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**Research article**

# Comparison of total amino acid compositions and total protein content in five wild mushrooms

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## Abstract

We studied the total amino acid compositions and total protein content in five wild mushrooms – *Artomyces pyxidatus*, *Caloboletus calopus*, *Hericium flagellum*, *Strobilomyces strobilaceus*, *Xerocomellus porosporus*. The highest total protein content is found in *Xerocomellus porosporus* (44.8% w/w) and *Strobilomyces strobilaceus* (42% w/w). In the fruiting bodies of the mushrooms, *Hericium flagellum* and *Artomyces pyxidatus*, the total protein content was the lowest, at 14.02% w/w and 17.7% w/w, respectively. The proportion of essential amino acids in the mushrooms was 42.26%, 44.98%, 46.99%, 39.33% and 40.97% in *Xerocomellus porosporus*, *Caloboletus calopus*, *Strobilomyces strobilaceus*, *Artomyces pyxidatus*, and *Hericium flagellum*, respectively. The proportion of hydrophobic amino acids ranged from 39.49% in *Xerocomellus porosporus* to 59.02% in *Hericium flagellum*. Therefore, the basidiomata of *X. porosporus* and *S. strobilaceus* are recommended as sources of protein, including essential amino acids, for the development of dietary supplements.

## Keywords

*Artomyces pyxidatus*, *Caloboletus calopus*, *Hericium flagellum*, *Strobilomyces strobilaceus*, *Xerocomellus porosporus*, wild basidiomata

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## Introduction

By 2050, the global population is estimated to grow to 10 billion, and as a result, the demand for food will increase by 50%, while the demand for animal-derived protein will nearly triple (Upcraft et al., 2020). Animal protein can be replaced or supplemented with protein from fungi. Fungi are rich in amino acids, high in protein, low in fat, and almost cholesterol-free (Li et al., 2023). Wild edible mushrooms are treasured ingredients with delicious taste and high nutritional value, and they are loved by consumers (Chen et al., 2023). Fungi are also an important source of protein for vegetarians (Teke et al., 2020). The evaluation of the chemical composition and biological activities of edible



mushrooms has recently been an active area of research. This type of research facilitates the identification of fungal species that possess high nutritional value and important biological activities (Liu et al., 2016).

Many fungal species have been studied in terms of their macromorphological and microscopic characteristics, ecology, taxonomy, nutritional value, distribution, and conservation. However, there is significantly less information about the chemical composition of many fungi species, particularly concerning biologically active substances and primary synthesis compounds. Most of the existing research focuses on well-known, popular edible species or those that have been cultivated for a long time. At the same time, there is also an interest in other species of fungi, which are undoubtedly also a source of valuable biologically active substances and primary synthesis substances. Therefore, studies were conducted on wild basidiomata of *Artomyces pyxidatus* (Pers.) Jülich, *Caloboletus calopus* (Pers.) Vizzini, *Hericium flagellum* (Scop.) Pers., *Strobilomyces strobilaceus* (Scop.) Berk., *Xerocomellus porosporus* (Imler ex Watling) Šutara.

*Artomyces pyxidatus* is a lignicolous, coralloid basidiomycete (Lickey et al., 2002). The colour of *A. pyxidatus* basidiomata is rather variable, ranging from cream to very bright yellow to tan (Wu et al., 2014), with the branch tips remaining more or less the same color as the rest of the fruiting body (Fraiture et al., 2008). *Artomyces pyxidatus* is one of the few coral fungi that fruit on wood, making them relatively easy to identify. In the literature, there are common names for the fungus, such as the “Crown-tipped Coral fungus” or “Candelabra Coral” due to the crown-like shape of the branch tips. In the region of Hutsulshchyna, the local name of the species is “sorochachi lapky” which translates to “magpie’s feet”. The species is not listed on the IUCN Red List; however, it was long considered extinct in Great Britain and after its discovery in Addington, Kent, it was added to the Red List (BBC, 2024). The fungus is edible and has a faint odor of freshly dug potatoes. The taste can be mild or peppery (Kuo, 2007). Described attempts to grow basidiomata of the species in culture indicate that basidiomata developed after 50 days of cultivation on 2% malt agar (Dodd, 1972).

