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PROTEOLYTIC ACTIVITY OF EDIBLE SPRUCE *MORCHELLA ESCULENTA*

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ABSTRACT

This study focused on the determination of non-specific proteolytic activity of edible spruce *Morchella esculenta* in water extract, phosphate-buffered saline (PBS) solution (pH = 7.5) extract and a suspension prepared from 200 mg DW (dry weight) of edible spruce in PBS solution (pH 7.5). A clear casein solution was used as a substrate. The absorbances were measured in quartz cuvettes at the wavelength of 280 nm against a blank with zero concentration of trypsin. Non-specific proteolytic activity was expressed as trypsin equivalents per kilogram of mushroom dry weight (mg.kg^{-1} DW). All of the extracts demonstrated non-specific enzymatic activity. The highest activity was observed in the PBS suspension and the lowest enzymatic activity was measured in the water extract of the *Morchella esculenta* fungi. The non-specific proteolytic activity decreased in the following order: PBS suspension extract (pH 7.5; 22.9 mg.kg^{-1} DW), followed by PBS extract (pH 7.5; 13.6 mg.kg^{-1} DW) and finally the water extract (10.94 mg.kg^{-1} DW).

Key words: casein substrate; *Morchella esculenta*; mushrooms; non-specific proteolytic activity

INTRODUCTION

Mushrooms produce a wide range of intracellular and extracellular enzymes which are able to cleave various organic matter including wood and side-products of human activities. They have an invaluable role in the natural carbon cycle. Mushrooms are a rich source of bioactive molecules including compounds beneficial for human health. For centuries, they have been used in natural medicines. For their growth and functions, fungi acquire the necessary substances by decomposing waste by specific enzymes, e. g. hydrolytic enzymes, cellulases, xylanases and laccases [1, 10]. Lakhanpal et al. [4] studied the production of extracellular enzymes (amylase, cellulase, pectinase, protease and lipase) in four varieties of spruce, *Morchella angusticeps, Morchella conica, Morchella deliciosa* and *Morchella esculenta*. They found out that in all of the species there were

enzymes, but the yellow spruce variety showed a higher amount of enzymes than the black spruce species. None of their samples showed any lipase activity. Thakur [8, 9] observed that yellow spruce varieties produced higher concentrations of cellulase, amylase, pectinase and urease than black ones. Similarly, the higher activity of extracellular enzymes (protease, cellulase and amylase) were observed in the fruiting bodies of yellow spruce in comparison to the black spruce. These findings enable one to distinguish yellow and black varieties of spruce. The production of all of the enzymes suggests that *Morchella* species are capable of degrading and utilizing various substrates, such as: cellulose, starch, urea, pectins and proteins. Until now, it was not possible to artificially grow the fruiting bodies of spruce. The fungi of the genus *Morchella* have a delicious taste and a faint smell. These organoleptic properties are retained also by the cultivated mycelium which is widely used as a flavour enhancer. Proteins isolated from the mycelium have properties comparable to plant proteins and can be used as their substitutes [6]. *Morchella esculenta* is valuable from both, the nutritional and medicinal aspects due to the content of a number of bioactive compounds, such as polysaccharides, proteins, trace elements, dietary roughage and vitamins. *Morchella esculenta* has been shown to have anti-inflammatory and anticancer activities attributed to their polysaccharides [5]. The polysaccharides isolated from spruce fruiting bodies have demonstrated immunostimulatory effects and extracts from these fungi have exhibited antibacterial activity against: *Staphylococcus aureus, Salmonella typhimurium, Listeria monocytogenes, Escherichia coli* and *Enterobacter cloacae* [2, 3]. The fruiting bodies and mycelium of spruce contain a high amount of proteins and polypeptides which, under specific conditions, can serve as enzymes that are able to degrade organic substances complexes to soluble substances that can be used as food or building material for cell growth.

