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Agaricus bisporus and its *in vitro* culture as a source of indole compounds released into artificial digestive juices

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ABSTRACT

The popularity of *Agaricus bisporus* results not only from the quality of the flavors, but also from the content of many substances of therapeutic properties. This paper presents a study on RP-HPLC determination of the content of indole compounds released from the lyophilized biomass from *in vitro* cultures of *A. bisporus* into artificial digestive juices at 37 °C. *A. bisporus in vitro* cultures were grown on media enriched with zinc salts. The release of 5-hydroxy-L-tryptophan and L-tryptophan was found in the greatest number of samples. The content of 5-hydroxy-L-tryptophan in the investigated samples ranged from 86.62 to 531 mg/100 g d.w. The amount of L-tryptophan was determined within the range of 1.63–4.68 mg/100 g d.w. and for melatonin 0.43–0.64 mg/100 g d.w. It was demonstrated for the first time that *in vitro* cultures of *A. bisporus* release indole compounds in conditions simulating the human digestive tract.

1. Introduction

Agaricus bisporus (J.E. Lange) Imbach (white bottom mushroom) is one of the most popular and most widely consumed edible mushroom species in the world. The first reports on the cultivation of *A. bisporus* date from 1707, from France. Its popularity results not only from the quality of the flavors, but also from high concentration of substances with therapeutic properties. *A. bisporus* fruiting bodies demonstrate antioxidant, antibacterial, anti-inflammatory, antitumor, and immunomodulatory properties (Muszyńska, Kała, et al., 2015; Muszyńska, Krakowska, et al., 2015; Patel & Goyal, 2012; Yilmaz, Solmaz, Turkekul, & Elmastas, 2006). *A. bisporus* is a species characterized by antioxidant properties because of the presence of phenolic compounds, and above all ergothioneine (derivative of histidine) which can be found in the fruiting bodies. In a cell, this compound not only contributes as an antioxidant, but also as antimutagen, chemo- and radioprotectant (Ey, Schömi, & Taubert, 2007; Markowa, 2009).

This species is a valuable source of vitamins, especially riboflavin, ergocalciferol (successful experiments based on the enrichment of *A. bisporus* with vitamin D_2 via UV-B irradiation of fruiting bodies and zinc (Koyalamudi, Jeong, Song, Cho, & Pang, 2009; Roberts, 2008). An important class of compounds occurring in the edible fungal species, including *A. bisporus*, is the indole compounds. Between 2007 and 2014, Muszyńska et al. studied the composition of fruiting bodies of edible mushrooms and biomass from *in vitro* cultures of these mushrooms and demonstrated the presence of non-hallucinogenic indole compounds in the studied material (Muszyńska & Sułkowska-Ziaja, 2012; Muszyńska, Sułkowska-Ziaja, & Ekiert, 2011; Muszyńska, Sułkowska-Ziaja, Hałaszuk, Krężałek, & Łojewski, 2014; Muszyńska, Sułkowska-Ziaja, & Wójcik, 2013; Muszyńska, Kała, et al., 2015). Studies on the content of indole compounds were performed because of their interesting traits: they act as neurotransmitters and antioxidants, exhibit antitumor properties, retard aging, and regulate the blood coagulation processes (Bilski, Perz, & Perz, 2005; Muszyńska et al., 2013; Stępień, Walecka-Kapica, Błońska, & Klupińska, 2014).

In humans, L-tryptophan is an exogenous amino acid and must therefore be consumed with food. In the human body, particularly in the central nervous system, L-tryptophan is converted into serotonin and melatonin. L-tryptophan undergoes three major changes: decarboxylation leading to the formation of tryptamine, hydroxylation causing conversion to 5-hydroxy-L-tryptophan, which then leads to the formation of serotonin, and disruption of the indole ring leading to the formation of kynurenine. It is also a precursor of niacin (Esposito & Cuzzocrea, 2010; Jones, 1982; Juorio &

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Durden, 1984; Lewy, Emens, Sack, Hasler, & Bernert, 2002; Martin & Sloan, 1970; Martin, Sloan, Christian, & Clements, 1972; Reiter, Tan, Burkhardt, & Manchester, 2001; Wurst, Kysilka, & Flieger, 2002). The minimum daily amount currently in use for L-tryptophan is 4 mg/kg of human body weight (FAO/WHO/UNU, 2007) as recommended by the WHO (World Health Organization). Sedative, analgesic, and antidepressant properties of 5-hydroxy-L-tryptophan (as it passes through the blood–brain barrier and is metabolized into serotonin) have long been known (Morgan et al., 2001). According to the recent studies, this compound together with melatonin is a potential drug for the treatment of neurodegenerative diseases such as Alzheimer's or Parkinson's disease (Ouchi et al., 2009; Singhal, Srivastava, Agrawal, Jain, & Singh, 2012; Wang, 2009).

Because there are numerous reports on the content of bioactive compounds in the fruiting bodies of edible mushrooms, but no information about their availability in the human body, *in vitro* cultures of *A. bisporus* were used to study the release of indole compounds. Mycelial cultures are a good material to study because growing conditions and medium can be subjected to modifications which provide the mycelium of the desired composition.

