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CHEMICAL COMPOSITION OF AGARICUS BISPORUS AND PLEUROTUS OSTREATUS FRUITING BODIES AND THEIR MORPHOLOGICAL PARTS

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Abstract: *The chemical composition of two edible mushroom species was investigated. Agaricus bisporus is a saprotrophic spice. Pleurotus ostreatus belongs to the wood-rotting mushrooms. They are the most popular among mushrooms that are cultivated in Europe. It is found that mushrooms contain carbohydrates, protein, lipids, phenolic compounds and mineral elements. P. ostreatus has more carbohydrates than A. bisporus. Trehalose dominates among the low molecular weight carbohydrates. The main monosaccharide in the hydrolysates of easily hydrolysable polysaccharides is glucose. Only glucose and glucosamine are detected in the hydrolysates of hardly hydrolysable polysaccharides. Glucan and chitin are present in the composition of this polysaccharide fraction. A. bisporus contains 2.0 times more chitin than P. ostreatus. All essential amino acids are found in the mushroom protein hydrolysates. Nonprotein nitrogen compounds are present in the mushrooms. Lipid level is 1.4 times higher in A. bisporus than in P. ostreatus. Unsaturated fatty acids dominate in the lipid composition. Phenolic compounds of mushrooms include low molecular weight compounds and melanin. The low molecular weight phenolic compounds levels in the mushrooms are the same. A. bisporus contains significantly more melanin than P. ostreatus. Mushrooms differ in the mineral element content. Mushroom morphological parts have the different chemical composition. Carbohydrates are mainly concentrated in the stipes, and protein can be found in the caps. A bisporus caps contain more lipids than the stipes. Morphological parts of P. ostreatus do not significantly differ in this index. The phenolic compounds are mainly concentrated in A. bisporus caps.*

Keywords: *white button mushroom, oyster mushroom, cap, stipe, carbohydrate, protein, lipids, phenolic compounds, mineral elements*

1. Introduction

Increase in the number of people suffering from the so-called "diseases of civilization" has changed the attitudes to the foodstuff. Now they can be only an energy source but also a promising means of non-drug correction of certain physiological processes in a human body. It has caused the fact that a new type of food products – functional food was proposed. It often includes the foodstuff containing some functional ingredients. These components are able to correct some physiological processes in a human body [1].

Mushrooms are a promising source of such ingredients. Some ecotoxicants in the wild

mushrooms, the possibility of biological mutations due to the environmental pollution, seasonality and difficulties of prediction the stable amounts of provision interfere using these types of mushrooms. Cultivated mushrooms do not have these defects [2].

Agaricus bisporus (white button mushroom) and *Pleurotus ostreatu*s (oyster mushroom) are mainly cultivated in Europe [2]. The content of protein, lipids and mineral elements in mushrooms was evaluated [3-7]. However, determination of carbohydrate content as the difference between the total dry weight and weight of these substances does not give enough information about carbohydrate composition. It was not investigated what mono- and oligosaccharides, polysaccharides are present in the mushrooms. However, some researches pay attention to the mushroom polysaccharides because of their high physiological activity [8].

Mushroom fruiting bodies are composed of two morphological parts: caps and stipes. They probably have different chemical composition. However, few investigations have focused on the content of some compounds in the mushroom morphological parts.

Thus, information about the chemical composition of these mushrooms is not sufficient for an objective evaluation of prospects for using their as a source of biologically active compounds, extraction and making dietary supplements on their basis.

The purpose of this research was to determine the chemical composition of *Agaricus bisporus* and *Pleurotus ostreatus* fruting bodies and their morphological parts.

2. Experimental

Two spices of edible mushrooms: *Agaricus bisporus* (white button mushroom) and *Pleurotus ostreatus* (oyster mushroom) were purchased at the farm that is cultivated mushrooms and used in this research.

The fruit bodies were cleaned, separated into caps and stipes, dried and milled.

Analysis of total dry matter content was carried out at 65 °C using a vacuum oven [3].

