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# Levels of physiologically active indole derivatives in the fruiting bodies of some edible mushrooms (Basidiomycota) before and after thermal processing



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**ABSTRACT**

Indole compounds were found in the fruiting bodies of selected mushroom species both before and after thermal processing. On the basis of HPLC analyses the following indole compounds were detected: L-tryptophan, 5-hydroxytryptophan, 5-methyltryptophan, tryptamine, 5-methyltryptamine, serotonin, indole and melatonin. The compound that was present in the largest amounts in the methanolic extracts from unprocessed mushrooms was 5-hydroxytryptophan in the amount of 7.32 mg/100 g DW in the case of the fruiting bodies of *Auricularia polytricha*; 15.83 in *Suillus bovinus*; 22.94 in *Macrolepiota procera*, and 24.83 in *Lentinula edodes*. In the methanolic extracts from thermally processed mushrooms the amount of 5-hydroxytryptophan was: 3.52 mg/100 g DW in the case of *A. polytricha*; 5.65 for *Leccinum scabrum* and 10.11 for *M. procera*. In addition, serotonin was found in unprocessed fruiting bodies of two mushroom species: 1.03 mg/100 g DW in *L. edodes* and 13.99 in *L. scabrum*, and also in thermally processed mushrooms.

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Mushrooms are able to accumulate both primary and secondary metabolites. Some of them may play an antioxidant role, e.g. phenolic and indole compounds, flavonoids, terpenoids, sterols, ascorbic acid, ergothioneine, statins, carotenoids. Mushrooms are also a source of some bioelements, for example selenium (Puttaraju et al. 2006; Ferreira et al. 2009; Kalać 2010; Witkowska et al. 2011). Recent studies have demonstrated that edible mushroom species contain non-hallucinogenic indole compounds and their derivatives (Muszyńska et al. 2011a,b; Muszyńska and Sułkowska-Ziaja 2012). The indole skeleton is the basis of the substances serving important functions in the human body, such as

serotonin and melatonin. Indole compounds fulfill the role of neurotransmitters or their precursors, exhibit antioxidant, anticancer, anti-aging actions, regulate the diurnal cycle in humans and participate in blood coagulation (Kasuga et al. 1995; Reguła and Siwulski 2007). These compounds and their derivatives are also anti-inflammatory and analgesic therapeutics (Esposito and Cuzzocrea 2010).

In the central nervous system, serotonin is used for communication between neurons and it appears to enhance the perception of well-being and modulate the intensity of emotional states. Serotonin is also involved in some cognitive functions, including memory and learning (Turner et al. 2006).

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The main precursors of serotonin are tryptophan and 5-hydroxytryptophan. 5-Hydroxytryptophan, which is a direct precursor of serotonin, when ingested, easily crosses the blood–brain barrier into the central nervous system where it is efficiently converted to serotonin (Birdsall 1998). This metabolite is sold in some countries (Canada, the United Kingdom, and the United States) as a dietary supplement for use as an antidepressant and sleep aid. It is usually derived from the seeds of *Griffonia simplicifolia* (Turner et al. 2006).

Other indole derivatives are melatonin and tryptamine. In neurodegenerative diseases (for example, some types of Parkinson's and Alzheimer's disease), melatonin plays a neuroprotective role (Reiter et al. 2001; Wang 2009; Singhal et al. 2012). Tryptamine is found in trace amounts in the brains of mammals and is believed to play a role as a neuromodulator or neurotransmitter (Martin and Sloan 1970; Martin et al. 1972; Jones 1982; Juorio and Durden 1984).

The presented study is a continuation of and supplement to a widely conducted examination of the chemical composition of mushrooms growing in Poland and confirms their usefulness as a potential source of non-hallucinogenic indole compounds with medicinal properties (Muszyńska et al. 2007, 2009, 2011a,b; Muszyńska and Sułkowska-Ziaja 2012). Bioactive indole derivatives were described earlier in inedible species, for example, the genera *Amanita* (Kohlmünzer et al. 2001) and *Tricholoma* (Gartz 1994; Dobbs 2001).

