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# Determination of indole compounds released from selected edible mushrooms and their biomass to artificial stomach juice

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ABSTRACT

The study consisted of experiments using edible mushroom species: *Agaricus bisporus*, *Boletus badius* and *Cantharellus cibarius*. As far as we know, there is no information about the release of indole compounds from mushrooms in the human body. The rate of the release of indole compounds was determined by HPLC method and on this basis it was possible to ascertain new important information about the safety and benefits of adding edible mushrooms to the diet. The experiments to verify the release of indole compounds in the artificial gastric juice were performed using the fruiting bodies of the studied species, and the corresponding mycelium obtained by *in vitro* culture. The highest levels of indole compounds concentration after extraction for 15, 30, 60 and 90 min were observed for *in vitro* cultures of the examined mushrooms. These concentrations ranged from 0.01 to 109 mg/100 g dry weight for serotonin and 5-OH-L-tryptophan, respectively. The dominant indole compound was 5-OH-L-tryptophan both in extracts derived from fruiting bodies and in mycelium collected from *in vitro* culture with the addition of zinc hydroaspartate (28–109 mg/100 g dry weight). Generally, the lower levels of the released compounds were found in extracts from the fruiting bodies.



## 1. Introduction

Both wild grown and cultivated mushrooms are popular and consumed on a large scale worldwide, and they are also taken medicinally by many people (Roupas, Keogh, Noakes, Margarett, & Taylor, 2012; Wasser & Weis, 1999; Witkowska, Zujko & Mironczuk-Chodakowska, 2011). Selected edible mushroom species were used in the experiments: *Agaricus bisporus* (J.E. Lange) Imbach – White bottom mushroom (Basidiomycota), mainly because this species is widely used for commercial purposes in Poland and Europe, and is the most frequently consumed mushroom in Polish and European society. This is mainly due to its taste and health qualities. Furthermore, some of the most popular wild mushrooms were also used: *Boletus badius* Pers. (Bay bolete) and *Cantharellus cibarius* Fr. (the Chantarelle). *A. bisporus* is a highly valued source of vitamins (especially riboflavin, ergocalciferol) and bioelements (for example selenium, magnesium, copper, iron, calcium, zinc and potassium). *C. cibarius* is a perfect source of carotenoids (including β-carotene), ergosterol, vitamin $D_3$, and laccase (Barros, Venturini, Baptista, Estevinho, & Ferreira, 2008; Elmastas, Isildak, Turkekul, & Temur, 2007; Muszyńska, Sułkowska-Ziaja, & Ekiert, 2009; Rangel-Castro, Staffas, & Danell, 2002; Roberts, Teichert, & McHugh, 2008) *B. badius* is a species containing high antioxidant substance concentrations (Elmastas et al., 2007). A notable factor about the fruiting bodies of mushrooms and their mycelium from *in vitro* culture is their ability to synthesize and to accumulate organic substances (such as indole compounds). However, there is no information about the release of indole compounds from mushrooms in the human body, and on the dependence of the amounts and types of substances introduced to culture media on the rate of release. For example, the addition of zinc can build a complex with an organic substance and influence its ability to be released from the fruiting bodies of mushrooms. As a result, the fruiting bodies of *A. bisporus*, *B. badius* and *C. cibarius* were used as the experimental material to enable the study of the release of organic compounds, i.e. indoles, in comparison to their biomass from an *in vitro* culture and to their biomass from an *in vitro* culture with an addition of zinc hydroaspartate. The group

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of indole compounds that were examined in fruiting bodies of edible mushrooms are non-hallucinogenic indole compounds. As a result of previous research was obtained that edible mushrooms are a good source of these compounds (Muszyńska, Maślanka, Sułkowska-Ziaja, & Ekiert, 2011; Muszyńska, Maślanka, Sułkowska-Ziaja, & Krzek, 2007; Muszyńska, & Sułkowska-Ziaja, 2012; Muszyńska, et al., 2009; Muszyńska, Sułkowska-Ziaja, & Ekiert, 2011; Muszyńska, Sułkowska-Ziaja, & Ekiert, 2013; Muszyńska, Sułkowska-Ziaja, & Wójcik, 2013). Taking into consideration the function of indole derivatives – such as L-tryptophan, 5-hydroxy-L-tryptophan, 5-methyltryptophan, serotonin, melatonin, tryptamine – that are neurotransmitters and their precursors, possess cardioprotective, anti-inflammatory and analgesic activity it is well justified to examine their ability to be released from mushroom materials to artificial stomach juice (Esposito & Cuzzocrea, 2010; Morgan et al., 2001).

The difficulty in obtaining research material (due to the temporary and unpredicted occurrence of fruiting bodies from natural sites) led to the use of mycelia from *in vitro* cultures for further experiments (Muszyńska et al., 2009). In closed laboratory conditions it is easier to control the accumulation and release of selected metabolites and elements. The obtained information's on the accumulation and release metabolisms of these substances might be used in the future to develop specialized cultivations of mushrooms.