For determination easily hydrolysable polysaccharides (EHP) content mushroom samples were being hydrolyzed with 2 % HCl solution at the boiling temperature for 4.0 hours. Then the residues after hydrolysis were washed with water up to neutral value pH, dried up to constant weight. For determination hardly hydrolysable polysaccharides (HHP) content these residues were being treated with 72 % H_2SO_4 solution at the ambient temperature for 2.5 hours. The water was added. Then they were being hydrolyzed for another 5 hours at the boiling temperature [9]. In the all hydrolyzates the total neutral carbohydrate was determined by the anthrone method with glucose as standard [10], the glucoasamine content was evaluated with 3-methyl-2-benzothiazolone-hydrazone-hydrochloride according to [11].

For identification of monosaccharides the sugars were converted into their corresponding alditol acetates [12] and identified by gas-liquid chromatography. Gas-liquid chromatography was performed on Hewlett-Packard 5890 A chromatograph with a flame ionization detector and integrator 3393 A with a Ultra-1 capillary column (25 m x 0.2 mm) from 175 °C to 270 °C at 10 °C/min in a stream of nitrogen.

Extraction of the mannitol was carried out according to [13]. Sample was suspended in the water and being thoroughly homogenized for 30 min. The suspension was being boiled for 15 min. After freeze-drying, the material was redissolved in the pyridine and being heated for 30 min. The mannitol solution in pyridine was thoroughly mixed with arabitol solution. This solution was mixed with a derivatizing agent (the mixture of N,O-bistrimethylsilyl trifluoroacetamide and trimethylchlorosilane). The mixrure was vortexed and allowed to stand at room temperature for 15 min. Then it was injected into the gas-liquid chromatograph.

Analysis of total nitrogen content was done according to the Kjeldahl method [3].

For determination non protein nitrogen 0.3 g of sample was transferred to the glass. Then 25 cm^3 of distilled water was added while stirring. It was heated up to the boiling temperature and 5 cm^3 of 50 % trichloroacetic acid solution was added stirring thoroughly. This mixture was left for 0.5 – 1 hour, filtered through ashless filter. The precipitate was washed with small portions of 2 % trichloroacetic acid solution. The filtrate was mineralized with sulphuric acid and nonprotein nitrogen content was determined by the Kjeldahl method [3].

Chitin nitrogen level in mushroom was obtained by multiplying the chitin content by the coefficient 0.069 (in the natural chitin there is 6.9 % N) [14].

Total protein nitrogen content was calculated as the difference between total nitrogen level and nonprotein nitrogen, chitin nitrogen values. Total protein content was calculated by multiplying the protein nitrogen content by 6.25 [3].

The level of amino acids was determined with using the T 339 chromatographic amino acid analyser. Liquid-phase hydrolysis of sample was being performed in 6M HCl solution at 110 °C for 24 h in an argon atmosphere [15]. The hydrolysate was lyophilised, dissolved in an appropriate volume of dilution buffer and filtered. The determination of the sulphur-containing amino acids (methionine and cysteine) was carried out by means of oxidative hydrolysis, using a mixture of formic acid and hydrogen peroxide (9:1) at 4 °C for 16 h, followed by standard hydrolysis procedure with HCl. Alkaline hydrolysis for tryptophan determination was done according to [15]. The calculations were carried out with reference to external standards.

The composition of amino acids was expressed as grams per 100 g of protein. On the basis of the amino acid composition, the chemical score index was calculated [16].

The lipid content was determined by Soxhlet extraction [17]. Fatty acids, transmethylated with 5 % sulphuric acid in methanol, were analyzed by gas liquid chromatography (HP 6890 Plus) equipped with a flame ionization detector [18].

The level of the total phenolic compounds was estimated in the ethanol extracts using Folin–Chiocalteu method [19]. For preparation these extracts sample (2 g) was being extracted by stirring with 20 cm^3 of 70 % ethanol solution at 65 ºC for 30 min and filtered. This operation was repeated one time again. Then 1 cm^3 of the combined extract was added to 10 cm^3 of distilled water and 2 cm^3 of Folin-Ciocalteu phenol reagent. The mixture was allowed to stand at room temperature for 5 min and then 2 cm^3 of 20 % sodium carbonate solution was added to the mixture. The resulting blue complex was measured at 730 nm. Chlorogenic acid from Sigma was used as a standard for the calibration curve.

