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Morphological and biochemical study of Bulgarian species *Agaricus bohusii*

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Abstract

The study is first report on the biochemical composition of *Agaricus bohusii* Bon. The results obtained contribute to the knowledge of the chemical composition of mushrooms belonging to the *Agaricus* genus. There has been isolation, identification and analysis of the polar fraction, lipid and mineral content, as well as of pigments. Proteins contained 18 amino acids. Amino acid profile showed *A. bohusii* proteins are rich in valin, glutamic acid, aspartic acid, glycine, isoleucine and lysine. A detailed macro– and microscopic description is given. The species are described and illustrated on the basis of Bulgarian specimens.

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Introduction

The chemical composition and biologically active substances in the fruiting bodies, and mycelia culture of edible *Agaricus* species has taxonomic and economic importance. Data on chemical composition and nutritional value of European edible mushroom species, including the genus *Agaricus*, were reviewed (Didukh *et al*., 2003; Bernaš *et al*., 2006; Foret and Arpin, 1991; Wasser and Weis, 1999; Bonzom *et al*., 1999; Bernando *et al*., 1999; Wasser *et al*., 2000; Dabbour and Takruri, 2002; Brondz *et al*., 2004; Kalač, 2009, 2012; Uzun *et al*., 2009; Reis *et al*., 2012). The authors establish the existence of a number of polysaccharide, lipid and protein fractions, and amino acids.

Agaricus bohusii Bon is very rare in Europe and the Balkan Peninsula species, therefore, has long been denied in the mycological literature. The species described by only in 1983 (Bohus, 1971; Bon, 1983). It is known only from Czech Republic, France, Hungary and Italy (Bohus, 1971; Capelli, 1983, 1984; ODONAT (Coord.), 2003). In Balkan Peninsula the species has been recorded in Serbia (Reis *et al*., 2012), was also found in Bulgaria (Lacheva, 2006, 2011a, 2012c, 2013). Included in the Red List of Bulgarian fungi and in the Red Data book of Republic of Bulgaria under category *Critically Endangered* (Gyosheva *et al*., 2006; Peev *et al*., 2011).

This *Agaricus* species is an edible and interesting mushroom and in this respect were set some of its chemical parameters related to nutrition. So far, the chemical composition of this species has not been studied.

The aim of present investigation was to study some morphological and biochemical properties of Bulgarian species *Agaricus bohusii*.

Material and methods

Collection and keeping of the samples

The samples are documented with color photographs, concise description. Fruiting bodies of both species were photographed with SONY Cyber-shot 5.1Mpix. and CANON PowerShot A460 5.0Mpix in standard JPEG format. Microscopic features are observed and measured in lactofenol under *Amplival* LM, with magnification × 1000. Schäeffer reaction was tested by aniline and 65% HNO_3 acid (Schäeffer and Møller, 1938) on dried samples.

The taxonomic decisions in the article have been made in conformity with the researches of Bohus (1971), Cappelli (1984), Parra (2005), Lacheva (2006).

The carpophores of *Agaricus bohusii* were collected from author in Bulgaria, Mt Strandzha, Burgas distr, near the dam of Malko Sharkovo, in communities of *Acer campestre* L., *Quercus frainetto* Ten. and *Q. cerris* L., under *Acer campestre* L., in August 2012. Air-dried studied specimens of the fungi are kept in the Mycological collection of the Agricultural University, Plovdiv (SOA).

Studies on the chemical composition of fruiting bodies were conducted in laboratory of the Agrochemistry, Agricultural University, Plovdiv.

Isolation, identification and analysis of the polar fraction

Analysis used properly cleaned and dried at 60°C fruiting bodies, as analyzed following indicators: total nitrogen – was conducted according to the method of Kjeldal; pure protein – was conducted according to the method of Barnshtayn (the amount of amino acids less the amount of the hydrolysis attached of the peptide ties of water – 15%); the extractions of proteins, their purification and electrophoretic fractionation were made by the method described in (Lowry *et al*., 1951); raw fiber – was conducted according to the method of Kyurshner and Hanak as modified by Kogan (Marh and Krzhevova, 1962); total lipid extraction was conducted according to the method of Koksle.

Amino acid extractions were conducted in two

stages – at first the extraction of free amino acids, and then the hydrolysis of dried sediment with 6 N HCl in vacuum for 24 hours at 105°C. Amino acid content was determined on amino acid analyzer (Hd-1200 E). For the determination of the amino acids is used colorimetric method of Horn and Jones as modified by Sawickii (1967).

Free mono- and disaccharides soluble in ethanol were extracted from both the caps and the stipes. Storage polysaccharides were hydrolysed by α-amylase for 24 hours at 37°C. Hemicellulose type structural polysaccharides were hydrolysed by 3% H_2SO_4 for 3 hours and cellulose type ones with 5% H_2SO_4 for 5 hours.

Identification and analysis of lipid and mineral content

Lipid content was determined by gravimetric method. Total mineral content (ash) – by burning and ashing at 650 °C.

