooking procedure, the sum of arsenic concentrations in both fractions (boiled mushroom and boiling water) was statistically compared with the arsenic content in the raw mushroom. ANOVA *p-values* were 0.2876, 0.5057 and 0.6552 for *L. edodes, A. bisporus*, *P. ostreatus*, respectively and were higher than *p>0.05* (at 95% confidence interval), indicating that there was no statistically significant difference between variance values. Therefore, the arsenic concentration in raw mushroom and the sum of arsenic concentrations in boiled mushroom and water were statistically equal.

4. Conclusions

For the first time, a study of arsenic bioaccessibility in raw and cooked mushroom using a PBET method is reported, enabling assessment of the potential health risk involved in consumption of the most commonly consumed mushrooms

worldwide. Detection and quantification limits, repeatability and accuracy of both total arsenic and PBET methods were satisfactory assessed by analysing several CRMs.

Boiling mushrooms decreased arsenic content which is released into the cooking water. This indicates that for further reliable and accurate studies of risk assessment, mushrooms must be analysed in the same form as ingested by the consumer. Special care is required in the case of *L. edodes*, where total arsenic in raw, griddled and boiled mushroom exceeded the maximum limit established by Chinese legislation.

Even when a cooking process led to a decrease in As content, the bioaccessibility of arsenic remained high, with values of 83% and 92% for the G and GI fractions, respectively. Therefore, a GI phase should be included in further studies so as not to underestimate the bioaccessible arsenic and to ensure the highest conservative estimation.

Further studies on the bioaccessibility of arsenic species in mushrooms which consider the effect of cooking should be conducted in order to improve the risk assessment process. Analytical tools for validation and quality control purposes, such as a Certified Reference Material with a bioaccessible arsenic content, should also be available. Lastly, it should be noted that more studies on *in vivo* bioavailability measurements are required to demonstrate the suitability of and validate *in vitro* bioaccessibility methods.

Acknowledgments

23

References

Agency for Toxic Substances and Disease Registry (ATSDR) (2007). Toxicological profile for arsenic. United States: Department of Health and Human Services, Public Health Service.

Calatayud, M., Bralatei, E., Feldmann, J., & Devesa, V. (2013). Transformation of Arsenic Species during in Vitro Gastrointestinal Digestion of Vegetables. *Journal of Agricultural and Food Chemistry, 61*, 12164–12170.

Cocchi, L., Vescovi, L., Petrini, L. E., & Petrini, O. (2006). Heavy metals in edible mushrooms in Italy. *Food Chemistry, 98*(2), 277–284.

Commission EC (2006). Commission regulation (EC) No 1881/ 2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of European Communication, L364*, 5–24.

Commission EC (2015). "Commission regulation (EU) 2015/1006 of 25 June 2015 amending Regulation (EC) No 1881/2006 as regards maximum levels of inorganic arsenic in foodstuffs." Off. J. Eur. Union. 2015.

Cordeiro, F., Llorente-Mirandes, T., López-Sánchez, J. F., Rubio, R., Sánchez Agullo, a, Raber, G., et al. (2015). Determination of total cadmium, lead, arsenic, mercury and inorganic arsenic in mushrooms: outcome of IMEP-116 and IMEP-39. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment, 32*(1), 54–67.

Cubadda, F., Raggi, a, Zanasi, F., & Carcea, M. (2003). From durum wheat to pasta: effect of technological processing on the levels of arsenic, cadmium, lead and nickel--a pilot study. *Food Additives and Contaminants, 20*(4), 353–60.

Devesa, V., Vélez, D., & Montoro, R. (2008). Effect of thermal treatments on arsenic species contents in food. *Food and Chemical Toxicology, 46*(1), 1–8.

European Food Safety Authority (EFSA) (2009). Scientific opinion on arsenic in food. Panel on Contaminants in the Food Chain (CONTAM). *EFSA Journal, 7*(10), 1351–1549.

European Food Safety Authority (EFSA) (2014). Dietary exposure to inorganic arsenic in the European population. *EFSA Journal, 12*(3), 3597–3665.

Falandysz, J., & Borovicka, J. (2013). Macro and trace mineral constituents and radionuclides in mushrooms: Health benefits and risks. *Applied Microbiology and Biotechnology, 97*(2), 477–501.

Feldmann, J., & Krupp, E. M. (2011). Critical review or scientific opinion paper: Arsenosugars-a class of benign arsenic species or justification for developing partly speciated arsenic fractionation in foodstuffs? *Analytical and Bioanalytical Chemistry, 399*(5), 1735–1741.

Funes-Collado, V., Rubio, R., & López-Sánchez, J. F. (2015). Does boiling affect the bioaccessibility of selenium from cabbage? *Food Chemistry, 181*, 304–309.