Melanin from mushrooms was being extracted by 2 % NaOH solution for

2 hours at the boiling temperature. The extract was cooled, acidified to pH 2.0. Concentrated pigment was separated by centrifugation at 8000 g for 15 min [20]. The precipitate was dissolved in the 2 % NaOH solution. Melanin content was calculated from the calibration curve on the basis of the photometry of the solution at the wavelength of 490 nm. Melanin from Sigma was used for the calibration curve.

Ash content was evaluated by weighing before and after treatment of the dried mushrooms in a chinaware crucible at 550 °C to constant weight. Total ash content was expressed in percentage of dry weight [3].

Analytical determinations were conducted in three simultaneous replications.

3. Results and Discussion

The water content in mushrooms and in their morphological parts was 91.5-92.1 %. Chemical composition of the mushroom morphological parts is given in Table. 1. Carbohydrates are one of the important mushroom components. They include mono- and oligosaccharides, easily and hardly hydrolysable polysaccharides (EHP and HHP respectively). Notably, their content in *P. ostreatus* is 2.1 times higher than in *A. bisporus*.

Table 1

The chemical composition of mushroom morphological parts (% of the dry weight)

Components	*Agaricus bisporus*		*Pleurotus ostreatus*	
	Cap	**Stipe**	**Cap**	**Stipe**
Mono-and oligosaccharides	1.2	1.4	16.0	11.0
Mannitol	30.4	28.9	7.5	5.2
Polysaccharides including easily hydrolysable	13.7	21.9	25.0	27.0
hardly hydrolysable	7.2	9.2	8.3	24.0
including chitin	4.0	7.9	2.6	2.9
Total nitrogen	5.3	4.7	4.8	2.9
including nonprotein nitrogen	2.0	1.7	1.9	1.1
Protein	18.9	15.3	17.0	10.0
Lipids	4.0	1.8	2.5	2.0
Phenolic compounds	8.3	4.0	4.9	3.2
including melanins	6.4	2.1	3.1	1.6
Mineral elements	9.0	7.6	7.9	6.7

EHP dominate among the carbohydrates. Their content in *A. bisporus* is 64.7 % of the total carbohydrate level, in *P. ostreatus* is 47.2 % respectively. Glucose is a main monosaccharide in their hydrolysates (Table 2). The levels of mannose, fucose and xylose that are present in the small amounts are almost the same in the studied mushrooms. However, the galactose content is higher in *A. bisporus* than in *P. ostreatus*. It is possible that EHP contain glucan and heteropolysaccharides.

Table 2

Monosaccharide composition of the easily hydrolysable polysaccharides hydrolysates of mushroom morphological parts (% of the monosaccharide residues)

Monosaccharide	*Agaricus bisporus*		*Pleurotus ostreatus*	
	Cap	**Stipe**	**Cap**	**Stipe**
Galactose	4.6	3.2	1.0	0.5
Glucose	79.5	84.0	82.5	88.0
Mannose	3.9	4.8	6.5	4.5
Xylose	5.2	3.3	5.0	5.0
Fucose	6.8	4.7	5.0	2.0

P. ostreatus has 1.9 times more HHP than *A. bisporus*. Only glucose and glucosamine are identified in their hydrolysates, so HHP contain glucan and chitin. However, the ratio of these polysaccharides depends on the mushroom type. Chitin dominates in *A. bisporus* (69.5 % of HHP), glucan prevails in *P. ostreatus* (82.0 % of HHP). This difference is associated with the mushroom nutrition type: saprotrophic spice (*A. bisporus*) synthesizes more chitin than wood-rotting one (*P. ostreatus*) [7, 21].

The trehalose dominates among the low molecular weight carbohydrate. *P. ostreatus* has 10.8 times more trehalose than *A. bisporus*. Probably this disaccharide makes reserve function. The reserve substance in *A. bisporus* is polyol – mannitol [22]. Its content is 4.6 times higher than in *P. ostreatus*.

It is shown that morphological parts of mushrooms contain different quantity of carbohydrates. The total level of polysaccharides is 1.3-1,5 times higher in stipes in comparison with caps. However, the caps have more low molecular weight carbohydrates and mannitol than stipes. It can be explained by the different biological roles of mushroom morphological parts. Stipes makes the transport and support function. That is why they contain well-developed transport and mechanical tissue structures. Polysaccharides are their main components. Caps accumulate a large amount of storage nutrition compounds that are necessary for forming mushroom reproductive organs – basidiospores [7, 21].

