IJM - Italian Journal of Mycology
ISSN 2531-7342 - Vol. 52 (2023): 112-125
Journal homepage: https://italianmycology.unibo.it/

Research article

Comparison of total amino acid compositions, total phenolic compounds, total flavonoid content, β-carotene content and hydroxyl radical scavenging activity in four wild edible mushrooms

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Article info
Received 14/2/2023; accepted 7/4/2023
https://doi.org/10.6092/issn.2531-7342/16457

Abstract
The total amino acid compositions, total phenolic compounds, total flavonoid content, β-carotene content and hydroxyl radical scavenging activity in four wild edible mushrooms − *Hericium erinaceus*, *Laetiporus sulphureus*, *Polyporus umbellatus*, *Sparassis nemecii* have been studied in this work. Maximal indexes of total phenolic compounds in 41.28-43.82 mg of gallic acid equivalents per gram of dry weight were revealed for ethanol extracts of *L. sulphureus* and *H. erinaceus*. Maximal index of total flavonoid content in 7.91 mg of quercetin per gram of dw was revealed for ethanol extract of *H. erinaceus*. The content of β-carotene from ethanol extracts of studied mushrooms ranged from 2.1 to 3.1 μg β-carotene per gram of dw. The most intensive ·OH scavenging activity is characteristic for ethanol extract of *H. erinaceus*. The fraction of essential amino acids in mushrooms was 44%, 41.25%, 43.49% and 47.30% in *S. nemecii*, *L. sulphureus*, *P. umbellatus* and *H. erinaceus*, respectively. The fraction of hydrophobic amino acids ranged from 38.19% in *L. sulphureus* to 49.5% in *H. erinaceus*. Therefore, the ethanol extracts of *H. erinaceus* can be used as a source of compounds with high antioxidant potential. The basidiomata of *S. nemecii* and *P. umbellatus* can be recommended for use as a source of amino acids.

Keywords
Hericium erinaceus, *Laetiporus sulphureus*, *Polyporus umbellatus*, *Sparassis nemecii*, wild basidiomata

Introduction

This study is directed at discovering natural sources of antioxidants and for the correction of various pathological states which is typically accompanied by the intensification of free radical processes. Fungi – organisms that are the second most species-rich organism group (Dai et al., 2015) occupy an important place in these kinds of studies. Therefore, the search for mushroom species whose basidiomata/extracts could become a reliable source of antioxidants is an important practical aspect of mycological science (Kozarski et al., 2015; Boonsong et al., 2016). Mushrooms can be also an inexhaustible source of essential amino acids, which is important in the development of special nutritional supplements for people with various metabolic disorders and/or specific dietary

https://doi.org/10.6092/issn.2531-7342/16457

nutrition (Yuwa-Amornpitak et al., 2020). Therefore, the study of the amino acid composition of these organisms is of great interest. Although most macromycetes have antioxidant properties and are a source of amino acids, we give preference to species whose basidiomata are edible, (i.e. non-poisonous for humans), currently cultivated or on the way to the development of artificial cultivation methods, and have recorded medical and biological properties. For the reasons highlighted above, we chose the species *Laetiporus sulphureus* (Bull.) Murrill, *Polyporus umbellatus* (Pers.) Fr., *Sparassis nemecii* Pilát & Veselý (Polyporales) and *Hericium erinaceus* (Bull.) Pers. (Russulales).

Laetiporus sulphureus is a fungus with positive medicinal and biological properties; it is widely distributed across North America and Europe and occasionally a weak parasite. This species is edible when young; its basidiomata grow striking golden-yellow shelf-like structures on tree trunks and branches. This mushroom produces the *L. sulphureus* lectin, which exhibits haemolytic and haemagglutination activities (Wang et al., 2018). Commercial cultivation of *L. sulphureus* occurs but at a much smaller scale than the oyster mushroom and *Agaricus bisporus* (J.E. Lange). The resulting mature basidiomata can reach a weight of 200–300 g (Pleszczyńska et al., 2012).

The chemical composition of the fungus was studied partly. Kovács and Vetter (2015) reported macrocomponents (crude protein, -fibre, -fat, -ash contents, calculated carbohydrate concentration, organic ingredients and the energy level), digestible protein, non-protein nitrogen and free amino acid contents (not amino acid list) of basidiomata of *L. sulphureus*. Mineral components, different protein fractions, soluble oligo- and polysaccharides of *L. sulphureus* were studied too. Sułkowska-Ziaja et al. (2018) reported proteins and fats content, total carbohydrates, malic-, citric- and ascor-bic acid of the basidiomata. The selected compounds isolated from *L. sulphureus* revealed anticoagulant, hypoglycemics, hypolipemic, anticancer, cytotoxic, anti-inflammatory, acetylcholinesterase inhibiting, antioxidants, antifungal and reverse transcriptase of HIV-virus, inhibitors activities. Therefore, the study of amino acid content and antioxidant activity of ethanol extracts of basidiomata of *L. sulphureus* will complement data about the biological value of the fungus.

Polyporus umbellatus is an edible and medicinal mushroom. The young basidiomata growing on the ground are edible, while its underground part has medicinal properties (Jong and Birmingham, 1990; Zhao and Zhang, 1992). This fungus is widely distributed in Europe, North America, and East Asia (Bandara et al., 2015). This mushroom is considering promising for cultivation due to its medicinal, and nutritional value. It is popular in China's fungal market and is called "Zhu Ling". Numerous publications are devoted to the development of methods of cultivation of *P. umbellatus*, in particular sclerotia and/or basidiomata of the fungus (Huang and Liu, 2007; Xing et al., 2013; Pasailiuk, 2020).