García Sartal, C., Barciela-Alonso, M. D. C., & Bermejo-Barrera, P. (2012). Effect of the cooking procedure on the arsenic speciation in the bioavailable (dialyzable) fraction from seaweed. *Microchemical Journal, 105*, 65–71.

García-Sartal, C., Romarís-Hortas, V., Barciela-Alonso, M. D. C., Moreda-Piñeiro, A., Dominguez-Gonzalez, R., & Bermejo-Barrera, P. (2011). Use of an in vitro digestion method to evaluate the bioaccessibility of arsenic in edible seaweed by inductively coupled plasma-mass spectrometry. *Microchemical Journal, 98*(1), 91–96.

He, Y., & Zheng, Y. (2010). Assessment of in vivo bioaccessibility of arsenic in dietary rice by a mass balance approach. *The Science of the Total Environment, 408*(6), 1430–6.

IARC (International Agency for Research on Cancer), 2012. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. A Review of Human Carcinogens: Arsenic, Metals, Fibres, and Dusts, vol. 100C. Lyon, France: International Agency for Research on Cancer, pp. 41-93.

26

Juhasz, A. L., Smith, E., Weber, J., Rees, M., Rofe, A., Kuchel, T., et al. (2006). In Vivo Assessment of Arsenic Bioavailability in Rice and its Significance for Human Health Risk Assessment. *Environmental Health Perspectives, (12)*, 1826–1832.

Juhasz, A. L., Smith, E., Weber, J., Rees, M., Rofe, A., Kuchel, T., et al. (2008). Application of an in vivo swine model for the determination of arsenic bioavailability in contaminated vegetables. *Chemosphere, 71*(10), 1963–1969.

Kalač, P. (2010). Trace element contents in European species of wild growing edible mushrooms: A review for the period 2000-2009. *Food Chemistry, 122*(1), 2–15.

Kalač, P. (2013). A review of chemical composition and nutritional value of wild-growing and cultivated mushrooms. *Journal of the Science of Food and Agriculture, 93*(2), 209–18.

Koch, I., Dee, J., House, K., Sui, J., Zhang, J., McKnight-Whitford, A., & Reimer, K. J. (2013). Bioaccessibility and speciation of arsenic in country foods from contaminated sites in Canada. *Science of the Total Environment, 449*, 1–8.

Koch, I., McPherson, K., Smith, P., Easton, L., Doe, K. G., & Reimer, K. J. (2007). Arsenic bioaccessibility and speciation in clams and seaweed from a contaminated marine environment. *Marine Pollution Bulletin, 54*(5), 586–594.

27

Laparra, J. M., Vélez, D., Barberá, R., Farré, R., & Montoro, R. (2005). Bioavailability of inorganic arsenic in cooked rice: practical aspects for human health risk assessments. *Journal of Agricultural and Food Chemistry, 53*(22), 8829–33.

Laparra, J. M., Vélez, D., Montoro, R., Barberá, R., & Farré, R. (2003). Estimation of arsenic bioaccessibility in edible seaweed by an in vitro digestion method. *Journal of Agricultural and Food Chemistry, 51*(20), 6080–5.

Leufroy, A., Noël, L., Beauchemin, D., & Guérin, T. (2012). Bioaccessibility of total arsenic and arsenic species in seafood as determined by a continuous online leaching method. *Analytical and Bioanalytical Chemistry, 402*(9), 2849–59.

Llorente-Mirandes, T., Barbero, M., Rubio, R., & López-Sánchez, J. F. (2014). Occurrence of inorganic arsenic in edible Shiitake (Lentinula edodes) products. *Food Chemistry, 158*, 207–15.

Llorente-Mirandes, T., Ruiz-Chancho, M. J., Barbero, M., Rubio, R., & Lopez-Sanchez, J. F. (2010). Measurement of arsenic compounds in littoral zone algae from the Western Mediterranean Sea: Occurrence of arsenobetaine. *Chemosphere, 81*(7), 867–875.

Maihara, V. A., Moura, P. L., Catharino, M. G., Castro, L. P., & Figueira, R. C. L. (2008). Arsenic and cadmium content in edible mushrooms from São Paulo, Brazil determined by INAA and GF AAS. *Journal of Radioanalytical and Nuclear Chemistry, 278*(2), 395–397.

MHC (2012). National Food Safety Standard. Maximum levels of contaminants in foods, GB2762-2012. Beijing: Ministry of Health of the People's Republic of China.

Moreda-Piñeiro, J., Alonso-Rodríguez, E., Romarís-Hortas, V., Moreda-Piñeiro, A., López-Mahía, P., Muniategui-Lorenzo, S., et al. (2012). Assessment of the bioavailability of toxic and non-toxic arsenic species in seafood samples. *Food Chemistry, 130*(3), 552–560.