Here, we present for the first time the content of indole compounds released from the lyophilized biomass of an *in vitro* culture *of A. bisporus* into artificial digestive juices (gastric and intestinal juice) in the human body (temperature 37 °C). The determination of indole compounds in the digestive juices was conducted using reversed-phase high-performance liquid chromatography (RP-HPLC). Indole compounds were subjected to qualitative and quantitative analysis. Optimization of the medium used for commercial culture of *A. bisporus* resulted in the most favorable composition in terms of the content of indole derivatives. Mushrooms, including *A. bisporus*, have several ways of accumulating metals in fruiting bodies from their surroundings; among these are the induction and binding of metals such Zn to cysteine, their proteins being rich in the metallothioneins (MT) (Rabinovich, Figlas, Delmastro, & Curvetto, 2007). As an additive to the modified Oddoux liquid medium, on which *in vitro A. bisporus* cultures were grown, we used zinc(II) ions in inorganic (zinc sulfate) and organic (zinc hydroxyaspartate) complexes. In order to compare which complex will most effectively increase the synthesis and release of indole compounds into artificial digestive juices, we used inorganic and organic compounds. Both zinc salts were used in calculated quantities such that the additive amount of zinc in all the culture media was the same. Control cultures constituted *in vitro* cultures on Oddoux medium grown without the addition of zinc compounds.

2. Materials and methods

2.1. Reagents

Zinc hydroaspartate was obtained from Farmapol (Poland); zinc sulfate was from OUM-7 Łódź. Citric acid, ammonium acetate, NaOH, K_2HPO_4, Na_2HPO_4, and $KHCO_3$ were bought from Polish Company of Chemistry (Gliwice, Poland), all of analytical grade. $NaHCO_3$ and NaCl were acquired from PPH Golpharm (Kraków, Poland); $MgCl_2$ was from Chempur (Kraków, Poland); $CaCl_2$ was from Pharma Zentrale GmbH (Germany). Bile salts and pepsin were procured from BTL (Łódź, Poland). Pancreatic extract, HCl, KCl, HNO_3 concentrated Suprapur®, KNO_3 Suprapur® H_2O_2 30% were derived from Merck (Darmstadt, Germany). Standards of indole compounds: 5-hydroxy-L-tryptophan, L-tryptophan, serotonin, melatonin, tryptamine, 5-methyltryptamine, 6-methyl-D,L-tryptophan, and methanol came from Sigma–Aldrich St Louis, MO, USA, all of HPLC grade. Quadruple-distilled water with a conductivity of less than 1 μS/cm was received using S2-97A2 distillation apparatus, Chemland (Stargard Szczecinski, Poland).

2.2. Mushroom material

Fruiting bodies of *A. bisporus* (J.E. Lange) Imbach (white bottom mushroom) of commercial origin were used for the study. In order to conduct the experiment, *in vitro* cultures of *A. bisporus* were grown on a modified liquid medium with a composition according to Oddoux and on the same medium supplemented with zinc(II) compounds in inorganic (zinc sulfate) and organic (zinc hydroxyaspartate) complexes (Oddoux, 1957).

2.3. Mycelial cultures of A. bisporus

Mycelial cultures were grown from previously prepared explants of *A. bisporus* fruiting bodies of commercial origin. Therefore, parts of the hymenium were first degreased and then sterilized. For the degreasing process, 70% ethyl alcohol was used for several seconds, while the sterilization was conducted in 15% sodium hypochlorite for 5 min. Afterwards, fragments of the fruiting bodies were rinsed several times with redistilled water and transferred to Oddoux solid medium under laminar flow. After several weeks, *in vitro* culture from the solid medium was passed under laminar flow up to 250 ml of liquid Oddoux medium supplemented with zinc sulfate or zinc hydroxyaspartate, or Oddoux medium without the addition of zinc salt the experimental cultures were grown using liquid medium to obtain the highest biomass increase for use in further measurements. The cultures were shaken at a rotational speed of 140 rpm (Altel shaker, Łódź) and incubated at 25 ± 2 °C in a cycle simulating the natural conditions of light (16 h in 900 lx lighting, as during the day time, and 8 h in dark, like in night). Mycelial liquid cultures of *A. bisporus* were shaken for four weeks and were later passaged under laminar flow.

2.3.1. Experiments using mycelial cultures of A. bisporus

Zinc salts were added to the Oddoux medium at two concentrations, in particular, as follows: 100 and 200 mg/l for zinc hydroxyaspartate and 87.23 and 174.47 mg/l for zinc sulfate. These concentrations of zinc compounds were used so that the amount of the element was the same per weight of the salts used. The content of Zn(II) ions used was 20 and 40 mg/l for the lower and higher concentrations of salts were used, respectively. For each medium (control and enriched with zinc salts), five *in vitro* cultures of *A. bisporus* were grown. After four weeks since initiation of *in vitro* cultures on liquid medium, the biomass was separated from the medium and rinsed several times with quadruple-distilled water. The resulting biomass was frozen and then dried via lyophilization.

2.4. Preparation of artificial gastric juice solutions

Solutions of artificial saliva, artificial gastric juice, and artificial intestinal juice, defined by the composition and pH simulating natural conditions occurring in the human digestive tract, were prepared using quadruple-distilled water.

2.4.1. Artificial saliva

Artificial saliva of pH 6.7 was prepared by mixing 100 ml 25 mM KH_2PO_4, 100 ml 150 mM $KHCO_3$, 100 ml 24 mM Na_2HPO_4, 100 ml 100 mM NaCl, 100 ml 1.5 mM $MgCl_2$, 100 ml 15 mM $CaCl_2$, and 6 ml 25 mM citric acid with quadruple-distilled water (Arvidson & Johasson, 1985).

2.4.2. Artificial gastric juice

2.0 g of NaCl and 3.2 g of pepsin were dissolved in quadruple-distilled water. Then, 80 ml of 1 M HCl was added and filled up with quadruple-distilled water to the final volume of 1000 ml