The medical and biological properties of *P. umbellatus* are well studied. Food and dietary supplements have been developed based on the mycelium and sclerotia of this fungus. Medical attributes of this fungus include enhanced liver function (Complexe Hepato Bio), relief of inflammatory conditions, supporting immune function (Polyporus MRL preparation), supporting white blood cell production and providing antioxidant protection (Mushroom 6 Immune Support Complex), strengthing the immune system (Mycophyto ® Complex), enhancing the body's immune response to an antigen (*P. umbellatus* Extract); it is also suitable for diabetics and people who suffer from celiac disease (*P. umbellatus* HdT), those who are diuretic, and enhances anticancer activity (Zhu Ling Hot-water extract).

The basidiomata of *P. umbellatus* are a source for biological active supplements such as "*P. umbellatus* 180 capsules" which have antitumor effects, immune system enhancement, hair growth, antioxidant and free-radical scavenging activity, diuretic effects, and antiviral effects (Bandara et al., 2015). Basidiomata of fungus are rich in important bioactive substances such as fatty acids, 2-hydroxytetracosanoic acid (Yosioka and Yamamoto, 1964), (1→3)-β-D-glucan (Lee

https://doi.org/10.6092/issn.2531-7342/16457

and Park, 2001), polysaccharides (Dai et al., 2012). Lu et al. (1985) isolated four components, viz. ergosta-5,7,22-trien-3-ol (ergosterol), ergosta-7,22-dien-3-one, ergosta-7,22-dien-3-ol, and 5α,8α-epidioxyergosta-6,22-dien-3-ol, from the basidioma of *P. umbellatus*. Ohsawa et al. (1992) identified seven polyporusterones from the basidiomata of *P. umbellatus* and named them as A, B, C, D, E, F and G. However, the amino acid content of *P. umbellatus* basidiomata has not been studied. Since protein content has a direct effect on free radical scavenging activity, we decided to study amino acid composition and antioxidant activity of wild *P. umbellatus* basidiomata.

Sparassis nemecii is an edible mushroom. It was included to the Red Lists of some European and Asian countries under an Endangered status. Examples of the studied fungus were found on the territory Hutsulshchyna National Nature Park (Hutsulshchyna NNP). It is the only place of fungus growth in Hutsulshchyna NNP and in Ukraine. Fungi were identified by Heluta et al. (2016). Pure cultures of this fungus are stored in the IBK Mushroom Culture Collection (Bisko et al., 2016, https://ccinfo.wdcm.org/details?regnum=1152) and in the mycological laboratory of Hutsulshchyna NNP. Cultivation of the fungus was done by Pasailiuk (2019). Suitable substrates for growing mycelium and small size basidiomata of *S. nemecii* were established. The mechanical properties of the substrate were also important factors for the growth of *S. nemecii* mycelium.

Sparassis spp., contribute a significant economic and industrial role as a source of health supplement therapeutics, pharmaceutics, and biotechnological products. There is a close relationship between *Sparassis* species and *L. sulphureus*, which also produces brown rot, and was suggested by sequence data from rDNA (including the ITS region) and the partial gene coding RNA polymerase sub-unit II (RPBII) (Wang et al., 2004). However, the chemical composition of *S. nemecii*, its medicinal value and commercial value were not studied. Consequently, it is important to study amino acid compositions and antioxidant activity of ethanol extracts of wild basidiomata of fungus, which we do in this article.

Hericium erinaceus is an edible and medicinal mushroom that belongs to the Russulalles order. This mushroom is very popular in Eastern medicine and the methods of its cultivation have been studied (Figlas et al., 2007; Hassan, 2007; Julian et al., 2018). For this reason, some of its properties and bioactive substances have already been studied. From the basidiomata of the mushroom, *H. erinaceus,* biological active components were isolated as meroterpenoid, named hericenone K, astradoric acid C, 3,4-dihydro-5-methoxy-2-methyl-2-(4'-methyl-2'-oxo-3'-pentenyl)-9(7H)-oxo-2H-furo[3,4-h] benzopyran, ergosterol peroxide, 3β,5α,9α-trihydroxy-ergosta-7,22-dien-6-one, cerevisterol, inoterpene A, ursolic acid, betulin, hemisceramide, and oleanolic acid (Zhang et al., 2015). The ability of *H. erinaceus* mycelia to inhibit bacterial growth was reported in Julian et al. (2018). This fungus (both the mycelia and basidiomata) has antidiabetic, antihyperlipodemic, hepatoprotective, anti-inflammatory, anticarcinogenic, antioxidative, antihypertensive, cardioprotective, antisenescence, antifatigue, antibiotic, immunostimulating, nephroprotective, and neuroprotective properties. It can improve anxiety, cognitive function, and lower depression levels (Friedman, 2015). In Eastern Asia, *H. erinaceus* is used traditionally to improve memory (Zhang et al., 2017). Considering the high medical and edible value of the mushroom, information about its amino acid composition and antioxidant activity of alcohol extracts of the wild *H. erinaceus* basidiomata of is an important aspect for understanding the nutritional value of the mushroom.