The aim of the study was to conduct, for the first time, qualitative and quantitative HPLC analyses of indole compounds in methanolic extracts from the fruiting bodies of selected edible mushroom species belonging to the phylum Basidiomycota: *Leccinum scabrum* (Bull.) Gray, *Macrolepiota procera* (Scop.) Singer and *Suillus bovinus* (Pers.). Roussel collected from their natural habitats, and cultivated mushrooms *Auricularia polytricha* (Mont.) Sacc. and *Lentinula edodes* (Berk.) Pegler of commercial origin. The levels of indole metabolites in those species were determined in unprocessed and thermally processed fruiting bodies. Thermal processing of edible mushrooms decreases the amount of indole compounds. For that reason, the thermal processing of mushrooms was so designed as to simulate the cooking conditions in which mushroom dishes (like sauces, soups) are normally prepared, since only a few edible mushroom species can be eaten raw. Moreover, not all indole compounds are stable at higher temperatures (among indole compounds the most stable is L-tryptophan because it is resistant to a temperature of 90 °C) (Cuq and Cheftel 1983), so it is appropriate to determine the actual amounts of these compounds in dishes in order to assess whether mushrooms are a good source of these compounds to consumers.

All of the species chosen for analysis have a well-documented medicinal and dietary value (Kiyotaka et al. 2002; Dembitsky et al. 2010; Sun et al. 2010; Song and Du 2012). However, this paper presents, for the first time, the results of analyses of physiologically active indole compounds in these mushroom species.

The materials for analyses were fresh fruiting bodies of *L. scabrum* (*Boletus scaber*) – the birch bolete, *M. procera* – the parasol mushroom, *S. bovinus* – the Jersey cow mushroom, which were collected between 2009 and 2011 from selected regions of southern Poland (mixed forests near Kraków). The

test materials also included fresh fruiting bodies of cultivated mushrooms *L. edodes* – the shiitake and *A. polytricha* – the black (or cloud ear) fungus, which were of commercial origin.

Fresh material, after removal of plant debris and after taxonomic identification according to Knudsen and Vesterholt (2008), was frozen and immediately dried by lyophilization (Freezone 4.5, Labconco, Kansas City, MO, USA; temperature: –40 °C). Voucher specimens: UJCMBF12 – 16, are deposited in the Department of Pharmaceutical Botany, Jagiellonian University, Medical College in Kraków.

Lyophilized fruiting bodies were weighed (5 g of each species) and ground in a mortar, and then subjected to extraction with petroleum ether in a percolator in order to remove the lipid fraction according to the procedure developed in our laboratory (Muszyńska et al. 2007). After the extraction with ether, the material was dried and subjected to extraction with methanol placed in a percolator (which was kept in the dark). The oil fractions were discarded and the remaining biomass was dried and again subjected to extraction with methanol in a percolator for 24 h. The extracts were concentrated by distillation in a vacuum evaporator under reduced pressure (200 mBa) at 40 °C. The residues were quantitatively dissolved in methanol, filtered through Whatman No. 3 paper and purified by TLC. For the purification of the extracts, we used TLC on aluminum-backed silica gel 60 plates (Merck, Darmstadt, Germany, Art. No. 1.055540001), onto which the methanol extracts were loaded. Chromatograms were developed in a mobile phase which was found to be optimal for the separation of indole compounds: *n*-propanol/ethyl acetate/water (7:1:2 v/v/v). Spots containing indole compounds were identified under a UV lamp at  $\lambda = 254$  nm. The obtained fractions were extracted from the chromatograms with methanol, filtered through a syringe-driven filter unit (Millex Millipore, Billerica, MA, 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 methanol, were subjected to HPLC analysis.

For sample preparation by thermal processing, dry materials after lyophilization were weighed (5 g of each species) and ground in a mortar; 80 ml of distilled water was added to each sample and the mixtures were boiled in a thermostated water bath (Labart, Gdansk, Poland) at 100 °C for 60 min in a Soxhlet apparatus. After that time, the whole mixtures containing the fruiting bodies with the aqueous extracts from mushrooms were frozen and lyophilized again. After lyophilization, the crushed dry mass (fruiting bodies and aqueous extracts) was treated by the same procedure as the unprocessed samples.