*Caloboletus calopus* is commonly referred to as the bitter beech bolete. It can often be found under both oak and beech trees, but it is almost always located on limestone soil (Miller and Watling, 1968). *Caloboletus calopus* seems a mushroom that shows promise as a source of antioxidant (for therapeutics or food preservation). Three extracts had antioxidant capacity – namely, the chloroform extract in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, the ethanolic extract in the Folin-Ciocalteu (F-C) assay, and the aqueous extract in the oxygen radical antioxidant capacity (ORAC) assay. The mushroom contains a mix of both nonpolar and polar antioxidant compounds. Moreover, the bioactive fractionation of a methanol extract of this mushroom led to the isolation of calopin, calopin B, and cyclocalopin A, which have antioxidant properties (Morel et al., 2018). *Caloboletus calopus* is rarely consumed because of the bitter taste imparted by calopins and cyclocalopins (Hellwig et al., 2002). According to the conclusions of Chinese researchers, *C. calopus* was classified as E2 in terms of its edibility (psychoneurological disorder poisoning) (Chen et al., 2023). It belongs to “fungi with conflicting reports on edibility”. Although *C. calopus* is not typically considered edible despite the fact that the mushroom does not lose its bitter taste during cooking, there are reports of it being consumed in Eastern Europe (Boa, 2004).

*Hericium flagellum* is found only on the wood of coniferous species, particularly on silver fir *Abies alba* Mill. (Kujawska et al., 2021). Basidiomata of the *H. flagellum* are edible when young. This fungus is among the most praised medicinal and edible mushrooms. It has been established that

they produce secondary metabolites that are potentially effective in the treatment of neurodegenerative diseases (Rupcic et al., 2018). The species is currently under assessment for the Global Fungal Red List and is considered rare in Austria, Croatia, Czech Republic, Germany, Poland, and Italy. However, it is not listed in the IUCN Red List. *Hericium flagellum* is well known in the eastern parts of North America (Gierczyk et al., 2019). According to GBIF – the Global Biodiversity Information System – specimens of the mushroom have been recorded only 193 times in the world (GBIF, 2024).

*Strobilomyces strobilaceus*, also known as “the old man of the woods”, is an ectomycorrhizal species that forms mutualistic relationships with host plant families such as Fagaceae (Sato et al., 2007), Pinaceae (Sato, 2016), Dipterocarpaceae, Myrtaceae, and Casuarinaceae (Han et al., 2020). It is native to Europe and North America. It is edible when young. It is listed in the Red Data Book of Ukraine (Didukh, 2009). There is currently no information on the nutritional composition of the mushroom; however, some sources mention possible antitumor properties of the species (Deng et al., 2023).

*Xerocomellus porosporus*, also known as “the sepia bolete”, is a small mushroom that can be found in mixed deciduous woods, often with oak, hornbeams, and beech trees (Phillips, 1988). While *X. porosporus* is edible, it doesn't have a strong “mushroom” flavor and has a spongy texture (Săndulescu et al., 2022). Currently, there is not much information about *X. porosporus*, and all of it relates to inventory processes describing the ecology of the fungus (Ďuriška et al., 2012; Moreno et al., 2016) and the identification of the species with closely related, similar species (Gherghel, 2009; Farid et al., 2021). There is also information about research on the antioxidant properties of wild specimens of the species in Portugal (Leal et al., 2013).

Therefore, it is worthwhile to supplement the research with information about the protein and amino acid composition of the listed species to identify new mushroom species that are valuable in terms of their nutrient content. The purpose of our work is to study the amino acid composition and total protein content in wild basidiomata of *A. pyxidatus*, *C. calopus*, *H. flagellum*, *S. strobilaceus*, and *X. porosporus* mushrooms.