The purpose of the work is to study the amino acid composition and antioxidant activity the ethanol extracts of wild basidiomata of *S. nemecii*, *L. sulphureus*, *P. umbellatus* and *H. erinaceus* mushrooms.

https://doi.org/10.6092/issn.2531-7342/16457

Materials and Methods

Source of the basidiomata

The fungi studied, *S. nemecii, L. sulphureus, P. umbellatus* and *H. erinaceus*, were collected on the territory of Hutsulshchyna NNP. Fruiting body samples of the fungi were included in the collection of dry and wet prepaprations at Hutsulshchyna NNP. *Sparassis nemecii* was introduced to a pure culture and transferred to the IBK mushroom culture collection at the M.G. Kholodny Institute of Botany, National Academy of Sciences (NAS) of Ukraine, Kyiv, Ukraine (Bisko et al., 2016; https://ccinfo.wdcm.org/details?regnum=1152). Mushroom samples were dried, minced, and stored in glass containers prior to the extraction process.

Preparation of mushroom extracts

Extraction was performed according to the method outlined in Boonsong et al. (2016) with some modifications. For 70% (v/v) ethanol extraction, each powdered sample (5 g) was mixed with 50 mL of 70% (v/v) ethanol and shaken at 150 rpm at room temperature for 24 h; it was then centrifuged at 12,000 rpm for 15 min. The supernatant was filtered with Whatman filter paper and the filtrate was collected. The residue was re-extracted under the same conditions. The obtained extract was concentrated under vacuum at 40 ºC using the rotary evaporator. The obtained sample was stored at 4 ºC.

Determination of total phenolic compounds in mushroom extracts

Phenolic compounds in the sample extracts were estimated by using Folin-Ciocalteu assay, based on procedures described by Gan et al. (2013). One milliliter of sample from ethanol extract was mixed with 1 mL of Folin-Ciocalteu's phenol reagent (1:9; Folin-Ciocalteu's reagent: distilled water). After 5 min, 1 mL of 13% sodium carbonate solution was added to the mixture and adjusted to 10 mL with distilled water. The reaction was kept in the dark for 90 min and its absorbance was read at 725 nm. A calibration curve was constructed with different concentrations of gallic acid as standard. The results were expressed as mg of gallic acid equivalents per gram of dry weight (mg GAE g^{-1} dw).

Determination of total flavonoid content

The content of total flavonoid of each ethanolic mushroom extract was determined using an aluminum chloride colorimetric assay, as described previously (Kumari et al., 2011). One mL of extract was diluted using 70% aqueous ethanol. 100 µL 10% aluminum nitrate and 100 µL, 1 M aqueous potassium acetate were mixed into the solution and incubated at room temperature for 40 min. The absorbance was recorded at 510 nm using a spectrophotometer (Agilent Technologies, USA). Quercetin was used as a standard to calculate the concentration of total flavonoids. Data were calculated as milligrams of quercetin equivalents per gram of dry weight (mg QE g^{-1} dw).

Determination of β-carotene

β-Carotene was determined from the dried ethanol extract according to Kumari et al. (2011). 100 mg of extract was mixed with 10 mL of acetone-hexane mixture (4:6) for 1 min and filtered. The absorbance was recorded at three different wavelengths (453, 505 and 663 nm). The β-carotene content was calculated by:

$$\beta\text{-carotene } (\mu g\ g^{-1}\ dw) = 0.216 \times A_{66\,3} - 0.304 \times A_{505} + 0.452 \times A_{453}$$

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of the extractives was determined by the method of Halliwell and

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Gutteridge (1989) with minor modifications (Rahman et al., 2015). Hydroxyl radical was generated by the Fe^{3+}-ascorbate-EDTA-H_2O_2 system (Fenton reaction). The assay is based on the quantification of the 2-deoxy-D-ribose degradation product, which forms a pink chromogen upon heating with TBA at low pH.

The reaction mixture contained deoxyribose (2.8 mM), KH_2PO_4-NaOH buffer, pH 7.4 (0.05 M), $FeCl_3$ (0.1 mM), EDTA (0.1 mM), H_2O_2 and mushroom extracts in a final volume of 2 mL. The mixture was incubated at 37°C for 30 min followed by the addition of 2 mL of trichloroacetic acid (2.8% w/v) and thiobarbituric acid. Thereafter, it was kept for 15 min in a boiling water bath, and cooled. The absorbance of solution was measured at 532 nm with a spectrophotometer. The hydroxyl radical scavenging capacity was evaluated with the inhibition of percentage of 2-deoxy-D-ribose oxidation on hydroxyl radicals.

The percentage of hydroxyl radical scavenging activity was calculated according to the following formula:

$$\% \text{ hydroxyl radical scavenging activity} = [A_0 - (A_1 - A_2)] \times 100 / A_0$$

Where:
A_0 is the absorbance of the control without a sample,
A_1 is the absorbance after adding the sample and 2-deoxy-D-ribose,
A_2 is the absorbance of the sample without 2-deoxy-D-ribose.

AAs analysis

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

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 ± SD (n = 3).

Results

Determination of total phenolic compounds

The obtained results showed significant differences in the content of total phenolic compounds in the ethanol extracts of the studied mushrooms. The maximum indicators of total phenolic compounds range from 41.28 to 43.82 mg GAE g^{-1} dw and were registered for *L. sulphureus* and *H. erinaceus* samples. A significantly lower content of total phenolic compounds was noted in samples of *P. umbellatus* and *S. nemecii.* The minimum values of total phenolic compounds recorded in *S. nemecii* were 6.67 mg GAE g^{-1} dw, which is about 6.2-6.5 times less compared to the established maximum values in the samples of *H. erinaceus* and *L. sulphureus*. The content of total phenolic compounds in mushroom extracts is shown in Figure 1.