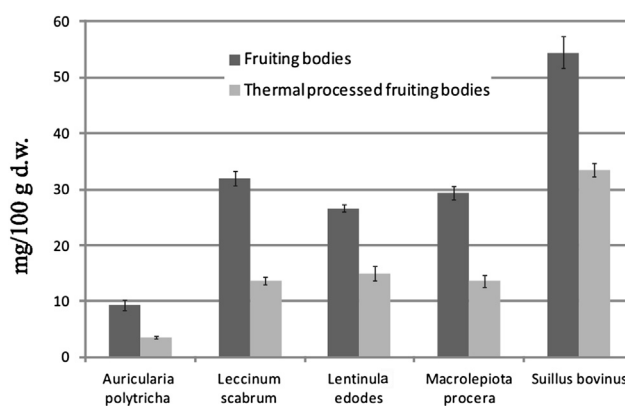
The obtained extracts were analyzed for the presence of L-tryptophan, 5-hydroxytryptophan, 5-methyltryptophan, serotonin, melatonin, tryptamine, 5-methyltryptamine, kynurenine, indoleacetic acid, indoleacetonitrile, indole and indoleacetamide (standards from Sigma–Aldrich (St. Louis, MO, USA)). The analyses 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 with pump: L-7100; column: Purospher® RP-18 (4 × 200 mm, 5  $\mu$ m) thermostated at 25 °C. The solvent system used was: methanol/water/ammonium acetate (15:14:1 v/v/v); flow rate: 1 ml/min. Detection

was carried out in a UV detector at  $\lambda = 280$  nm. The identification of indole compounds was made by comparing the retention times of sample peaks with those of the standards. The results were expressed in mg/100 g of dry weight (DW), calculated by internal normalization of the chromatographic peak area. An example chromatogram is presented in Fig. 1.

For each mushroom species, three samples were used for the determination of every compound and all the analyses were carried out in triplicate. The results were presented as mean values with the standard deviation (SD).

The method of extraction applied to the fruiting bodies of mushrooms and the HPLC analysis proved to meet the optimal conditions for the qualitative and quantitative determination of indole compounds in the test material. The analyses showed that all the tested species of mushrooms contained non-hallucinogenic indole compounds. The highest total amounts of indole compounds were found in the fruiting bodies of *S. bovinus*, both in the unprocessed and thermally processed ones (51.42 and 33.44 mg/100 g DW, respectively) (Fig. 2). The lowest amounts of indole compounds were found in the extracts from the fruiting bodies of *A. polytricha* (10.44 mg/100 g DW in the unprocessed fruiting bodies and 3.59 in the cooked ones).

