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Original Research Article

Study of mercury content in wild edible mushrooms and its contribution to the Provisional Tolerable Weekly Intake in Spain

Q1 Carlos Ostos, Fernando Pérez-Rodríguez *, Baldomero Moreno Arroyo, Rafael Moreno-Rojas

Department of Food Science and Technology, University of Córdoba, Campus Rabanales, Darwin-Anexo Building, 1014 Córdoba, Spain

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ABSTRACT

The study determined total mercury (Hg) content of 10 wild edible mushroom species collected in southern Spain. Results indicated that the highest Hg level corresponded to *Boletus aereus* with 10.28 ± 2.92 mg/kg DW (dry weight), while the lowest Hg level was found in *Terfezia arenaria* with 0.09 ± 0.08 mg/kg DW. Regarding the anatomic parts of the mushrooms, caps showed significantly higher Hg concentrations than stems in *B. aereus*, *Amanita caesarea* and *Macrolepiota procera*. The percentage of contribution to the Provisional Tolerable Weekly Intake (PTWI) for Hg was also calculated. Based on consumption data in Spain (0.011 kg/person/week), %PTWI was calculated ranging between 0.06 and 3.5% for mean Hg levels. When a high level of consumption was assumed (0.100 kg/person/week), %PTWI ranged between 0.58 and 31%. Results suggest that wild edible mushrooms in southern Spain could contribute with high Hg levels to the Spanish diet. However, the lack of consumption data for wild edible mushrooms made it difficult to give more definitive conclusions; hence further studies including specific consumption data will be needed.

1. Introduction

Spain possesses a great diversity in mushrooms and truffles with more than 2500 species (Moreno et al., 1996; Gómez et al., 1993). Picking wild edible mushrooms is an important activity in Spain, and it involves large numbers of consumers. Mushroom consumption has grown1 noticeably in Spain over the past few years, probably as a direct result of, among other factors, recent studies about the health benefits associated with certain compounds found in mushrooms. For instance, *Boletus edulis* contains a powerful antioxidant compound, ergothioneine, which can be found at high concentrations, reaching levels of 528 mg/kg WW (Ey et al., 2007). Moreover, it has been reported that eating certain mushroom species can have potentially beneficial effects in the reduction of cardiovascular diseases (Guillamón et al., 2010).

It is well known that mushrooms are able to assimilate and accumulate compounds and nutrients from the environment (Alonso et al., 2004). Mushrooms can accumulate metals in the fungi epithelium, which can persist and even increase in some parts of the fruitful body, in some cases reaching higher concentrations than in the environment where they grow (Campos et al., 2009). There are many factors that can influence the presence of metals in mushrooms, such as climate, geographic location, environmental conditions, and concentration of macromolecules in the cellular wall of each specific species. In the case of total mercury (Hg), several studies have highlighted the importance of mushrooms as significant sources of this metal. According to the review by Kalač and Svoboda (2000), total mercury content of the examined wild mushrooms was between 0 and 20 mg/kg. Similarly, high levels of total mercury have been found in *Boletus* spp. and *Agaricus* spp. with concentration ranges of 2–5 and 10–20 mg/kg, respectively (Kalač, 2010). Moreover, Hg concentration in wild mushrooms could be higher as suggested by the study by Falandysz and Szajek (1994), in which average Hg concentration in wild growing *Agaricus* spp. was significantly higher than the

* Corresponding author. Tel.: +34 957 21 20 04; fax: +34 957 21 20 00.
E-mail address: b42perof@uco.es (F. Pérez-Rodríguez).

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concentration found in cultivated *A. bisporus*. These data demonstrate that the toxicological risk associated with Hg content might be higher in wild edible mushrooms. In addition, mushroom picker populations are usually associated with high consumption levels of wild mushrooms during the picking season.

Exposure to mercury has been identified as a serious threat to the development of the child in utero and early in life in addition to producing toxic effects in the nervous, digestive and immune systems, and in the lungs, kidneys, skin and eyes (Tchounwou et al., 2003). Hence the World Health Organization (WHO, 2010) has considered this heavy metal as one of the top ten elements of major public health concern.

To date, no previous studies have been carried out in the southern Spain on the Hg content of the most consumed wild edible mushroom species in this region, which has one of the biggest mushroom-picker populations in Spain, with a wide consumption of wild edible mushrooms. There is therefore a need to determine the Hg levels in the most-consumed wild edible mushrooms in southern Spain in order to assess the exposure level to this metal in picker populations. Accordingly, the aim of this study was to determine the Hg content in 10 different species of wild edible mushroom collected in the southern Spain and to assess the exposure level of mushroom picker populations by using the well-recognized safety criterion Provisional Tolerable Weekly Intake (PTWI) for Hg.