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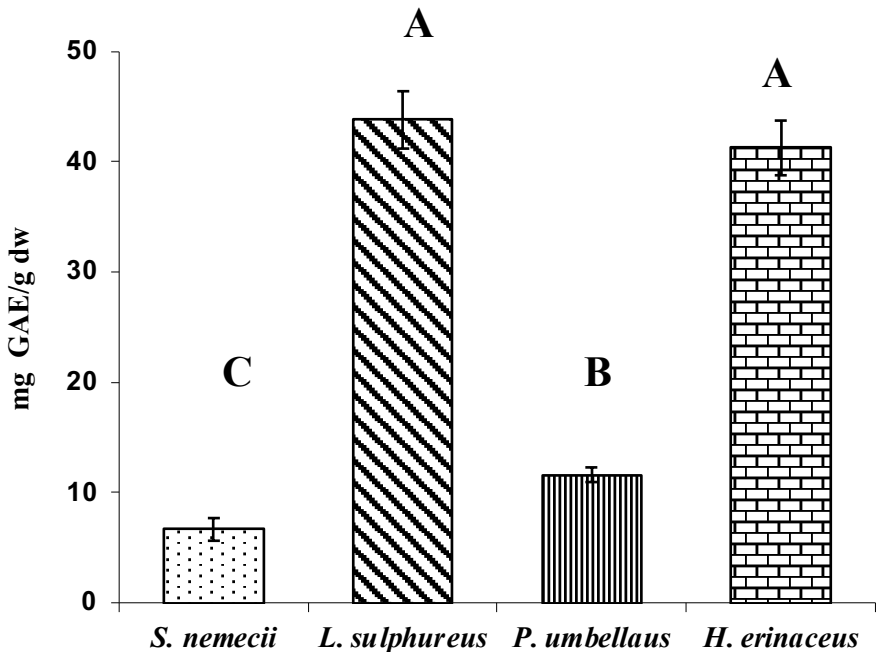

Fig. 1 – Total phenolic compounds in ethanol extracts of *Sparassis nemecii*, *Laetiporus sulphureus*, *Polyporus umbellatus* and *Hericium erinaceus* basidiomata. All values are Mean ± SD (n = 3). Values bearing different letters in the same column are significant at P<0.05.

Determination of total flavonoid content

Total flavonoid content in the studied mushrooms is shown in Figure 2. Ethanol extracts of *P. umbellatus* and *S. nemecii* revealed that samples of these fungus had a lower level of total flavonoid content (4.3-4.6 mg QE g^{-1} dw.) than other studied mushrooms. The maximal total flavonoid content was registered in ethanol extract of *H. erinaceus*– 7.91 mg QE g^{-1} dw.

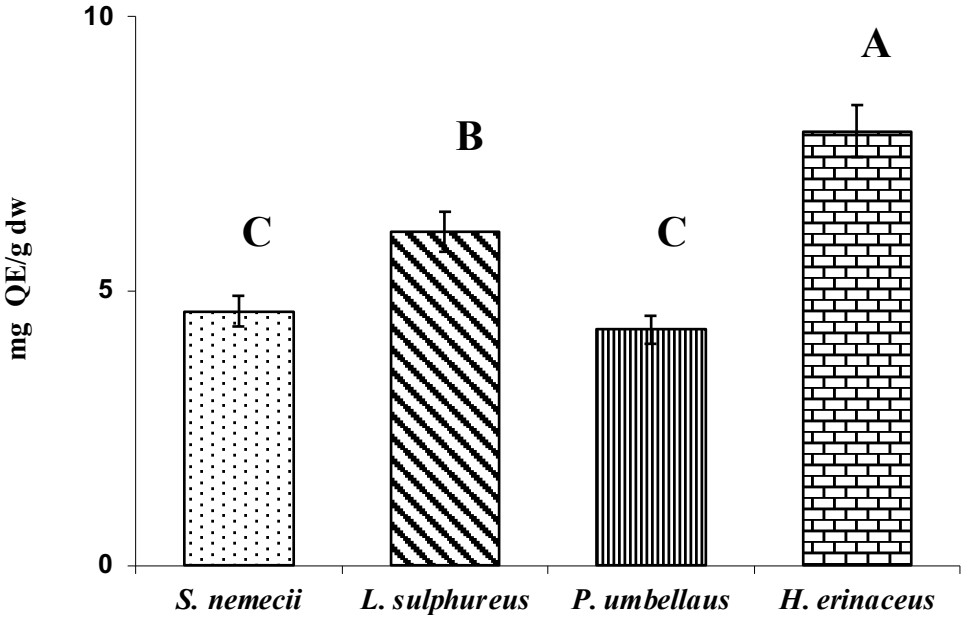

Fig. 2. – Total flavonoid content in ethanol extracts of *Sparassis nemecii*, *Laetiporus sulphureus*, *Polyporus umbellatus* and *Hericium erinaceus* basidiomata.

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Determination of β-carotene

The content of *β-carotene* in extracts of the basidiomata of the studied mushrooms did not differ significantly and ranged from 2.1 to 3.1 μg g^{-1} dw (Fig. 3).