Moreda-Piñeiro, J., Moreda-Piñeiro, A., Romarís-Hortas, V., Moscoso-Pérez, C., López-Mahía, P., Muniategui-Lorenzo, S., et al. (2011). In-vivo and in-vitro testing to assess the bioaccessibility and the bioavailability of arsenic, selenium and mercury species in food samples. *TrAC Trends in Analytical Chemistry, 30*(2), 324–345.

Oomen, A. G., Hack, A., Minekus, M., Zeijdner, E., Cornelis, C., Schoeters, G., et al. (2002). Comparison of five in vitro digestion models to study the bioaccessibility of soil contaminants. *Environmental Science & Technology, 36*(15), 3326–34.

Raab, A., Baskaran, C., Feldmann, J., & Meharg, A. A. (2009). Cooking rice in a high water to rice ratio reduces inorganic arsenic content. *Journal of Environmental Monitoring, 11*(1), 41–44.

Reeder, R. J., Schoonen, M. a. a., & Lanzirotti, a. (2006). Metal Speciation and Its Role in Bioaccessibility and Bioavailability. *Reviews in Mineralogy and Geochemistry, 64*(1), 59–113.

Vetter, J. (2004). Arsenic content of some edible mushroom species. *European Food Research and Technology, 219*(1), 71–74.

WHO (World Health Organization), (2011). *Guidelines for Drinking-water Quality, 4th edition*, 564 pp. Available online:

http://whqlibdoc.who.int/publications/2011/9789241548151_eng.pdf

WHO (World Health Organization). (2011). Evaluation of certain contaminants in food: Seventy-second meeting of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series 959.

Wuilloud, R. G., Kannamkumarath, S. S., & Caruso, J. a. (2004). Speciation of essential and toxic elements in edible mushrooms: Size-exclusion chromatography separation with on-line UV-inductively coupled plasma mass spectrometry detection. *Applied Organometallic Chemistry, 18*(4), 156–165.

Figure captions

Figure 1. Bioaccessibility (%) of arsenic in the gastric (a) and gastrointestinal (b) fractions in raw, griddled and boiled mushroom determined by a PBET method. Error bars denote combined standard uncertainty.

Table 1

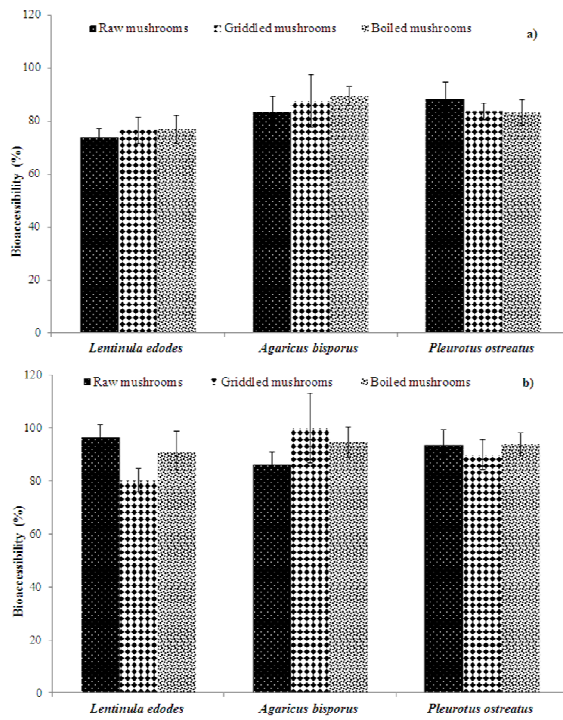ICPMS operating parameters.

Tuning parameters

General

	RF power	1550 W
	RF matching	1.76 V
	Peristaltic pump speed	0.1 rps
	Stabilization delay	30 s
	Sampler and skimmer cones	Nickel
	Nebuliser	BURGENER Ari Mist HP
	Number of replicates	3
	Spray chamber (type and temperature)	Scott-type and 15 ℃

Gas flows

	Carrier gas flow, Ar	0.75 L min^{-1}
	Make up gas flow, Ar	0.39 L min^{-1}

Torch alignment

	Sampling depth	7.5 mm

Ion lenses

	Extract 1	0 V
	Extract 1	-130 V
	Omega Bias-ce	-18 V
	Omega Lens-ce	0.8 V
	Cell entrance	-26 V
	QP Focus	-15 V
	Cell exit	-36 V

Quadrupole and

Octopole parameters

	QP/OctP bias difference	2 V

Reaction cell

	Collision cell	ON
	He gas	3.6 mL min^{-1}

Mass-to-ratio

	As	m/z 75
	Be, Rh and Tl (internal standard)	m/z 9, m/z 103 and m/z 205, respectively

Table 2

Accuracy and repeatability values of the total arsenic method. Total arsenic content in certified reference materials (CRM) is expressed as µg As kg^{-1} dry mass (mean ± SD, n = 6). Repeatability is expressed as (RSD %, n=6) and the CRMs were analysed within a day and by the same analyst.