## Materials and Methods

### *Source of the basidiomata*

The studied fungi, *A. pyxidatus*, *C. calopus*, *H. flagellum*, *S. strobilaceus*, *X. porosporus*, were collected in the territory of the Hutsulshchyna National Nature Park (Hutsulshchyna NNP) in the mycological reserve “Kamenystyi” (Table 1). The Park is located on the eastern side of the Carpathian with an absolute altitude of 290-1472 m asl. The basidiomata samples (five for each species) were included in the “Collection of dry and wet preparations at Hutsulshchyna NNP”. This collection is maintained by the scientific department of Hutsulshchyna NNP. The mushrooms were first thoroughly washed to remove dirt and other foreign substances, then cut into pieces and dried at temperature  $45 \pm 2$  °C. The samples were pre-dried, minced, and stored in glass containers.

**Table 1.** Information about the analysed basidiomata.

Mushroom	<i>X. porosporus</i>	<i>C. calopus</i>	<i>S. strobilaceus</i>	<i>A. pyxidatus</i>	<i>H. flagellum</i>
Collection date	Sept. 2023	Sept. 2023	Aug. 2023	Sept. 2023	Sept. & Oct. 2023
Substrate	Brown mountain-forest soil	Loamy brown forest soil	Brown mountain-forest soil	Decaying <i>F. sylvatica</i>	Decaying <i>A. alba</i>
Forest type	<i>Fagus sylvatica</i> plantation mixed with <i>Abies alba</i>	<i>F. sylvatica</i> plantation mixed with <i>Picea abies</i>	<i>F. sylvatica</i> plantation mixed with <i>A. alba</i>	<i>F. sylvatica</i> plantation mixed with <i>A. alba</i>	<i>F. sylvatica</i> plantation mixed with <i>A. alba</i>

#### *Determination of total protein content*

A 100 mg of the dried sample was weighed into two 25 mL falcon tubes. Five mL of 5% SDS was added, and the mixture was left for 2 hours at room temperature and vortexed every 30 minutes. After two hours, tubes were placed in a centrifuge and centrifuged at 2000 rpm for 10 minutes. A 50 $\mu$ L aliquot of the sample was then diluted with 950  $\mu$ L of distilled water. A 100  $\mu$ L aliquot of the diluted sample was subsequently extracted for analysis (using Folin-Lowry's method) (Teke et al., 2020).

#### *Amino acids (AAs) analysis*

For the analysis of hydrolyzed AA, the samples were hydrolyzed in 6 N hydrochloric acid in vacuum-sealed tubes at 106 °C for 24 hours. The content of AAs in different hydrolysates was determined using an automatic amino acid analyzer T-339 (Microtechnology, Czech Republic, based at the Palladin Institute of Biochemistry of the NAS of Ukraine) with postcolumn derivatization using ninhydrin. The concentrations of the amino acids (in g/100 g protein) were calculated from external standards for the different AAs (Lazarus 1973).