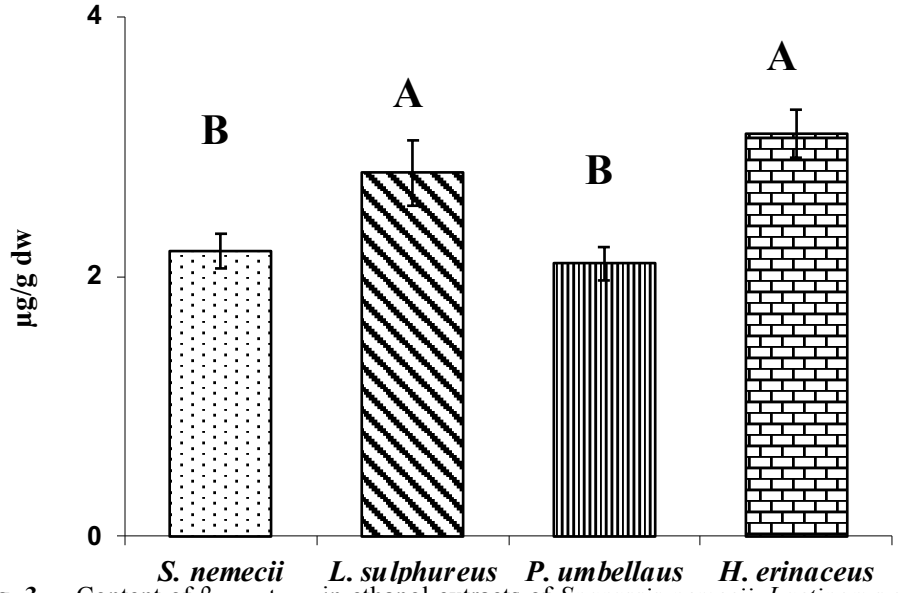

Fig. 3. − Content of β-carotene in ethanol extracts of *Sparassis nemecii*, *Laetiporus sulphureus*, *Polyporus umbellatus* and *Hericium erinaceus* basidiomata.

Hydroxyl radical scavenging activity

Investigation of the hydroxyl radicals scavenging activity of ethanol extracts of mushroom basidiomata showed that the most intense absorption of ·OH radicals is caused by ethanol extracts of *H. erinaceus* which inhibits ·OH radicals by 67.7%. Indicators of hydroxyl radical scavenging activity of mushroom extracts are shown in Figure 4.

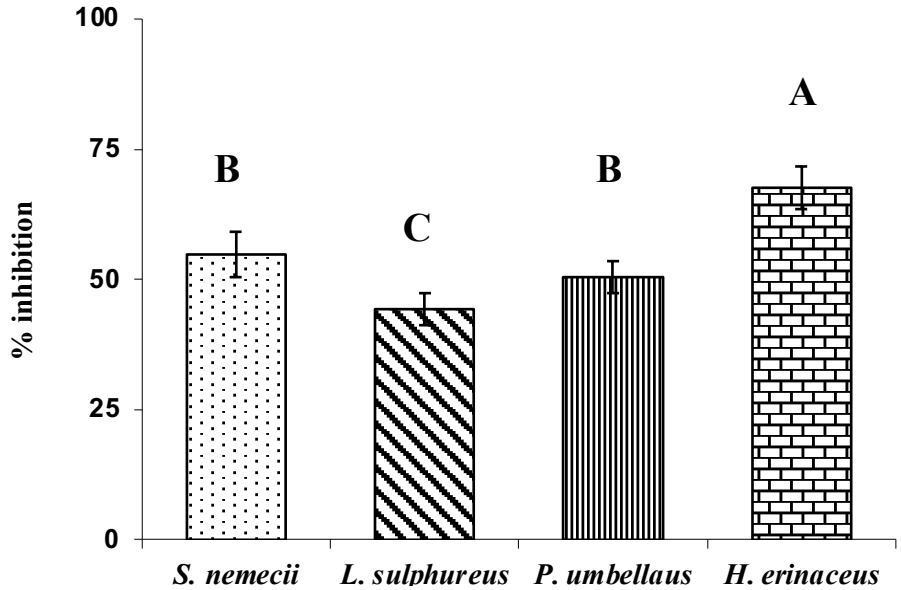

Fig. 4. − Hydroxyl radical scavenging activity of ethanol extracts of *Sparassis nemecii, Laetiporus sulphureus, Polyporus umbellatus* and *Hericium erinaceus* basidiomata.

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AAs analysis

Amino acid content was analyzed in the studied mushrooms. Table 1 shows the amino acid profile of the four mushroom species in g/100g of protein. For *S. nemecii* and *P. umbellatus*, we recorded the highest content of five amino acids, in particular lysine, aspartic acid, glutamic acids, leucine, and isoleucine. Our research shows that *H. erinaceus* has the lowest amount of all amino acids except for three (aspartic acid, glutamic acids, leucine). In *L. sulphureus*, the content of amino acids is lower compared to *S. nemecii* and *P. umbellatus*, but significantly higher than in *H. erinaceus*.

Table 1 - Amino acid composition of *Sparassis nemecii, Laetiporus sulphureus, Polyporus umbellatus* and *Hericium erinaceus* mushrooms.