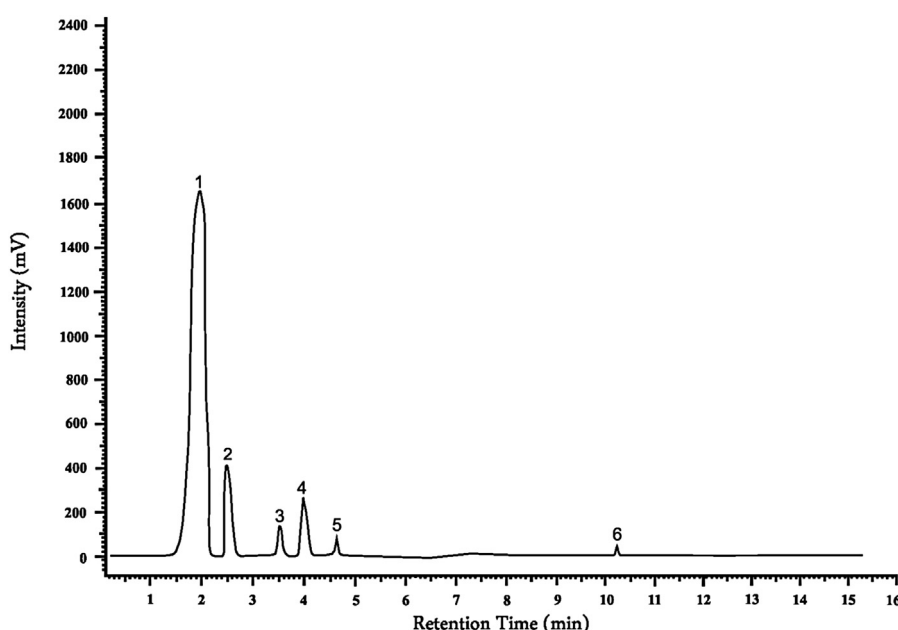
The greatest variety of indole compounds was found in the extract from the thermally unprocessed fruiting bodies of *M. procera*. The extract contained six out of all the indole compounds present in the studied mushrooms. Each of the species contained L-tryptophan, which in the human body is a precursor of neurotransmitters and hormones with the structure of indole. The highest amounts of L-tryptophan were determined in the extracts from the thermally unprocessed fruiting bodies of *S. bovinus* – 25.90 mg/100 g DW (Table 1). Among the extracts from the heat-treated fruiting bodies, those from the fruiting bodies of *S. bovinus* also contained the highest amount of L-tryptophan – 17.71 mg/100 g DW (Table 2).



**Fig. 2 – Total amounts of indole compounds in the fruiting bodies of edible mushrooms before and after thermal processing.**

The smallest amount of L-tryptophan was found in the fruiting bodies of *A. polytricha* (0.16 mg/100 g DW). L-Tryptophan is an exogenous amino acid, and it must be therefore supplied with food. Processed edible mushrooms, especially *S. bovinus*, can constitute a source of it and an alternative to other foods that contain L-tryptophan. The amounts of tryptophan determined in previous analyses of processed fruiting bodies of *Boletus edulis*, *Lactarius deliciosus* and *Pleurotus ostreatus* (Muszyńska and Sułkowska-Ziaja 2012) were approximately half of the amount found in the currently analyzed *S. bovinus*.

Relatively large quantities of another indole derivative, 5-hydroxytryptophan, were found in the tested mushrooms. This fact deserves attention because 5-hydroxytryptophan is the direct precursor of biologically active compounds that occur naturally in the human body, such as serotonin and melatonin. 5-Hydroxytryptophan was present both in



**Fig. 1 – HPLC chromatogram of the extract from the fruiting bodies of *Macrolepiota procera*: (1) 5-hydroxytryptophan, (2) L-tryptophan, (3) tryptamine, (4) 5-methyltryptamine, (5) melatonin, (6) indole.**

**Table 1 – Amounts (mg/100 g DW) of indole compounds in the fruiting bodies of selected Basidiomycota species. Data are presented as the mean  $\pm$  SE of three series.**

Species	<i>Auricularia polytricha</i>	<i>Leccinum scabrum</i>	<i>Lentinula edodes</i>	<i>Macrolepiota procera</i>	<i>Suillus bovinus</i>
Indole compounds	mg/100 g DW				
L-Tryptophan	0.16 $\pm$ 0.05	9.56 $\pm$ 0.04	0.58 $\pm$ 0.02	3.47 $\pm$ 0.05	25.90 $\pm$ 0.2
5-CH <sub>3</sub> -Tryptophan	— <sup>a</sup>	8.32 $\pm$ 0.05	— <sup>a</sup>	— <sup>a</sup>	— <sup>a</sup>
5-OH-Tryptophan	7.32 $\pm$ 0.2	— <sup>a</sup>	24.83 $\pm$ 0.3	22.94 $\pm$ 0.5	15.83 $\pm$ 0.5
Tryptamine	2.77 $\pm$ 0.05	— <sup>a</sup>	0.04 $\pm$ 0.01	0.92 $\pm$ 0.04	3.15 $\pm$ 0.05
5-CH <sub>3</sub> -Tryptamine	0.19 $\pm$ 0.01	— <sup>a</sup>	— <sup>a</sup>	2.54 $\pm$ 0.05	6.51 $\pm$ 0.3
Serotonin	— <sup>a</sup>	13.99 $\pm$ 0.4	1.03 $\pm$ 0.02	— <sup>a</sup>	— <sup>a</sup>
Indole	— <sup>a</sup>	— <sup>a</sup>	— <sup>a</sup>	0.02 $\pm$ 0.03	0.03 $\pm$ 0.01
Melatonin	— <sup>a</sup>	— <sup>a</sup>	0.13 $\pm$ 0.01	0.07 $\pm$ 0.02	— <sup>a</sup>