The various nutrition types of studied mushrooms cause the difference in the quantity of protein in their composition. *A. bisporus* has 1.3 times more protein than *P. ostreatus*.

18 amino acids are found in the mushroom protein hydrolysates (Table 3).

Table 3

Amino acid composition of protein of mushroom morphological parts (% of total protein content)

Amino acid	*Agaricus bisporus*		*Pleurotus ostreatus*	
	Cap	**Stipe**	**Cap**	**Stipe**
Lysine	5.7	5.7	5.6	6.0
Histidine	2.8	2.8	2.1	2.1
Arginine	4.9	4.8	5.6	5.4
Aspartic acid	11.8	10.9	11.4	10.4
Threonine	4.9	4.6	4.5	3.7
Serine	5.8	5.9	5.4	5.2
Glutamic acid	20.0	18.1	19.2	17.2

Proline	5.3	5.9	4.1	5.7
Glycine	5.0	5.7	4.3	4.0
Alanine	5.7	5.7	6.8	4.3
Cysteine	3.7	4.4	3.9	3.8
Valine	3.9	4.4	5.4	5.9
Methionine	1.1	1.8	2.3	1.9
Isoleucine	3.6	3.6	3.7	3.8
Leucine	7.2	7.3	6.7	9.2
Tyrosine	3.4	2.7	3.5	4.9
Phenylalanine	4.2	4.2	4.3	5.0
Tryptophan	1.2	1.4	1.3	1.4

The most abundant amino acid is glutamic acid, which plays an important role in the organism metabolism. The part of essential amino acids gets 39.4 – 43.2 % of the total amino acids level. Leucine is the main essential amino acid. The levels of methionine and cysteine, phenylalanine and tyrosine, tryptophan, threonine, leucine, lysine of both spices of mushrooms are higher than in the ideal protein (Table 4). Lysine is a deficient amino acid for the most food raw-materials. Blood circulation is disrupted and the number of red blood cells reduces while it lacks in a human body [23]. *P. ostreatus* contains more valine and isoleucine than *A. bisporus*.

P. ostreatus has less nonprotein nitrogen compounds than *A. bisporus* (1.2 times). These intermediate products of protein metabolism include free amino acids, urea, organic and purine bases, ammonia nitrogen that is free ammonia and ammonium-magnesium salts of phosphoric acid [7, 21].

Table 4

Amino acid chemical score of protein of mushroom morphological parts (%)

Amino acid	*Agaricus bisporus*		*Pleurotus ostreatus*	
	Cap	**Stipe**	**Cap**	**Stipe**
Lysine	103.6	104.0	100.9	109.5
Threonine	121.5	114.3	111.5	93.3
Methionine + cysteine	137.4	177.1	176.9	162.3
Valine	78.4	80.7	108.4	118.8
Isoleucine	89.8	90.3	93.0	96.0
Leucine	102.9	104.3	96.3	131.1
Phenylalanine + tyrosine	126.3	115.3	130.7	164.5
Tryptophan	120.0	140.0	130.0	140.0

Mushroom morphological parts differ in the total amount of protein and the intermediate products of protein metabolism. They get the highest level in caps. It can be explained by their biological role. A significantly difference in the quantity of protein and nonprotein compounds is found in *P. ostreatus* morphological parts.

Stipes contain more essential amino acids in the protein hydrolysates than caps. This is typical for *P. ostreatus* and *A. bisporus.*

The amount of lipids in *A. bispo*rus is 1.4 times higher than the one in *P. ostreatus. A. bisporus* caps have more these compounds than stipes. However, the morphological parts of *P. ostreatus* contain almost the same quantity of lipids. It is shown that unsaturated fatty acids dominate in the lipid composition. Their

level in *A. bisporus* is 82.8 % of the total fatty acids, in *P. ostreatus* is 71.5 %. *A. bisporus* and olive oil have almost the same level of unsaturated fatty acids [24]. Their content in *P. ostreatus* is approximately equal to the level of these acids in soybean and cotton oil [24]. Mushrooms are also different in abundant unsaturated fatty acids. The oleic acid is in *A. bisporus*, linoleic one is in *P. ostreatus*. The latter together with the linolenic acid are the essential acids. They are involved in the synthesis of cell membranes, prostaglandins, in the regulation of cells metabolism [24].