AAs	Mushroom			
	S. nemecii	***L. sulphureus***	***P. umbellatus***	***H. erinaceus***
Lysine*	1.74±0.065^{a}	1.10±0.028^{b}	1.47±0.107^{a}	0.22±0.011^{c}
Histidine*	0.50±0.046^{a}	0.27±0.033^{b}	0.42±0.021^{a}	0.06±0.002^{c}
Arginine	1.29±0.061^{a}	0.75±0.054^{b}	1.23±0.037^{a}	0.14±0.019^{c}
Aspartic acid	2.37±0.058^{a}	1.53±0.102^{b}	2.01±0.116^{a}	0.37±0.044^{c}
Threonine*	1.18±0.041^{a}	0.67±0.037^{b}	0.97±0.124^{a}	0.14±0.023^{c}
Serine	1.07±0.085^{a}	0.68±0.058^{c}	0.87±0.112^{b}	0.15±0.037^{d}
Glutamic acid	3.15±0.073^{a}	2.02±0.016^{b}	2.98±0.212^{a}	0.53±0.071^{c}
Proline	0.99±0.066^{a}	0.54±0.048^{b}	0.84±0.131^{a}	0.27±0.012^{c}
Glycine	1.12±0.078^{a}	0.71±0.069^{c}	0.97±0.086^{b}	0.18±0.017^{d}
Alanine	1.54±0.083^{a}	1.09±0.099^{b}	1.34±0.089^{a}	0.24±0.031^{c}
Cystine	0.47±0.049^{a}	0.29±0.032^{b}	0.23±0.041^{c}	0.07±0.009^{d}
Valine*	1.15±0.120^{a}	0.81±0.062^{b}	1.06±0.105^{a}	0.17±0.041^{c}
Methionine*	0.22±0.014^{a}	0.15±0.011^{b}	0.10±0.009^{c}	0.09±0.005^{c}
Isoleucine*	2.12±0.113^{a}	1.08±0.071^{b}	1.93±0.141^{a}	0.77±0.066^{c}
Leucine*	1.90±0.077^{a}	1.15±0.027^{c}	1.64±0.096^{b}	0.31±0.014^{d}
Tyrosine	0.51±0.047^{a}	0.14±0.011^{c}	0.35±0.073^{b}	0.09±0.011^{d}
Phenylalanine*	1.06±0.056^{a}	0.55±0.048^{b}	0.90±0.094^{a}	0.16±0.012^{c}
Gamma-Aminobutyric Acid (GABA)	0.04±0.006^{d}	0.49±0.052^{a}	0.23±0.019^{b}	0.09±0.011^{c}
TAA, total amino acid	22.41	14.01	19.52	4.06
EAA/TAA	44.00 %	41.25 %	43,49 %	47.30 %
HAA/TAA	40.00 %	38.19 %	40.00 %	49.50 %

Notes – All values are means ± SD 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.

Based on the results of this study, the four mushroom species can be ranked in order of decreasing amino acid content: *S. nemecii* > *P. umbellatus* > *L. sulphureus* > *H. erinaceus*. In addition, studies have shown that content of amino acids such as aspartic acid and glutamic acids is in maximum quantities, while the lowest content is methionine, tyrosine, histidine and cystine. The glutamic acid content varied from 3.15 g/100 g in *S. nemecii* to 0.53 g/100 g in *H. erinaceus*, while the methionine content varied from 0.22 g/100 g in S. *nemecii* to 0.09 g/100 g in *H. erinaceus*. *Laetiporus sulphureus* is enriched in GABA (0.49 g/100 g) compared to other studied mushroom species.

Italian Journal of Mycology 52 (2023): 112-125

https://doi.org/10.6092/issn.2531-7342/16457

The studied species of mushrooms contain all essential amino acids. The fraction of essential amino acids in mushrooms was 44.00%, 41.25%, 43.49%, and 47.30% in *S. nemecii*, *L. sulphureus*, *P. umbellatus*, and *H. erinaceus*, respectively. At the same time, leucine and isoleucine predominate among essential amino acids in all studied mushrooms. The proportion of hydrophobic amino acids (Ala, Pro, Val, Met, Phe, Ile, and Leu) ranged from 38.19% in *L. sulphureus* to 49.50% in *H. erinaceus*.

Discussion

Currently, the issue of finding natural sources of antioxidants that can be used to correct various pathological conditions accompanied by the intensification of free radical processes remains relevant. Therefore, the demand for discovering novel compounds with good antioxidant activity is understandable (Zhou et al., 2010). In the context of this problem, mushrooms are increasingly being considered as potential producers of antioxidants. Mushrooms are also important as a nutraceutical and dietary support (Turfan et al., 2020). In this study, we evaluated/analyzed the amino acid composition, total phenolic compounds, total flavonoid content, β-carotene and antioxidant activity of ethanol extracts of four mushroom species *S. nemecii*, *L. sulphureus*, *P. umbellatus* and *H. erinaceus*. Ethanol as an extractant was chosen for the reason established by Jiang et al. (2016); anhydrous ethanol appears to be the most efficient extraction solvent that can be used to extract the effective antioxidant compounds in the pharmaceutical and food industries.

A number of studies have demonstrated the close association between the prevention of reactive oxygen species-associated diseases (ROS-associated diseases) and intake of food rich in antioxidants, including mushrooms (Abdullah et al., 2012). A vast body of evidence indicates that wild edible mushrooms contain many biologically active compounds disclosing antioxidant and other properties (Sun et al., 2017). Among the components of mushrooms that exhibit high antioxidant activity, it is worth noting carotenoids, in particular β-carotenes, polyphenols, including flavonoids (Podkowa et al., 2021).

Carotenoids protect fungi from oxidative stress and non-ionizing irradiation such as UV light (Lin and Xu, 2020). Therefore, they act as photoprotectors against the lethal combination of light and as antioxidants (Ribeiro et al., 2011). Carotenoids are known as singlet oxygen quenchers and lipid peroxidation chain breakers (Robaszkiewicz et al., 2010). Additionally, carotenoids are among the bioactive products with significant medical value (Mata-Gómez et al., 2014). In 1976, Valadon reported the carotenoids are additional taxonomic characters in fungi. In a number of cases, they are very good taxonomic markers. According to Zhang et al. (2018), the content of the carotenoids is one of the indicators of the quality of commercial basidiomata, for example *Cordyceps militaris* (L.) Fr. Information on carotenoids in mushrooms is fragmentary. Robaszkiewicz et al. (2010) reported the content of β-carotene differed considerably between the thirteen analyzed edible mushroom species from 0.233 (*Tuber mesentericum* Vittad.) to 18.649 μg g^{-1} (*Tricholoma equestre* (L.) P. Kumm.) of dried body. For dried vegetables, for examples carrot and pumpkin β-carotene reveals 26.57 and 773.66 μg g^{-1} dw (Piyarach et al., 2020). The content of β-carotene in extracts of the fungi in our study showed that basidiomata of the mushrooms ranged from 2.1 to 3.1 μg g^{-1} dw. These indexes are higher than, for example, indexes of β-carotene methanolic extract of *Boletus edulis* Bull. estimated by the same method (Robaszkiewicz et al., 2010).