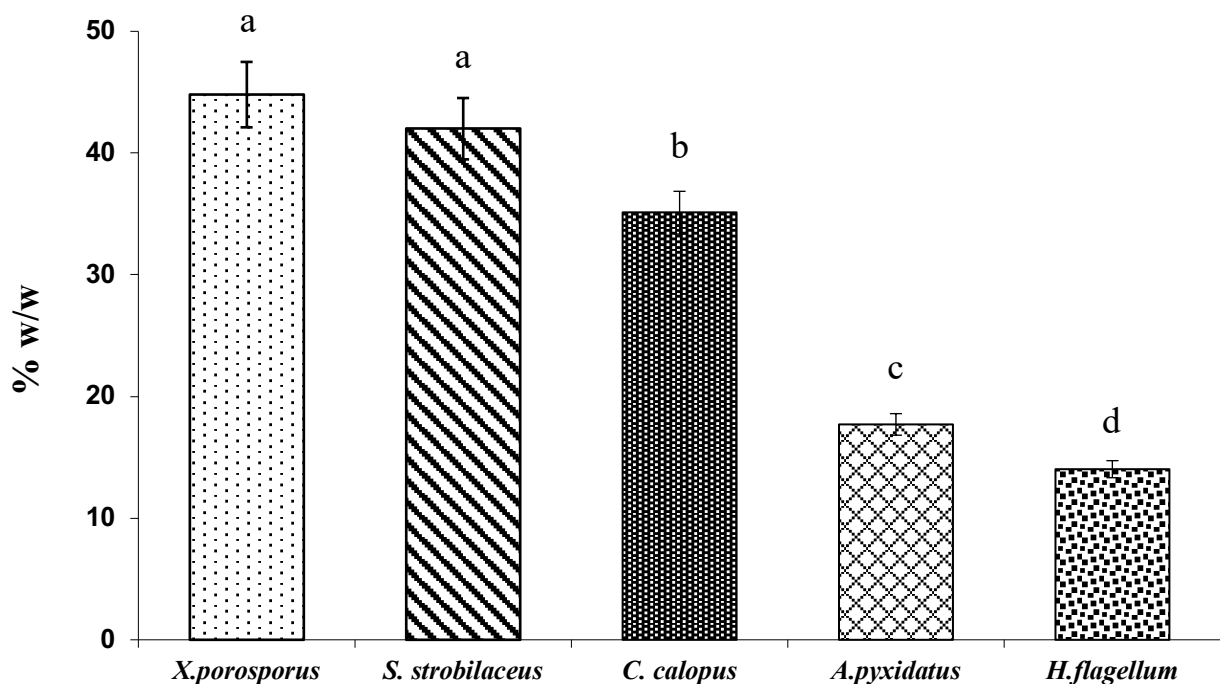
#### *Data analysis*

The results were processed using Statistica 8.0 (StatSoft Inc., Tulsa, Oklahoma, USA). All experiments were conducted using three biological replicates. All values are mean  $\pm$  SE (n = 3).

## Results

#### *Determination of total protein content*

The results of the research showed that the highest total protein content among the studied species of fungi was *X. porosporus* (44.8% w/w) and *S. strobilaceus* (42% w/w) respectively (Fig. 1). In contrast, the lowest total protein content was found for basidiomata of *H. flagellum* (14.02% w/w) and *A. pyxidatus* (17.7% w/w).



**Fig. 1.** – Total protein contents of basidiomata of *Xerocomellus porosporus*, *Strobilomyces strobilaceus*, *Caloboletus calopus*, *Artomyces pyxidatus*, *Hericium flagellum*. All values are mean  $\pm$  SE (n = 3). Values bearing different letters in the same column are significant at  $P < 0.05$ .

#### AAs analysis

The amino acid content was analyzed in the studied mushrooms. Table 2 shows the amino acid profile of the five mushroom species in g/100 g of protein. The results of the research showed that the highest content of amino acids is found in the mushroom *X. porosporus*, which is almost twice as high as the amino acid content in the mushrooms *C. calopus* and *A. pyxidatus*, and three times higher than that in *H. flagellum* (Table 2). Based on the results of this study, the five mushroom species can be ranked in order of decreasing amino acid content: *X. porosporus* > *S. strobilaceus* > *C. calopus* > *A. pyxidatus* > *H. flagellum*.

In the five samples of studied mushrooms, the highest content of isoleucine and glutamic acid was found. The basidiomata of *X. porosporus* are also enriched in arginine, aspartic acid, and leucine. The content of arginine in *X. porosporus* was 2.7-4.5 times higher, and the content of leucine was 1.5-3.5 times higher compared to other studied mushroom species. Furthermore, the study showed that the lowest content in the analyzed mushroom samples was found for methionine, tyrosine, histidine, and cystine. The content of isoleucine varied from 2.41 g/100 g in *X. porosporus* to 0.90 g/100 g in *H. flagellum*, while the content of methionine ranged from 0.18 g/100 g in *X. porosporus* to 0.04 g/100 g in *H. flagellum*. In *C. calopus*, histidine was absent.

The content of aromatic amino acid (tyrosine and phenylalanine) in *C. calopus*, *S. strobilaceus* and *A. pyxidatus* did not differ significantly and was more than 2.5 times lower compared to *X. porosporus*. Additionally, *X. porosporus* was rich in gamma-aminobutyric acid (0.98 g/100 g) compared to other studied mushroom species.