Table 5

Fatty acid composition of lipids of mushroom morphological parts (% of the total fatty acids)

Fatty acids	*Agaricus bisporus*		*Pleurotus ostreatus*	
	Cap	**Stipe**	**Cap**	**Stipe**
Tridecanoic	traces	traces	2.8	10.5
Myristic	0.4	traces	traces	19.1
Pentadecanoic	1.1	2.0	1.9	traces
Palmitic	8.7	7.6	11.2	7.1
Stearic	5.9	9.1	1.5	3.2
Oleic	54.8	38.7	18.4	17.6
Linoleic	24.1	41.9	61.9	36.6
Linolenic	5.0	0.7	0.7	5.2

The amount of certain types of fatty acids and their ratio in the products are important for the normal functioning of a human body. *A. bisporus* and *P. ostreatus* contain 34.4 % and 53.5 % polyunsaturated, 48.4 % and 18.0 % monounsaturated, 17.2 % and 27.3 % saturated acids respectively. The ratio of linoleic and linolenic acids is 9.6 for *A. bisporus* and 18.9 for *P. ostreatus*. That is approximately equal to the recommended norms [24]. This fact allows us to offer mushrooms as food ingredients to the elderly and patients with cardiovascular disease.

However, mushroom morphological parts have the different levels of these acids. It is found the level of monounsaturated acids is 1.4 times higher in *A. bisporus* caps than in stipes. But they contain less polyunsaturated acids. The level of saturated acids is almost the same in caps and stipes of this mushroom. Morphological parts of *P. ostreatus* have approximately the same content of monounsaturated acids. The amount of polyunsaturated in caps is 1.5 times more than in stipes. The level of saturated acid is 2.3 times less.

Phenolic compounds in mushrooms include high molecular weight compounds – melanin and low molecular weight substances. The latter content is approximately the same for mushrooms and their morphological parts. They show high antioxidant and antimicrobial properties [25].

Identification of melanin was carried out by qualitative reactions and the pattern of absorption in the UV and visible regions of the spectrum. They are oxidized by H_2O_2, $KMnO_4$ solutions and formed a precipitate reacting with $FeCl_3$ solution. It is characteristic for all pigments of this nature, regardless of their origin. The absorption spectrum of mushroom melanins is typical [20]. The highest absorption intensity is observed in the UV

region of the spectrum and then with increasing wavelength it gradually decreases.
The amount of melanin in *P. ostreatus* is far less than in A. *bisporus*. Caps have higher levels of these polymers.
It is known [7, 21] that the level of mineral elements in mushrooms depends on the type of mushrooms. The studied mushrooms differ in their content. Mushroom morphological parts contain various amounts of mineral elements. Caps have them more than stipes.

4. Conclusion

The various nutrition types of studied mushrooms cause the differences in their chemical composition.
It is shown, that carbohydrates are dominating macrocomponent in the mushrooms. *P. ostreatus* contains 2.1 times more these compounds than *A. bisporus*. Trehalose level in *P. ostreatus* is 10.8 times higher than that in *A. bisporus*. The latter contains more mannitol. The easily hydrolysable polysaccharides mainly compose of glucose units. Hardly hydrolysable polysaccharides contain glucan and chitin. *P. ostreatus* is richer in this fraction than *A. bisporus*. The level of the essential amino acids is 39.4 – 43.2 % of the total amino acids. The content of total protein and nonprotein nitrogen compounds is higher in *A. bisporus* in comparison with *P. ostreatus*. *A. bisporus* contains 1.4 times more lipid than *P. ostreatus*. The part of unsaturated fatty acids is 71.5-82.8 %. The oleic acid dominates in *A. bisporus*, the linoleic one is the main fatty acid in *P. ostreatus*. The total amount of phenolic compounds is higher in *A. bisporus* than in *P. ostreatus*. Melanins dominate among these substances. The studied mushrooms contain the different amount of mineral elements.
It is shown that among mushroom morphological parts stipes have higher level of carbohydrates. Caps contain more mannitol, protein, nonprotein nitrogen compounds, lipids, phenolic compounds.
Attention should be paid to difference in chemical composition of mushrooms and their morphological parts while the technologies for obtaining a number of functional components from them are developed.

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