Isolation and identification of pigments

Extractions of pigments were carried out in three different ways: with acetone, 1% HCl in ethanol, 50% acetone in water (V/V) (Klyshev *et al*., 1978). Visible absorption spectra were recorded on Specord M-40.1% HCl-ethanol exctracts were chromatographed on paper with butanol: acetic acid : water (4:1:5).

Extraction and isolation of carotene compounds were as follows. Biomass of both the caps and the stipes were extracted with 80% acetone (first portion) and 100% acetone (next portions). Acetone extracts were chromatographed on paper with petroleum ether : acetone : benzene (12,5:17,5: 42) and on thin layer with n-hexane : diethyl ether : acetic acid (80:20:1) (Lowry *et al*., 1951).

Results and discussion

Description of the species

Agaricus bohusii Bon, Doc. Mycol., 13(49): 56, 1983 (Fig. 1).

Pileus up to 6–12(-18) cm in diameter, thick-fleshed, initially hemispherical, subsequently convex to flat-convex, seldom flat or slightly depressed, dry, pale-brown, dark-brown in the center, with radial, triangular, light-brown to brownish-dark fibrous scales on whitish background. Margin even, on drying undulate, often with remnants of the veil. Margin at first with involute, thin inrolled, finally straight margin with conspicuous, 5 mm thick, with fibrillose brownish scales, often with remnants of the veil. Pileal cuticle consisting of brown thick-walled cylindrical hyphae, without clamps, 5–15 µm in diameter. *Gills* free, thin, crowded, narrowing toward the margin of pileus, with an even edge, whitish-pink, subsequently pale-red to red-brown, with pale, sterile edge. Gill trama in young carpophores initially regular, latter irregular, consisting of cylindric, thin-walled hyphae, 5–12 µm in diam. *Stipe* up to 8–17.5 × 1.5–3 cm, central, fusiform, inflated in the center, strongly narrowing at the base, initially white, latter pale-brown, the touch red-brown, smooth to silky-fibrillose. *Flesh* whitish, on cutting becoming light reddish-brown, firm. *Ring* narrow apical, free standing, with a thin, whitish, the touch upper layer red tinge and thick, delicate, often cracked, brown bottom layer. *Smell* non-distinctive. *Taste* non-distinctive. *Basidiospores* 4.5– (4.2 ± 0.01) –5.5 × 3.8– (4.1 ± 0.01) –4.6 µm, (n = 50), brown, ovoid-ellipsoid, with apiculus and 1–2 refractive droplets. Spore print dark-brown. *Basidia* 19–23 × 6.5–7.5 µm, 4-sterigmate, clavate. *Sterigmata* 2–3 µm long. *Cheilocystidia* 18–35 × 6.5–9.5 µm, numerous, clavate to cylindrical, lageniform, thin-walled, hyaline or brownish. *Macrochemical reactions:* Cross reaction with Schaeffer's reagent negative.

Data of biochemical study

The studies of biochemical parameters showed that the total content of proteins in biomass of the caps of *Agaricus bohusii* is 16.7% of dry weight, meanwhile in the stipes only 8.6% (Table 1).

Available data on the content of proteins in carpophores of certain species of mushrooms show

them to vary over a broad range: from 4.2 to 55.1%. For example, fruit bodies of some *Agaricus* L.: Fr. emend. Karst. species contain 11.5–43.7% of protein (Buhalo, 1988; Chang and Hayes, 1978; Kalač, 2009, 2012). The micelium of some Agaricomycetes (e.g. *Oligoporus caesius, Stereum hirsutum*, *Trametes versicolor*) contain 34.5– 36.7% of protein. Sixteen amino acids were found in the protein composition, including 6 essential ones (Oleshko and Babickaya, 1991).

Table 1. Amino acids content in proteins of *A. bohusii* basidiomata (% of dry weight).

Amino acid	*Agaricus bohusii* (%)
Lisine	1.5
Histidine	0.3
Arginine	0.7
Aspartic acid	1,9
Threonine	0.9
Serine	0.4
Glutamic acid	2.5
Proline	0.9
Glycine	1.9
Alanine	0.8
Cistine	0.1
Valine	3.0
Methionine	0.1
Isoleucine	1,8
Leucine	2.7
Tyrosin	1.4
Phenyialanine	0.6
Triptophan	1.4
Total	25.3

Proteins of carpophores of *Agaricus bohusii* were found to contain 18 amino acids, with especially high amounts of valin, glutamic acid, aspartic acid, glycine, isoleucine and lisine (Table 1). From this it can be assumed, that proteins of this fungus in its digestibility could be attributed to the proteins from plant origin (Staheev, 1978). The fruiting bodies of *Agaricus bisporus* (J. Lge) Imbach contained 19 amino acids comprising 17.9–39.8% of dry biomass, and by *A. geesteranii* Bas. & Heinem., contained 19 amino acids (Wasser *et al*., 1999, 2001). Despite the low content of sulfur-containing amino acids from a biological point of view, this mushroom protein represents of interest, although research on amino acid content and extent of absorption of protein in the fruiting bodies of *Agaricus macrosporus* (F.H. Møller & J. Schaeff.) Pilát, reveal protein digestibility (80.5%) and a high content of essential sulfur-containing amino acids (Dabbour and Takruri, 2002). The electrophoretic study of soluble proteins of *Agaricus bohusii* carpophores showed a marked heterogeneity.