**Table 2.** Amino acid composition of *Xerocomellus porosporus*, *Caloboletus calopus*, *Strobilomyces strobilaceus*, *Artomyces pyxidatus* and *Hericium flagellum* mushrooms. Values are reported as g/100 g protein.

AAs	<i>X. porosporus</i>	<i>C. calopus</i>	<i>S. strobilaceus</i>	<i>A. pyxidatus</i>	<i>H. flagellum</i>
Lysine*	0.90±0.073 <sup>a</sup>	0.33±0.028 <sup>d</sup>	0.49±0.037 <sup>b</sup>	0.41±0.029 <sup>c</sup>	0.26±0.028 <sup>f</sup>
Histidine*	0.33±0.042 <sup>a</sup>	-	0.22±0.019 <sup>b</sup>	0.16±0.022 <sup>c</sup>	0.11±0.063 <sup>c</sup>
Arginine	1.30±0.044 <sup>a</sup>	0.35±0.041 <sup>c</sup>	0.49±0.056 <sup>b</sup>	0.38±0.031 <sup>c</sup>	0.29±0.001 <sup>d</sup>
Aspartic acid	1.61±0.106 <sup>a</sup>	0.78±0.081 <sup>c</sup>	1.15±0.099 <sup>b</sup>	0.92±0.084 <sup>c</sup>	0.65±0.099 <sup>d</sup>
Threonine*	0.91±0.088 <sup>a</sup>	0.43±0.037 <sup>c</sup>	0.55±0.041 <sup>b</sup>	0.40±0.037 <sup>c</sup>	0.28±0.056 <sup>d</sup>
Serine	0.92±0.101 <sup>a</sup>	0.48±0.034 <sup>c</sup>	0.62±0.057 <sup>b</sup>	0.52±0.044 <sup>c</sup>	0.33±0.007 <sup>d</sup>
Glutamic acid	1.86±0.151 <sup>a</sup>	1.04±0.093 <sup>b</sup>	1.21±0.118 <sup>b</sup>	1.23±0.151 <sup>b</sup>	0.90±0.079 <sup>c</sup>
Proline	0.81±0.063 <sup>a</sup>	0.69±0.058 <sup>b</sup>	0.43±0.007 <sup>c</sup>	0.43±0.039 <sup>c</sup>	0.23±0.002 <sup>d</sup>
Glycine	0.81±0.071 <sup>a</sup>	0.45±0.062 <sup>c</sup>	0.59±0.019 <sup>b</sup>	0.41±0.043 <sup>c</sup>	0.26±0.072 <sup>d</sup>
Alanine	0.98±0.086 <sup>a</sup>	0.65±0.056 <sup>c</sup>	0.75±0.055 <sup>b</sup>	0.58±0.064 <sup>c</sup>	0.37±0.049 <sup>d</sup>
Cystine	0.17±0.014 <sup>a</sup>	0.08±0.002 <sup>b</sup>	0.15±0.011 <sup>a</sup>	0.11±0.041 <sup>b</sup>	0.04±0.001 <sup>c</sup>
Valine*	0.62±0.070 <sup>a</sup>	0.25±0.029 <sup>d</sup>	0.43±0.024 <sup>b</sup>	0.33±0.016 <sup>c</sup>	0.17±0.055 <sup>f</sup>
Methionine*	0.18±0.021 <sup>a</sup>	0.06±0.004 <sup>c</sup>	0.10±0.077 <sup>b</sup>	0.12±0.009 <sup>b</sup>	0.04±0.016 <sup>c</sup>
Isoleucine*	2.41±0.151 <sup>a</sup>	2.22±0.167 <sup>a</sup>	2.33±0.167 <sup>a</sup>	1.42±0.111 <sup>b</sup>	0.90±0.051 <sup>c</sup>
Leucine*	1.21±0.033 <sup>a</sup>	0.53±0.062 <sup>c</sup>	0.82±0.074 <sup>b</sup>	0.56±0.073 <sup>c</sup>	0.36±0.099 <sup>d</sup>
Tyrosine	0.59±0.062 <sup>a</sup>	0.21±0.023 <sup>c</sup>	0.29±0.030 <sup>b</sup>	0.24±0.017 <sup>b</sup>	0.15±0.051 <sup>d</sup>
Phenylalanine*	0.76±0.069 <sup>a</sup>	0.30±0.018 <sup>c</sup>	0.37±0.032 <sup>b</sup>	0.32±0.045 <sup>b</sup>	0.23±0.002 <sup>d</sup>
GABA	0.98±0.087 <sup>a</sup>	0.32±0.022 <sup>b</sup>	0.31±0.040 <sup>b</sup>	0.35±0.061 <sup>b</sup>	0.17±0.066 <sup>c</sup>
TAA	17.32	9.16	11.30	8.90	5.82
EAA/TAA	42.26%	44.98%	46.99%	39.33%	40.97%
HAA/TAA	39.49%	52.29%	47.35%	41.91%	59.02%