Certified Reference Materials	Matrix	Accuracy		Repeatability (RSD %)
		Certified Value	Measured value	
NIST SRM 1568a	Rice	290 ± 30 [a]	286.7 ± 6.1	2.1
NIST SRM 1570a	Spinach leaves	68 ± 12 [a]	68.5 ± 4.1	5.9
NMIJ CRM 7503-a	Rice	98 ± 7 [a]	97.8 ± 3.8	3.9
ERM-BC211	Rice	260 ± 13 [a]	256.1 ± 6.7	2.6
WEPAL IPE-120	Mushroom *(Agaricus bisporus)*	137 ± 67 [b]	170.2 ± 8.2	4.8

[a] Certified value: mean ± uncertainty.

[b] Indicative value: mean ± standard deviation.

Table 3

Quality control results of bioaccessibility study. Bioaccessible total arsenic in gastric (G) and gastrointestinal (GI) fractions and repeatability of PBET extraction method in certified reference materials (CRMs). Concentrations are expressed as µg As kg^{-1} dry mass (mean ± SD, n = 6). Repeatability is expressed as (RSD %, n=6) and the CRMs were analysed within a day and by the same analyst.

CRM	Matrix	Bioaccessibility [a]				Repeatability (RSD %, n=6)	
		As in G fraction	BA (%) in G fraction [b]	As in GI fraction	BA (%) in GI fraction [b]	G fraction	GI fraction
ERM-BC211	Rice	249.9 ± 7.5	98 ± 4	268 ± 16	105 ± 7	3.0	6.0
WEPAL IPE-120	Mushroom *(Agaricus bisporus)*	149.4 ± 8.5	88 ± 7	177 ± 15	104 ± 10	5.7	8.4

[a] Acceptance criterion: values accepted only when results were 85–115% of the established value.

[b] Bioaccessibility= [(Total As in bioaccessible fraction, G or GI)/ (Total As in sample)] x 100.

Table 4. Concentration of total arsenic in raw, griddled and boiled mushrooms and in boiling water. Concentrations expressed as µg As kg^{-1} dry mass (mean ± SD, n = 3).

Sample	Total arsenic			
	Raw	**Griddled**	**Boiled**	**Boiling water**
Lentinula edodes	1393 ± 61	1316 ± 45	568 ± 23	879 ± 27
Agaricus bisporus	185.0 ± 9.0	167.7 ± 7.7	86.2 ± 1.8	103.4 ± 6.1
Pleurotus ostreatus	335 ± 19	298.7 ± 6.2	98.3 ± 3.1	242.1 ± 9.6

Table 5. Bioaccessible arsenic in gastric (G) and gastrointestinal (GI) fractions of PBET method expressed as µg As kg^{-1} dry mass (mean ± SD, n = 3).

Sample	Cooking treatment	Bioaccessible arsenic	
		Total As in G phase	Total As in GI phase
Lentinula edodes	raw	1028 ± 12	1346 ± 26
	griddled	1008 ± 57	1057 ± 45
	boiled	437 ± 25	516 ± 39
Agaricus bisporus	raw	154.0 ± 8.2	159.2 ± 4.1
	griddled	147 ± 15	168 ± 20
	boiled	77.1 ± 2.7	81.5 ± 4.7
Pleurotus ostreatus	raw	295 ± 15	313.5 ± 6.0
	griddled	250.1 ± 7.7	269 ± 16
	boiled	81.8 ± 4.1	92.3 ± 2.9

Assessment of arsenic bioaccessibility in raw and cooked edible mushrooms by a PBET method

Toni Llorente-Mirandes, Mariona Llorens-Muñoz, Virginia Funes-Collado, Àngels Sahuquillo and José Fermín López-Sánchez[*]

Department of Analytical Chemistry, University of Barcelona, Martí i Franquès 1-11,

Barcelona E-08028, Spain

HIGHLIGHTS

Arsenic content in mushrooms before and after being cooked is reported

The bioaccessible arsenic in raw and cooked mushrooms is assessed by an *in vitro* PBET

As decreased in boiled mushroom (53-71%) and less than 11% in griddled mushrooms

High As bioaccessibility (74-100%) in raw, boiled and griddled mushroom was obtained