a Content lower than 0.001 mg/100 g DW.

unprocessed and processed fruiting bodies. Its highest amounts were found in the extracts from the unprocessed fruiting bodies of *L. edodes* (24.83 mg/100 g DW), and among the heat-treated fruiting bodies in the extracts from *M. procera* (10.11 mg/100 g DW). The maximum amount of 5-hydroxytryptophan found by Muszyńska et al. (2011b) in the uncooked fruiting bodies of *P. ostreatus* was 2.08 mg/100 g DW, while in the currently analyzed unprocessed *L. edodes* and *M. procera* more than ten times higher amounts were determined.

Tryptamine and its derivative 5-methyltryptamine were also present in the extracts from the examined mushrooms. The amounts of tryptamine in the unprocessed fruiting bodies ranged from 0.04 to 3.15 mg/100 g DW and were comparable to those in the processed samples (0.36–2.50 mg/100 g DW). Comparing the results with those obtained previously from analyses of other species of edible mushrooms (Muszyńska et al. 2009 2011a,b; Muszyńska and Sułkowska-Ziaja 2012), what deserves attention is that 5-methyltryptamine was found for the first time in three (*A. polytricha*, *M. procera*, *S. bovinus*) out of the five currently tested species, both processed and unprocessed.

5-Methyltryptamine was determined in the fruiting bodies of *S. bovinus*, *M. procera* and *A. polytricha* (6.51, 2.54, 0.19 mg/100 g DW, respectively), but in the processed samples of the same species it occurred only in small amounts (0.12, 0.93, 0.06, respectively). The least frequently found compound was 5-methyltryptophan. It was present only in the extracts from the unprocessed as well as processed fruiting bodies of

*L. scabrum* (8.32 and 2.88 mg/100 g DW). Heat treatment caused a decrease in its content. 5-Methyltryptophan had been determined previously only in the processed fruiting bodies of four species: *B. edulis*, *C. cibarius*, *L. deliciosus* and *P. ostreatus* (Muszyńska and Sułkowska-Ziaja 2012) in amounts comparable to those found in *L. scabrum*.

An indole compound rarely found in the tested mushrooms was melatonin. It was present in small amounts in the extracts from the fruiting bodies of *L. edodes* and *M. procera* (0.13 and 0.07 mg/100 g DW, respectively), but, as in the case of 5-methyltryptophan, heat treatment was also probably a cause of the decrease in its content (0.04 mg/100 g DW in *L. edodes*). The amounts of melatonin and indole in the present study, compared to those in the previously analyzed species, are lower (Muszyńska et al. 2011b; Muszyńska and Sułkowska-Ziaja 2012).

The effect of high temperature on the presence of serotonin is ambiguous. Serotonin was determined in the extracts from the processed fruiting bodies of *L. edodes* and *S. bovinus*, whereas in the extracts from the unprocessed fruiting bodies of these mushroom species its presence was not revealed. On the other hand, in the case of *L. scabrum*, serotonin was found in the unprocessed fruiting bodies, but did not appear in the extracts from the fruiting bodies subjected to thermal processing. In the unprocessed *L. edodes*, a high amount of 5-hydroxytryptophan was observed, but no serotonin, the amount of which increased in this mushroom after thermal processing. 5-Hydroxytryptophan is an intermediate