All values are expressed as means ± SE of three determinations (in g/100 g protein). Means with the same letter across a row are not significantly different at 0.05 probability level. \*Essential amino acids (EAA); TAA, total amino acid; HAA, hydrophobic amino acids; GABA, Gamma-Aminobutyric Acid.

The proportion of essential amino acids in mushrooms was 42.26%, 44.98%, 46.99%, 39.33%, and 40.97% in *X. porosporus*, *C. calopus*, *S. strobilaceus*, *A. pyxidatus* and *H. flagellum*, respectively. Among the essential amino acids, leucine and isoleucine predominated in all studied mushrooms. The proportion of hydrophobic amino acids (Ala, Pro, Val, Met, Phe, Ile, and Leu) ranged from 39.49% in *X. porosporus* to 59.02% in *H. flagellum*. Therefore, the highest proportion of essential amino acids was characteristic of *S. strobilaceus* while the highest proportion of hydrophobic amino acids was found in *H. flagellum*.

## Discussion

Currently, there is a pressing issue in the world concerning the inadequate supply of complete dietary protein to the population (Kabat et al., 2001; Medek et al., 2017). Therefore, the search for potential new sources of protein and the production of high-protein food products are among the current tasks of modern science (Wu et al., 2014). Mushrooms play varied and important roles in human nutrition and health. Mushrooms are rich in protein and fiber, have low fat and carbohydrate content, contain no cholesterol, possess a low glycemic index, and have a heart-friendly sodium to potassium ratio. Additionally, they contain unique bioactive compounds, including ergothioneine and polysaccharides (Pandey et al., 2022). Mushrooms have high nutritional value, especially as a source of protein, which can enrich a person's diet, especially in some developing countries where animal protein may be unavailable and expensive (Teke et al., 2020).

The chemical composition of mushrooms depends on the substrate, the species of fungus, the time of harvest, and the conditions of post-harvest storage. Variations in protein content in mushrooms can also depend on the species/strain, the stage of development, the size of the pileus, and the method of analysis (Teke et al., 2020). The protein content in mushrooms is a reliable indicator of their biological value. At the same time, the amino acid contents in the protein must meet the needs of the human body for the synthesis of its own proteins (Tagkouli et al., 2020).

Although mushrooms are lower in protein content than meat, they are free from many harmful ingredients, such as high levels of saturated fatty acids, sodium and cholesterol that are found in meat. Mushrooms can be more filling than meat. Research has shown that consuming equal amounts of protein from mushrooms and meat has also shown that the protein from mushrooms increases satiety, reduces the feeling of hunger, and decreases prospective consumption (Hess et al., 2017). Some mushrooms are good sources of protein, with higher protein content of most vegetables (Uju and Obiakor-Okeke, 2014). For example, the protein content of 100 g of dried fruit bodies of *Pleurotus ostreatus* (Jacq.) P. Kumm., ranges from 17 grams to 42 grams (Kirbağ and Akyüz, 2010). Mushrooms contain all essential amino acids in higher proportions compared to most grains, legumes, and nuts. They are a better source of isoleucine, methionine, phenylalanine and tryptophan compared to fruits and vegetables (Pandey et al., 2022).

