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Agaricus bohusii from Serbia: chemical characterization, antioxidant potential and antifungal preserving properties in cream cheese

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ABSTRACT

In the present work, a detailed chemical characterization of *Agaricus bohusii* Bon was performed, including evaluation of nutritional value (e.g. macromolecules, free sugars and fatty acids), bioactive compounds (e.g. tocopherols, phenolic compounds and organic acids), and antioxidant activity of its methanolic extract (e.g. scavenging activity, reducing power and inhibition of lipid peroxidation). The antifungal preserving properties of the mentioned *A. bohusii* extract incorporated in cream cheese were also analyzed, using the fungus *Penicillium verrucosum* var. *cyclopium* as source of contamination. Comparison of sensory evaluation of cream cheese alone and enriched with *A. bohusii* extract was recorded. *A. bohusii* demonstrated to be a rich source of carbohydrates and proteins, containing γ-tocopherol as the only isoform of tocopherols. Polyunsaturated fatty acids predominated over mono and unsaturated fatty acids and the main sugars found were mannitol and trehalose. *p*-Hydroxybenzoic and *p*-coumaric acids were the phenolic acids identified in the studied sample and two related compounds were found in higher amounts: γ-L-glutaminyl-4-hydroxybenzene and cinnamic acid. Malic, oxalic and fumaric acids were the organic acids identified and quantified in *A. bohusii*. High concentration of total phenolics was in correlation with a relatively strong antioxidant capacity. Methanol extract successfully inhibited development of *P. verrucosum* var. *cyclopium* in cream cheese, tested at room temperature after 7 days of inoculation. Sensory evaluation showed cream cheese in combination with *A. bohusii* extract more acceptable to panelists than cream cheese alone.

1. INTRODUCTION

Mushrooms are widely appreciated all over the world for their nutritional and bioactive properties. They have been considered valuable health foods being a source of many different nutraceuticals, including antioxidant and antimicrobial compounds [1,2]. *A. bohusii*

is an edible and prized mushroom especially common in Serbia and southern Europe. However, as far as we know, there are no studies about this species, neither from Serbia nor from any other country. Thus, the work realized involves a detailed study of *A. bohusii*, contributing to increased knowledge of mushroom species throughout the world.

2. MATERIAL AND METHODS

2.1. Mushroom species

Agaricus bohusii Bon was collected during July of 2011 in Jabučki rid, Northern Serbia [3].

2.2. Chemical characterization

Nutritional value (moisture, proteins, fat, carbohydrates and ash) was assessed using the AOAC procedures [4]. Free sugars were determined by HPLC coupled to a refraction index detector (RI). Fatty acids were determined after a transesterification procedure using a gas chromatographer coupled to a flame ionization detector (GC-FID). Tocopherols were determined by HPLC and a fluorescence detector. Phenolic acids and organic acids were determined by ultra fast liquid chromatography (UFLC) [3].

2.3. Antioxidant potential

The samples (~1g) were dissolved in methanol (final concentrations of 20 mg/mL). The final solutions were further diluted to different concentrations to be submitted to distinct bioactivity evaluation *in vitro* assays. Trolox was used as standard [3]. Reducing power was evaluated by *Folin-Ciocalteu* and Ferricyanide/Prussian blue assays. Radical-scavenging activity was determined through DPPH radical-scavenging activity assay and lipid peroxidation inhibition through β-carotene/linoleate system and TBARS assay [3].

2.4. Antifungal preserving properties in cream cheese

Full-fat cream cheese “a la Kajmak” by Mlekara Šabac was purchased from a local supermarket (Serbia). The cheese mixture was contaminated (inoculated) with ~10^4 CFU of *Penicillium verrucosum* var. *cyclopium* that had been prepared by *in vitro* culture. The concentration of *P. verrucosum* var. *cyclopium* was then determined in the cheese on 0 hour, 2^{nd}, 4^{th} and 7^{th} day, using the serial dilution and spread plate technique [3].

2.5. Sensory evaluation

Sensory evaluation of cream cheese and cream cheese enriched with *A. bohusii* extract was assessed by a group of 10 untrained panelists. Overall acceptance was evaluated using a 5-point scale, where 1means extremely dislike and 5 extremely like [3].

3. RESULTS AND DISCUSSION

Agaricus bohusii species revealed to be a rich source of macromolecules (Table 1), namely carbohydrates and proteins, with a low content in ash and fat. The free sugars identified were mannitol and trehalose. Concerning tocopherols, γ-tocopherol was the only isoform identified (Table 1).

Table 1. Nutritional value, sugars and tocopherols composition of *A. bohusii* (mean ± SD).