**Table 2 – Amounts (mg/100 g DW) of indole compounds in thermally processed fruiting bodies of selected Basidiomycota species. Data are presented as the mean  $\pm$  SE of three series.**

Species	<i>Auricularia polytricha</i>	<i>Leccinum scabrum</i>	<i>Lentinula edodes</i>	<i>Macrolepiota procera</i>	<i>Suillus bovinus</i>
Indole compounds	mg/100 g DW				
L-Tryptophan	— <sup>a</sup>	2.74 $\pm$ 0.04	0.63 $\pm$ 0.02	0.03 $\pm$ 0.05	17.71 $\pm$ 0.2
5-CH <sub>3</sub> -Tryptophan	— <sup>a</sup>	2.88 $\pm$ 0.05	— <sup>a</sup>	— <sup>a</sup>	— <sup>a</sup>
5-OH-Tryptophan	3.52 $\pm$ 0.05	5.65 $\pm$ 0.2	— <sup>a</sup>	10.11 $\pm$ 0.5	— <sup>a</sup>
Tryptamine	— <sup>a</sup>	0.36 $\pm$ 0.02	— <sup>a</sup>	2.56 $\pm$ 0.04	2.50 $\pm$ 0.05
5-CH <sub>3</sub> -Tryptamine	0.06 $\pm$ 0.02	— <sup>a</sup>	— <sup>a</sup>	0.93 $\pm$ 0.05	0.12 $\pm$ 0.01
Serotonin	— <sup>a</sup>	2.07 $\pm$ 0.04	14.33 $\pm$ 0.4	— <sup>a</sup>	13.11 $\pm$ 0.2
Indole	0.01 $\pm$ 0.01	— <sup>a</sup>	— <sup>a</sup>	0.02 $\pm$ 0.01	— <sup>a</sup>
Melatonin	— <sup>a</sup>	— <sup>a</sup>	0.04 $\pm$ 0.01	— <sup>a</sup>	— <sup>a</sup>

a Content lower than 0.001 mg/100 g DW.



metabolite of L-tryptophan in the biosynthesis of serotonin (Birdsall 1998), which suggests that in *L. edodes* 5-hydroxytryptophan was converted to serotonin. Serotonin had been determined previously only in unprocessed fruiting bodies, with the highest amount, being almost twice as high as that in the currently analyzed *L. scabrum*, found in *S. luteus* (34.11 mg/100 g DW) (Muszyńska et al. 2011b).

It has been proven that thermal processing of edible mushrooms affects the composition of indole compounds and heat-treated fruiting bodies contain on average about half of the total amount of indole compounds (Fig. 2). The effect of elevated temperature during cooking on the levels of indole compounds is, however, not clear. Both a decrease and increase in the amount of some indole compounds can be observed. The decrease is probably due to the decomposition of these compounds under high temperature, because they are thermolabile. The increase in the amount of some compounds (indole, 5-hydroxytryptophan, L-tryptophan, tryptamine, 5-methyltryptamine) may be associated with thermally induced changes in other compounds.

In conclusion, it can be said that the method of preparing meals with mushrooms can affect the levels of indole compounds because of their sensitivity to elevated temperature. An increase in temperature causes a partial decrease in their amounts, but cooked mushrooms still remain an excellent source of these metabolites, particularly L-tryptophan and 5-hydroxytryptophan, which are the precursors of such physiologically active indole compounds as serotonin and melatonin. Attention should be drawn to the fact that serotonin does not cross the blood–brain barrier, while L-tryptophan and 5-hydroxytryptophan have this ability and are the precursors of serotonin, and have therefore an antidepressant action. Serotonin, which also occurs in edible mushrooms, when ingested, can regulate the peristaltic movement of the gut. Current research indicates that mushrooms are an alternative to animal meat, because they are a good source of L-tryptophan, which, as an exogenous amino acid, is important in the daily human diet. The results indicate the need for continuation and expansion of research on the accumulation of indole compounds in mushrooms.

## Disclosure

The authors declare no conflict of interest. All the experiments undertaken in this study comply with the current laws of Poland.

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