The high content of phenolic compounds in ethanol extracts of *L. sulphureus* and *H. erinaceus*, along with the high content of flavonoids and β-carotene, can be considered as an indicator of their potential antioxidant activity. This probably explains the high hydroxyl inhibitory activity of *H. erinaceus* mushroom ethanol extracts. The hydroxyl radical is the most reactive among ROS due to its high oxidizing activity. Therefore, the neutralization of hydroxyl radicals is considered as the most

https://doi.org/10.6092/issn.2531-7342/16457

effective way to protect against oxidative damage to cell biomolecules. Antioxidants of fungi can exert their effect in two ways: the first consists in the direct absorption of hydroxyl radicals, while the second is in the suppression of their generation (Zhang et al., 2011). The antioxidant activity of mushrooms is directly proportional to the amount of bioactive compounds (Elbatrawy et al., 2015). Antioxidant activity is mainly associated with the content of phenol, but the carotenoids, vitamins, and polysaccharides present in the extracts enhance the antioxidant effects (Palacios et al., 2011). The antioxidant activity of phenolic compounds depends on the number of functional groups and location. It is believed that an important factor affecting the antioxidant activity of phenolic compounds is the number of hydroxyl groups, because their ability to chelate metal ions depends on this (Hussain et al., 2016). The main mechanism of antioxidant action of phenolic compounds consists either in direct inhibition of free radicals or in strengthening of endogenous antioxidant activity. In addition, some phenolic compounds stimulate the synthesis of endogenous antioxidants through the activation of the Nrf/ARE pathway (Scalbert et al., 2005). The reaction of cells to these compounds is implemented through direct interaction with enzymes or receptors involved in signal transmission, which leads to modification of the redox state of the cell and can cause a number of redox-dependent reactions. As antioxidants, polyphenols increase the probability of cell survival, but under the conditions of their excessive intake, they can show pro-oxidant properties (Kozarski et al., 2011).

The mechanisms of antioxidant action of flavonoids include direct scavenging of free radicals, chelation of metal ions involved in free radical formation, inhibition of enzymes such as xanthooxidases and lipoxygenases involved in free radical production, and regeneration of membrane-bound antioxidants. such as α-tocopherol (Kozarski et al., 2011). It is asserted that the main mechanism of action of flavonoids on the absorption of radicals is the donation of a hydrogen atom (Amic et al., 2007). The ability of flavonoids to inhibit ROS depends on the location and number of hydroxyl groups in the molecule. The double bond and carbonyl function in the heterocycle or polymerization of the nuclear structure increases activity by affording a more stable flavonoid radical through conjugation and electron delocalization (Heim et al., 2002). Pan et al. (2016) show that flavonoids are powerful scavengers of hydroxyl and superoxide radicals and actively chelate transition metals. However, despite the high content of phenolic compounds, flavonoids and β-carotenes in *L. sulphureus*, we found low hydroxyl inhibitory activity for alcohol extracts of this fungus. Probably, the antioxidant compounds of this mushroom are involved in the process of neutralization of other ROS.

The high antioxidant capacity can be confirmed by the results of our amino acid analysis. Some studies have shown that hydrophobic amino acids (HAA) play an important role in the manifestation of antioxidant activity (Sun et al., 2017). A high content of HAA could enhance their antioxidant activity (Zhuang et al., 2009). Thus, it is possible to expect a high antioxidant activity of the studied mushrooms, especially mushrooms of the *H. erinaceus* species, which have the highest content of hydrophobic amino acids. The amino acid composition of mushrooms is a reliable indicator of the nutritional value of food (Sun et al., 2017). At the same time, the results of the amino acid analysis obtained by us do not provide grounds for asserting the high nutritional value of the *H. erinaceus* mushroom, since the samples of the basidiomata of this mushroom are characterized by a low total content of amino acids compared to other studied mushroom samples. With regard to the species *S. nemecii* and *P. umbellatus*, which are characterized by a low content of phenolic compounds, flavonoids and β-carotenes and a lower hydroxyl inhibitory activity compared to *H. erinaceus*, but we can state their high nutritional value based on the amino acid analysis based on our study. These mushrooms are characterized by a high total content of amino acids with a significant proportion of all essential amino acids.

https://doi.org/10.6092/issn.2531-7342/16457

Conclusion

Therefore, *H. erinaceus*, which ethanol extracts showed the highest content of antioxidant compounds and the highest hydroxyl inhibitory activity, can be used as a source of compounds with high antioxidant potential. Its antioxidant compounds may be prospective protective agents that will help prevent and treat oxidative stress-associated diseases. *Sparassis nemecii* and *P. umbellatus* can be recommended for use as a potential source of amino acids, including essential ones, as well as for the development of nutritional supplements for people with various metabolic disorders and/or specific dietary nutrition.

