Food Chemistry 194 (2016) 849–856

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

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 *

Department of Analytical Chemistry, University of Barcelona, Martí i Franquès 1-11, Barcelona E-08028, Spain

ARTICLE INFO

Article history:
Received 30 March 2015
Received in revised form 27 July 2015
Accepted 13 August 2015
Available online 14 August 2015

Keywords:
Arsenic
Edible mushrooms
Arsenic bioaccessibility
In vitro PBET
Cooking
ICPMS

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.

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 (2009, 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 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.

Due to the increasing mushroom consumption, 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

* Corresponding author.
E-mail address: fermin.lopez@ub.edu (J.F. López-Sánchez).

http://dx.doi.org/10.1016/j.foodchem.2015.08.047
0308-8146/

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 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 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 *A. 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 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

L. edodes, *A. bisporus* and *P. 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 −4 °C until analysis was performed (up to 24 h).

2.4. Cooking procedures of mushroom samples

Around 100 g of mushroom was boiled in approximately 700 mL of doubly deionised water for 10 min. Once the mushroom