The presence of indole compounds in the fruiting bodies of edible mushrooms was confirmed, but due to the dietary reasons, it is important to determine whether these compounds are released in the human body and in what quantities. So the main goal for this work was to use high performance liquid chromatography (HPLC) to determine the release rate of indole compounds from fruiting bodies of mushrooms to artificial stomach juice. Thus, new important information could be acquired about the safety and benefits of adding edible mushrooms to a diet. Determination of indole compounds will allow for an estimation of interaction possibilities between indole compounds found in mushrooms and drugs that influence the human metabolism of similar substances – MAO inhibitors (Muszyńska et al., 2007).

## 2. Materials and methods

### 2.1. Reagents

Acetic acid was from Polish Company of Chemistry (Gliwice, Poland); NaCl was from PPH Golpharm (Kraków, Poland); pepsin was from BTL (Łódź, Poland); HCl, Suprapur® was from Merck (Darmstad, Germany); zinc hydroaspartate was from Farmapol (Poland); standards of indole compounds: L-tryptophan, 5-OH-L-tryptophan, 5-$CH_3$-tryptophan, serotonin, melatonin, tryptamine, 5-$CH_3$-tryptamine, kynurenine sulfate, indoleacetic acid, indoleacetonitrile, indole, indoleacetamide and ethanol were from Sigma–Aldrich St Louis, Mo, USA, all of HPLC grade. Quadruple-distilled water with conductivity of less than 1 μs $cm^{-1}$, obtained with the use of S2-97A2 distillation apparatus, ChemLand (Stargard Szczecin, Poland).

### 2.2. Sample

Material for analysis consisted of edible mushrooms: *B. badius* and *C. cibarius* growing wild in mixed forests of southern Poland (near Kraków from 2012 to 2013); and *A. bisporus* of commercial origin, purchased at a supermarket in Poland (2013). Taxonomic identification of the young sporocarps was made according to online keys (http://www.mycokey.com) and to authors Knudsen and Vesterholt (2008). Representative voucher specimens were deposited at the Department of Pharmaceutical Botany, Jagiellonian University Collegium Medicum, Kraków, Poland. The young sporocarps were used to derive *in vitro* cultures, from which they obtained mycelium formed the material for further analysis. The procedure for conducting *in vitro* culture on agar-solidified Oddoux medium (1957) was developed by Muszyńska, Sułkowska-Ziaja, and Wójcik (2013).

#### 2.2.1. Experimental culture in vitro

Both agitated liquid cultures of selected edible mushrooms on Oddoux medium and cultures on the same medium but with the addition of 0.1 g/L of zinc hydroaspartate were maintained for three weeks. Next, the obtained fresh biomass of: mycelium from *in vitro* cultures, mycelium from *in vitro* cultures on the same medium but with zinc hydroaspartate was separated from the liquid medium using filter paper on a Büchner funnel, rinsed with redistilled water. The amount of 50 g of each of the materials were frozen and lyophilizated (Freezone 4.5. Labconco; temperature: −40 °C) to obtain the mushrooms samples for quantitative HPLC analyses.

### 2.3. Sample preparation

The lyophilized fruiting bodies and biomass from *in vitro* cultures were weighed and then 150 mg of mushroom materials were ground in a porcelain mortar and extracted in artificial stomach juice. In the stomach, pH value ranges from 1.0 to 3.5, but in most artificial gastric juice models, pH is 2.0. The solution of this artificial body fluid was prepared according to Polish Pharmacopoeia IX by dissolution of 2.0 g sodium chloride and 3.2 g pepsin in quadruple-distilled water. Then, 80 mL of mol/L hydrochloric acid was added to adjust the pH followed by supplementation with quadruple-distilled water to 1000 mL.

### 2.4. HPLC analysis

Samples once prepared were shaken for 15 min, 30 min, 60 min, 90 min, and centrifuged at 120 g × 10 min. The obtained fractions were filtered through a syringe-driven filter unit (Millex, Millipore Corporation, USA) and then concentrated by distillation in a vacuum evaporator under reduced pressure at 40 °C. The extracts, quantitatively dissolved in 1.5 mL of solvent system: ethanol/water/acetic acid (17.1:55.46:20.25 mol/L), were subjected to HPLC analysis and were analyzed for presence of indole compounds: L-tryptophan, 5-OH-L-tryptophan, 5-$CH_3$-tryptophan, serotonin, melatonin, tryptamine, 5-$CH_3$-tryptamine, kynurenine sulfate, indoleacetic acid, indoleacetonitrile, indole and indoleacetamide. The analyses were carried out according to the procedure developed by Kysilka and Wurst (1985) with our modifications (Muszyńska et al., 2009). HPLC analyses were performed using a Hitachi apparatus (Merck, Japan) with an L-7100 pump and a Purospher® RP-18 (4 × 200 mm, 5 μm) column and then thermostated at 25 °C. The solvent system used was: ethanol/water/acetic acid (17.1:55.46:20.25 mol/L); flow rate: 1 mL/min. Detection was carried out in a UV detector at λ = 280 nm. The identification of indole compounds was made by comparing the retention times of sample peaks with those of the standards. An example chromatogram is presented in Fig. 1.

### 2.5. Statistical analysis

The results were expressed in mg/100 g of dry weight, calculated by internal normalization of the chromatographic peak area. The statistical analyses were carried out using the Student's test. The results were expressed as mean values with standard