Table 2. Content of nitrogen, protein, fiber, lipids and ash of *A. bohusii* basidiomata (% of dry weight).

Index	*Agaricus bohusii* (%)
Total nitrogen	5.7
Protein nitrogen	4.6
Pure protein	25.3
Ash	3.5
Fiber	4.2
Lipids	3.7

The biomass of the caps and stipes of *Agaricus bohusii* contained the same concentrations of free mono- and disaccharides: in the pileus – 2.70% of dry weight, but different quantities of storage polysaccharides (2.47% and 1.2%).

Fig. 1. Macro- and microscopic features of *Agaricus bohusii* from Bulgaria: a, b – basidiomata of *A. bohusii* at different stages of development *in situ*, c – basidiospores (photos: M. Lacheva). Bar = 5μm.

The analysis of structural polysaccharides of the cellulose type showed that the content in the stipes was higher (6.77% of dry weight) than in the caps (3.97%). The same was established for easily hydrolyzing polysaccharides of the hemicellulose type in different morphological elements of *Agaricus bohusii* (Table 2).

Data analysis in *Agaricus bisporus* show that the greater the amount of ethanolamine and choline, as compared with the rest of a diacylglycerophospholipid, mono-, di-and triglycerides, sphingolipids and steroids. The sterols are presented primarily of ergosterol and are also high, and the fatty acids from the phospholipid predominates linolenic acid (Bonzom *et al.*, 1999; Pedneault, 2008; Kalač, 2012). Malic, oxalic and fumaric acids were the organic acids identified and quantified in *A. bohusii* (Kalač, 2012; Reis *et al.*, 2012).

The biomass of *Agaricus bohusii* has been established to have a low amount of lipids. So, the caps accumulated them to 3.71% of dry weight, while the stipes accumulated approximately half that amount. The lipid content of *Agaricus bohusii* was close to the low limit of the content of lipids in *Agaricus* reported by other authors (Wasser *et al.*, 1999; 2001; Ribeiro, 2009; Kalač, 2012; Reis *et al.*, 2012) (Table 2).

Histochemical reaction with Lugol's reagent resulted in rise of reddish-brown colour of the storage polysaccharides of *Agaricus bohusii,* that was characteristic of glycogen, evidence for their higher degree of branching. They have a similarity with the branching reserve polysaccharides of some Cyanophyta species (Shnyukova, 1994; Wasser *et al.*, 1999). In accordance with absorption spectra and with circular dichroism of polyiodic complexes of storage polysaccharides isolated by preparatory techniques, as well as the value of their β-amylolysis limit, the reserve polysaccharides of Cyanophyta proned to be high-branched polyglucans of the glycogen type with a great number of α-l,6-links (Sudina, 1978; Shnyukova, 1994; Wasser *et al.*, 1999). The pigments of fungi were divided on the basis of their chemical nature into: carotenoids, bensole derivative : quinones, anthraquinones, azulenes, and heterocyclic nitrogen-bearing pigments (Becker, 1988; Velíšek, 2011). As a rule, the fungi have some groups of pigments which determine their colour, which in many cases are used as a chemotaxonomic test. Carotenoids, porphyrin-like compounds, flavins, flavoproteins act as photoreceptors in many photosensory processes in fungi (Kamagai, 1988; Wasser *et al.*, 1999; Ribeiro, 2008a).

Paper chromatography of extracts showed, in addition to a good-coloured zone of anthocyanin, the pale-yellow zone which moved with the solute front. It is known that some fungi and lichens have yellow colour due to the presence of quinone pigments (Britton, 1983).

The pigment extracts from the pileus and stipe of *Agaricus bohusii* had different concentrations of pigments. The colour of the fruit body depends on anthocyanins and quinones. Suitable conclusion in relation to the absence of β-carotene was made by investigation of carpophores and vegetative mycelium of *Pleurotus ostreatus* (Jacq. : Fr.) P. Kumm. (Kamagai, 1988; Velíšek, 2011).

Conclusions

Certain morphological and biochemical properties of the rare for mycobiota of the Europe species *Agaricus bohusii* were studied. It was shown that the biomass of the caps contained 16.7% proteins on the dry weight, while the stipe – only 8.6%. Proteins contained 18 amino acids, with especially high amounts of valin, glutamic acid, aspartic acid, glycine, isoleucine and lysine. From the essential amino acids predominate lysine, threonine, valine and leucine. Despite the low content of sulfur-containing amino acids from a biological point of view, this mushroom protein represents of interest. The 24 electrophoretic fractions of soluble proteins of *Agaricus bohusii* carpophores were revealed. Mono- and disaccharides were uniformly distributed in caps and stipes. Storage polysaccharides and lipids are localised mainly in caps. The structural polysaccharides are distributed predominately in stipes. Characteristics of the visible absorption spectrum of pigment extracts from carpophores showed that the main pigments responsible for colour of this species are anthocyanins.

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