Nutritional value		**Individual compounds**	
Ash (g/100g dw)	9.04 ± 0.01	**Sugars**	
Proteins (g/100g dw)	18.06 ± 0.46	Mannitol (g/100g dw)	13.01 ± 0.85
Fat (g/100g dw)	3.12 ± 0.02	Trehalose (g/100g dw)	0.47 ± 0.03
Carbohydrates (g/100g dw)	69.79 ± 0.47	**Tocopherols**	
Energy (kcal/100g dw)	379.44 ± 0.10	γ-tocopherol (μg/100g dw)	27.82 ± 0.93

dw – dry weight

Polyunsaturated fatty acids (PUFA: 73.79%) predominated over mono (MUFA: 5.17%) and unsaturated fatty acids (SFA: 21.05%) [3].

The studied mushroom species revealed a high concentration of total phenolics as also the EC_{50} values obtained for all the antioxidant activity assays were ≤ 1.29 mg/ml, indicating that *A. bohusii* has a relatively high antioxidant potential. *p*-Hydroxybenzoic and *p*-coumaric acids were the phenolic acids identified and two related compounds were found in higher amounts: γ-L-glutaminyl-4-hydroxybenzene and cinnamic acid. Oxalic, malic and fumaric acids were also quantified (Table 2).

Table 2. Antioxidant potential of *A. bohusii* methanolic extract (mean ± SD).

Antioxidant activity		**Phenolic compounds and related compounds**		**Organic acids**	
Total phenolics (mg GAE/g extract)	89.59 ± 3.07	γ-L-glutaminyl-4-hydroxybenzene (μg/g dw)	32.44 ± 0.81	Oxalic acid (mg/g dw)	12.97 ± 0.56
Ferricyanide/Prussian blue (EC_{50}; mg/mL)	1.29 ± 0.01	*p*-Hydroxybenzoic acid (μg/g dw)	9.21 ± 0.21	Malic acid (mg/g dw)	43.89 ± 1.01
DPPH radical-scavenging activity (EC_{50}; mg/mL)	1.03 ± 0.04	*p*-Coumaric acid (μg/g dw)	1.28 ± 0.13	Fumaric acid (mg/g dw)	8.31 ± 0.06
β-carotene/linoleate system (EC_{50}; mg/mL)	0.21 ± 0.02	Cinnamic acid (μg/g dw)	3.96 ± 0.16		
TBARS inhibition (EC_{50}; mg/mL)	0.06 ± 0.02				

dw – dry weight. EC_{50}: Extract concentration corresponding to 50% of antioxidant activity or 0.5 of absorbance for the Ferricyanide/Prussian blue assay. GAE means gallic acid equivalents.

Methanol extract successfully inhibited development of *P. verrucosum* var. *cyclopium* in cream cheese, tested at room temperature after 7 days of inoculation (Figure 1).

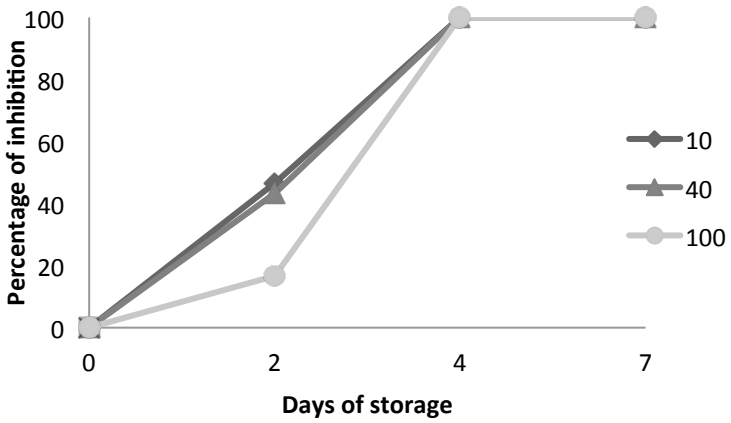

Figure 1. Inhibition percentage of *P. verrucosum* var. *cyclopium* with 10, 40 and 100 mg/mL of *A. bohusii* methanolic extract during 7 days of cream cheese storage, at 25°C.

Sensory evaluation showed that cream cheese in combination with *A. bohusii* extract is more acceptable to panelists than cream cheese alone (Table 3).

Table 3. Acceptability scores given by the panelist group for cream cheese and cream cheese + *A. bohusii* extract.

	Cream cheese	**Cream cheese + extract**
Acceptability*	4.1	4.8

*The results are expressed as the average of all grades.
1-extremely dislike; 2-dislike; 3-neither like nor dislike; 4-like; 5-extremely like.

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