The aim of our study was to determine the non-specific proteolytic activity of water extract, extract in phosphate-buffered saline (PBS) solution (pH = 7.5), and in a suspension prepared from 200 mg DW (dry weight) of spruce *Morchella esculenta* in PBS solution (pH 7.5) using a clear casein solution as a substrate.

MATERIALS AND METHODS

Spruce (*Morchella esculenta*) fruiting bodies (Figure 1) collected in mid-April, 2018, at Bahoň marsh in the village of Beša, near the thermal power plant in Vojany in the Slovak Republic, were investigated for their proteolytic activities. The moisture of the mushrooms was 93.21 % and their dry weight (DW) reached 6.79 %. The fruiting bodies were dried in a dryer (BINDER, Germany) at a temperature of 60 °C to a constant weight and then milled to obtain a fine powder (mixer STRAUME, Ukraine). The fine powder was stored in a closed dark container at room temperature until used for analysis.

The extracts for testing were prepared by re-suspending 200 mg of the dried powdered fruiting bodies in 4 ml of either water or PBS, by mixing at laboratory temperature for 2 hours and filtering the mixtures. The filtering was omitted to obtain PBS suspension.

The determination of the non-specific proteolytic activity was performed using a casein substrate according to the methodology published by Wu et al. [11], which was adapted to our laboratory conditions. The non-specific activities were determined using water extract, extract with 200 mmol.dm^{-3} PBS at pH 7.5, and a suspension prepared from 200 mg dried spruce powder and 4 ml of PBS at pH 7.5.

The casein substrate was always freshly prepared before its use. One hundred mg of casein was dissolved in 10 ml PBS (pH 7.5). Subsequently, the casein solution was heated at 60 °C for 30 minutes. After cooling, the observed precipitate was centrifuged and the supernatant was used as a substrate. We added 900 µl of casein substrate to 100 µl of sample extract, or trypsin standard, respectively, and after thorough vortex mixing, the reaction mixture was incubated for 15 to 120 minutes at 37 °C. The reaction was terminated by the addition of 1000 µl of 10 % trichloroacetic acid. A precipitate was allowed to form within 30 minutes at room temperature. The reaction mixture was then centrifuged (14 000 rpm) for 5 minutes to separate the formed precipitate and the absorbance of the clear supernatant was measured at a wavelength of 280 nm against a blank sample.

The blank sample was prepared separately for each assay as follows: into the test tube we pipetted first 1000 µl of 10 % trichloroacetic acid and subsequently added all of the reagents which were used at the determination of the non-specific activity of the samples.

For each assay, a trypsin calibration curve was prepared. We used the same procedure as before but instead of the sample we pipetted solutions with increasing concentrations of trypsin. The stock solution was prepared by dissolving 100 mg of trypsin (Sigma-Aldrich with an activity of 0.000—2.000 BAEE units.mg^{-1} solid, USA) in 100 ml of 0.001 mol.dm^{-3} HCl with 50 mmol.dm^{-3} $CaCl_2$. The absorbance of the calibration solutions was measured against the sample with null concentration of trypsin. The values were used to construct a calibration curve and the trypsin amount in the samples was calculated from the regression equation. The enzymatic activity of the fungi samples was expressed as trypsin equivalents, i.e. milligrams of trypsin per kilogram of mushroom dry weight (mg.kg^{-1} DW) (Fig. 1).

RESULTS AND DISCUSSION

The objective of this study was to investigate the proteolytic activity of a very popular wild growing mushroom *Morchella esculenta* that can be collected from March to late summer. Spruce is an economically important mushroom growing on all continents. So far, it has not been possible to domesticate this mushroom [4]. Only mycelium cultivation, rich in biologically active compounds, such as polysaccharides, exhibiting antitumour, antiallergic, anti-inflammatory and immunomodulatory effects has been well established [5]. Proteins isolated from spruce fruiting bodies and mycelium show enzymatic activity capable of the degradation of lignin, cellulose and hemicellulose [7]. Proteins from the mycelium have better properties than plant proteins, so they can serve as their substitution [6]. Extracts from fruiting bodies of *Morchella esculenta* exhibited antibacterial activity as well [3].