Despite the many advantages of using mushrooms as sources of primary and secondary synthesis substances with known medicinal effects, the consumption and use of wild mushroom fruiting bodies pose certain challenges for scientists due to their ability to accumulate toxic molecules, such as heavy metals. This issue has led to new scientific developments aimed at purifying the environment of heavy metals by fungi, such as in wastewater treatment (Tamjidi et al., 2023). The phenomenon of heavy metal accumulation is inherent in all currently studied mushroom species. The presence of heavy metals in the substrate does not hinder fungal growth. Moreover, in *Tuber borchii* Vittad., for example, a high tolerance to Pb has been established, with all tested strains capable of growing at Pb concentrations exceeding 4000 mg L<sup>-1</sup> (Sisti et al., 2022). Thus, before consuming wild fruiting bodies for any purpose, the question of their potential accumulation of heavy metals arises. It is known that the fruiting bodies of all wild mushroom species can accumulate heavy metals if the substrate on which they grow is contaminated. However, studies on wild xylotrophic fungi have shown that their fruiting bodies tend to accumulate fewer heavy metals compared to fungi growing on the forest floor. Notably, Mn, Sr, Ca, and Pb are usually excluded by these fungi, whereas K, Rb, and, to a lesser extent, Cd, Fe, Zn, Cu, Mg, and Na are accumulated (Tyler, 1982). In our experiment,

the wood-degrading species studied are *H. flagellum* and *A. pyxidatus*. To date, no research has been reported on the accumulation of heavy metals in *Hericium* species (Jianzhao et al., 2024) or in *A. pyxidatus*. However, the accumulation of heavy metals in edible mushrooms remains a significant challenge for the edible mushroom cultivation industry. Wild mushrooms often show high concentrations of heavy metals due to their efficient mechanisms for absorbing these elements from the soil. Such findings have been demonstrated in representatives of the Boletaceae family (Lalotra et al., 2016). Therefore, before collecting wild mushrooms for personal consumption, it is crucial to ensure that they are harvested from areas free from heavy metal contamination. Ideally, a monitoring system should be established to assess and publish data on heavy metal content in soil, water, and wood, as this is a vital safety concern for all who utilize forest resources, both wood and non-wood.

In this study, we evaluated the amino acid composition of 5 mushroom species *X. porosporus*, *C. calopus*, *S. strobilaceus*, *A. pyxidatus*, and *H. flagellum*. *Hericium flagellum* has been studied as a source of secondary metabolites (Jianzhao et al., 2024). The broad spectrum of compound types and their diverse biological activities present promising opportunities for developing new pharmaceuticals and edible products. The genus *Hericium* is considered a valuable source of dietary supplements in both nutritional and medicinal food applications (Fernandes et al., 2021; Gonkhom et al., 2021). In general, mushrooms contain approximately 90% water and 10% dry matter (Ho et al., 2020). Additionally, *Hericium* has a high overall nutritional profile (Atila et al., 2021). The cultivation methods for *Hericium* vary depending on the intended use, such as basidiomata production or chemical investigations (Gonkhom et al., 2021). The cultivation methods for *H. flagellum* fruiting bodies and the optimal conditions for mycelial growth have been reported by Ko et al. (2005). *Hericium flagellum* is an edible mushroom species, though it is not as widely known or commonly consumed as other *Hericium* species, such as *Hericium erinaceus* (Bull.) Pers. (Lion's Mane). It can be cooked and incorporated into various dishes. Our study showed that *H. flagellum* had the highest proportion of hydrophobic amino acids and the lowest nutritional value in terms of total amino acid content among the five mushrooms analyzed. The basidiomata of *H. flagellum* exhibited a lower total amino acid content compared to the other mushroom samples studied.