2. Materials and methods

2.1. Methods of sampling

Mushroom samples of 10 edible wild species were picked from different areas of southern Spain during the typical collection season for mushrooms (autumn–winter) in 2011 resulting in $n = 602$ samples. Only fruit bodies showing full development, with cap and stem, were collected. Old and injured fruit bodies were not used. Caps and stems were sampled separately to enable Hg determination for each anatomic part separately. Each sample consisted of one anatomic part, either the cap or stem from one or two individual specimens in order to obtain a representative amount for each species. After collection, (fresh) samples were weighed; weight ranged from 15 to 25 g depending on size and species. A major description of the number of samples for each species and sampling area is given in Table 1. Then, samples were washed with bi-distilled-deionized water in order to remove any possible trace of soil, which could affect mercury content. Next, samples were immediately frozen in individually packaged into plastic bags and labeled. Prior to analysis, water content was determined for each individual sample (AOAC, 2002), and then the samples were lyophilized.

2.2. Sample preparation

Pretreatment by wet digestion in a closed system was performed in a CEM Corporation MDS 2000 microwave (Spectralab Scientific, Ontario, Canada) with hermetic and pressure regulated Teflon reactors/vessels. A quantity of 0.2 g of lyophilized sample was deposited in the vessels (weighed on a precision balance), adding 3 mL of nitric acid (69%) and 0.5 mL of (33%) hydrogen peroxide (Panreac, Barcelona, Spain). Three droplets of potassium permanganate (5%) were added to enhance the oxidative digestion process of the organic matrix. To facilitate digestion, each sample was divided into two equal-sized portions (i.e. aliquots) so that content would fit the vessel size. Each sample aliquot was diluted to a final volume of 15 mL with deionized, bi-distilled water (>18 MΩ), obtained from an Optimum-Maxima Elga Option 3 Water Purifier deionization system (Thermofisher, Boston, USA). Reagents used in sample preparation were purchased from Panreac (Barcelona, Spain).

2.3. Analytical determination and calibration curve

Analytical determinations of Hg were performed by atomic absorption spectrophotometry (EAA) with cool vapor associated with a flow-injection analysis system (FIA) using a Perkin Elmer 2100 equipped with a quartz cell purchased from Perkin Elmer (Massachusetts, USA). The samples were measured in triplicate. The optimized parameters corresponded to wavelength 253.7 nm, with a slit of 0.7 nm using HCl as liquid carrier, Ar as gaseous carrier, and $BHNa_4$ as reducing agent stabilized with NaOH. The 0.75% (w/v) $BHNa_4$ was prepared daily by dissolution of the appropriate amount of the solid reagent in 1.0% (w/v) sodium NaOH. The liquid carrier corresponded to 3.0% (w/v) HCl obtained from a stock solution of (37%) HCl. Reagents used in analytical determination were purchased from Panreac (Barcelona, Spain).

Standard mercury solutions were prepared (0.1, 1, 10, and 100 μg/L) by stepwise solutions from 1000 mg/L stock solution purchased from Merck-Titrisol (Darmstadt, Germany). These solutions were used to construct a calibration curve, which was performed daily. In addition, confirmation tests for the calibration curves were carried out during experiments by assessing the calibration curve slope with central concentrations of the standard mercury solutions each 50 measurements and the whole calibration curve each 100 measurements.

2.4. Analytical method optimization

The optimization of the analysis procedure was performed with three parameters: limit of quantification (LOQ), limit of detection (LOD) and precision (Thomson et al., 2002). For precision, a total of 10 different samples were analyzed in different days, performing 10 readings for each sample. The obtained values for the above parameters after optimization corresponded to 0.03 mg/kg DW, 0.01 mg/kg DW and 4.5%, respectively. In order to estimate the recovery percentage of the used method, samples ($n = 10$) of a certified reference material consisting of *Cantharellus tubaeformis* provided by Livsmedels Verket (Uppsala, Sweden) were analyzed on different experimental days. The results indicated that recovery percentage for Hg corresponded to 101.4% (95% CI: 96.2–109.1%) while the repeatability relative standard deviation (RSDr), calculated on these data, was 4.8%

2.5. Statistical treatment

A minimum of 10 independent samples ($n \geq 10$) were analyzed per each mushroom species (Table 1). The number of analyzed independent samples depended on the availability of the type of picked mushroom. Three measurements were performed per each analyzed independent sample. Due to the subdivision of samples into two equal-sized subsamples for digestion, the value for each independent sample corresponded to the mean of the two subsamples.

Concentration data were evaluated statistically by descriptive parameters such as mean and standard deviation (SD). In order to determine statistical differences in the Hg content between different mushroom species or anatomic parts, a variance analysis and a post hoc test (i.e. Tukey's mean homogeneity test) were performed using STATISTICA® software (StatSoft Iberica, Portugal). The significance level used in this study corresponded to $p \leq 0.05$.

2.6. Provisional Tolerable Weekly Intake (PTWI) calculation

In order to assess the Hg intake risk associated with consumption of the studied mushroom species, a safety level or criterion was used. This criterion considered only inorganic Hg since recommendations for methyl-mercury are given only for