Our results showed that the spruce fruiting bodies exhibited protease activity. Casein substrate allowed us to analyse their non-specific proteolytic activity (Table 1 and Fig. 1).

The casein substrate allowed us to investigate the non-specific proteolytic activity of spruce *Morchella esculenta*. It was observed that less proteases were extracted into water than into phosphate-buffered saline with pH 7.5. The extraction with different extraction agents resulted in the release of non-identical proteases at different quantitative proportions. The highest proteolytic activity was detected in the PBS fungal suspension (pH 7.5). This indicated that all enzymes present in the mushrooms that favoured the respective enzymatic reaction conditions were involved in the enzymatic process.

Fig. 1. Trypsin calibration curve for determination of non-specific proteolytic activity of spruce fungi in an aqueous extract, PBS solution and PBS suspension, determined on a casein substrate at 280 nm

Table 1. Non-specific proteolytic activity of spruce (*Morchella esculenta*) measured in water extract, phosphate-buffered saline (pH 7.5) extract and in suspension with phosphate-buffered saline (pH 7.5)

	Extract of spruce Morchella esculenta		
	Water	PBS extract pH 7.5	PBS suspension extract pH 7.5
Absorbance at λ = 280 nm	0.282	0.377	0.487
Proteolytic activity mg trypsin.kg^{-1} DW	10.94	13.6	22.9

DW—dry weight; PBS—phosphate-buffered saline

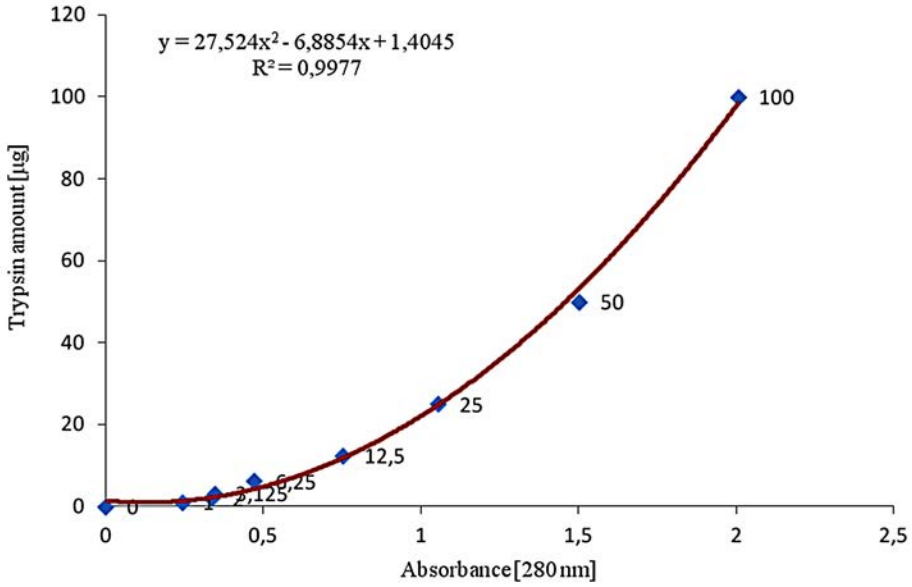

Fig. 1. *Morchella esculenta* (spruce)

CONCLUSIONS

Mushrooms carry a wide spectrum of enzymatic activity. Spruce fruiting bodies exhibit non-specific proteolytic activity. Our results allowed us to conclude that each of the three samples tested showed a non-specific proteolytic activity. The comparison of our results obtained by individual determinations conducted with an aqueous extract, the phosphate-buffered saline extract, and the suspension with phosphate-buffered saline solution revealed, that the non-specific proteolytic activity of spruce mushrooms was the highest in the phosphate-buffered saline suspension extract.

The results confirmed our assumption that mushrooms are nutritionally a valuable food and their potential in biotechnology processes in the food production, bakery products, or cheese and wine production, can also be highlighted.

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