*Xerocomellus porosporus* is an edible mushroom, though it is not highly regarded for culinary use. The protein content of *X. porosporus* has not been widely studied compared to other edible mushrooms. However, our research showed that *X. porosporus* had the highest total protein content among the studied fungal species (44.8% w/w). Additionally, *X. porosporus* was rich in gamma-aminobutyric acid (0.98 g/100 g) compared to the other studied mushroom species. The basidiomata of *X. porosporus* were also enriched in arginine, aspartic acid, and leucine. When comparing our results with known data on representatives of the Boletaceae family, particularly one of the most popular edible species, *Boletus edulis* Bull., the reported protein content for this mushroom ranges from 31.86% (Rana, 2016) to 32.23% (Durakova, 2020), while the total amino acid content varies from 2.529 g/100 g to 7.2 g/100 g (Rana, 2016; Tan et al., 2022). Thus, both the total protein content of *X. porosporus* (44.8% w/w) and the total amino acid concentration in its fruiting bodies (17.32 g/100 g) exceed the values reported for wild *B. edulis* fruiting bodies. Interestingly, for other studied species of the Boletaceae family, namely *S. strobilaceus* and *C. calopus*, a higher total protein content (42% w/w and 35.1% w/w, respectively) and amino acid content (11.30 g/100 g and 9.16 g/100 g, respectively) were also found compared to the values described above for *B. edulis*.



It is well known that the protein content, amino acid composition, and amino acid profile of the same species can vary depending on the collection region. For instance, *B. edulis* from Croatia contains the highest total amino acid content (7.2 g/100 g dry matter), followed by samples from Finland (2.66 g/100 g dry matter), Yunnan, China (2.15 g/ 100 g dry matter), and Taiwan, China (0.9 g/100 g dry matter) (Tan et al., 2022).

Mushrooms from three regions (Finland, Croatia, and Yunnan, China) contained nine essential amino acids, whereas those from Taiwan, China, contained only seven EAAs. Thus, the species found in Ukraine, within the territory of the Hutsulshchyna NNP, currently demonstrate higher levels of protein and amino acids compared to other Boletaceae species collected in Europe and Asia. Although the culinary value of *S. strobilaceus* and *X. porosporus* is generally considered low, our findings suggest that these species have potential as sources of amino acids and proteins. However, similar studies should be conducted on local wild *B. edulis* fruiting bodies to better assess the potential of Boletaceae representatives.

As for *C. calopus*, its extreme bitterness makes it inedible for most people. Research has identified specific compounds responsible for its bitterness. Notably, cyclopinol, along with related compounds such as cyclocalopin A and O-acetylcyclocalopin A, has been isolated from fresh fruiting bodies of this mushroom. These compounds contribute to its characteristic bitter taste (Schüffler, 2018). It would be beneficial to examine the properties of this species bitterness in more detail. Together with information on its protein and amino acid composition, its potential application in drug development, such as in veterinary medicine, should be considered. Both *A. pyxidatus* and *H. flagellum* exhibited the lowest total protein content and TAA – 17.7% w/w and 14.02% w/w, respectively. In our experiment, the fruiting bodies of fungi that grow on wood had lower levels of the studied parameters compared to non-wood-destroying species. When comparing these values with those of other species, such as *P. ostreatus*, we find that the protein content in dry samples varies across different sources, ranging from 17.06% w/w (Effiong et al., 2024) to 25.91% w/w (Tolera and Abera, 2017) and from 17% w/w to 42% w/w (Kirbağ and Akyüz, 2010). The variation in reported values for the same species can be attributed to differences in measurement approaches and methods of preparing fruiting bodies for analysis. Therefore, it cannot be definitively stated that wood-destroying fungi are characterized by a lower protein content than, for example, mycorrhizal species.

Among the studied mushrooms, *X. porosporus* and *S. strobilaceus* are the most valuable sources of total protein and amino acids, while *H. flagellum* has the lowest nutritional value in terms of total amino acid content. Mushrooms of the species *X. porosporus* and *S. strobilaceus* can be recommended for use as a source of protein and amino acids, including essential amino acids, for the development of a dietary supplement. *Hericium flagellum* and *A. pyxidatus* cannot be recommended as a potential source of amino acids. Further research is needed to explore *H. flagellum* and *A. pyxidatus* as a source of other primary metabolites and to continue studying the secondary metabolites of basidiomata. It is important to conduct similar research on other species of fungi to create a database on the nutritional value of mushrooms.

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