Acknowledgements

The authors express their sincere gratitude to Nina M. Fontana (Department of Native American Studies, University of California, Davis, California 95616) for editorial assistance with the manuscript.

References

Abdullah N, Ismail SM, Aminudin N, Shuib AS, Lau BF (2012) Evaluation of selected culinary-medicinal mushrooms for antioxidant and ACE inhibitory activities. Evidence-Based Complementary and Alternative Medicine 2012:464238. https://doi.org/10.1155/2012/464238

Amic D, Davidovic-Amic D, Beslo D, Rastija V, Lucic B, Trinajstic N (2007) SAR and QSAR of the antioxidant activity of flavonoids. Current Medicinal Chemistry 14:827–45. https://doi.org/10.2174/092986707780090954

Bandara AR, Rapior S, Bhat DJ, Kakumyan P, Sunita C, Xu J, Hyde KD (2015) *Polyporus umbellatus*, an edible-medicinal cultivated mushroom with multiple developed health-care products as food, medicine and cosmetics: a review. Cryptogamie, Mycologie 36(1): 3–43. https://doi.org/10.7872/crym.v36.iss1.2015.3

Bisko NA, Lomberg ML, Mytropolska NY, Mykchaylova OB (2016) The IBK Mushroom Culture Collection. Alter press, Kyiv.

Boonsong S, Wanwimol K, Pongtep W (2016) Antioxidant cctivities of extracts from five edible mushrooms using different extractants. Agriculture and Natural Resources 50:89−97. https://doi.org/10.1016/j.anres.2015.07.002

Dai YC, Cui BK, Si J, He SH, Hyde KD, Yuan HS, Liu XY, Zhou LW (2015) Dynamics of the worldwide number of fungi with emphasis on fungal diversity in China. Mycology Progress 14(62):1–9. https://doi.org/10.1007/s11557-015-1084-5

Dai H, Han XQ, Gong FY, Dong H, Tu PF, Gao XM (2012) Structure elucidation and immunological function analysis of a novel beta-glucan from the fruit bodies of *Polyporus umbellatus* (Pers.) Fries. Glycobiology 22:1673−1683. https://doi.org/10.1093/glycob/cws099

Elbatrawy EN, Ghonimy EA, Alassar MM, Wu FS (2015) Medicinal mushroom extracts possess differential antioxidant activity and cytotoxicity to cancer cells. International Journal of Medicinal Mushrooms 17(5):471−479. https://doi.org/10.1615/intjmedmushrooms.v17.i5.70

Figlas D, González M, Ramiro CN (2007) Cultivation of culinary-medicinal Lion's Mane mushroom *Hericium erinaceus* (Bull.: Fr.) Pers. (Aphyllophoromycetideae) on substrate containing sunflower seed hulls. International Journal of Medicinal Mushrooms 9:67−73. https://doi.org/10.1615/IntJMedMushr.v9.i1.80

https://doi.org/10.6092/issn.2531-7342/16457

Friedman M (2015) Chemistry, nutrition, and health-promoting properties of *Hericium erinaceus* (Lion's Mane) mushroom fruiting bodies and mycelia and their bioactive compounds. Journal of Agricultural and Food Chemistry 63(32):7108−7123. https://doi.org/10.1021/acs.jafc.5b02914

Gan CH, Nurul AB, Asmah R (2013) Antioxidant analysis of different types of edible mushrooms (*Agaricus bisporous* and *Agaricus brasiliensis*). International Food Research Journal 20(3):1095−1102. http://www.ifrj.upm.edu.my/20%20(03)%202013/8%20IFRJ%2020%20(03)%202013%20Asmah%20(306).pdf

Halliwell B, Gutteridge JMC (1989) Free radicals in biology and medicine. Clarendon Press 3:617–683.

Hassan F (2007) Cultivation of the monkey head mushroom (*Hericium erinaceus*) in Egypt. Journal of Applied Sciences Research 3(10):1229−1233.

Heim KE, Tagliaferro AR, Bobilya DJ (2002) Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. The Journal of Nutritional Biochemistry. 13(10):572-584. https://doi.org/10.1016/S0955-2863(02)00208-5

Heluta VP, Fokshei SI, Derzhypilskyi LM (2016) The first finds in Ukraine of the rare mushroom *Sparassis nemecii* (Sparassidaceae). In: Rare plants and fungi of Ukraine and adjacent areas: implementing conservation strategies (Didukh, ed), 16–20 May 2016, Palivoda, Kyiv, Ukraine, pp 182−183. [in Ukraine]

Huang HC, Liu YC (2007) Acceleration of mycelia growth by adding *Grifola umbellata* broth concentrate in solid-state and submerged cultures. Biochemical Engineering Journal 37:139−143. https://doi.org/10.1016/j.bej.2007.04.003

Hussain T, Tan B, Yin Y, Blachier F, Tossou M, Rahu N (2016) Oxidative stress and inflammation: What polyphenols can do for us? Oxidative Medicine and Cellular Longevity 2016:7432797. https://doi.org/10.1155/2016/7432797

Jiang S, Wang Y, Zhang X (2016) Comparative studies on extracts from *Hericium erinaceus* by different polarity reagents to gain higher antioxidant activities. Experimental and Therapeutic Medicine 12(1):513−517. https://doi.org/10.3892/etm.2016.3279

Jong S, Birmingham J (1990) The medicinal value of the mushroom *Grifola*. World Journal of Microbiology and Biotechnology 6:227−235. https://doi.org/10.1007/BF01201290

Julian A, Wright C, Reyes R (2018) Prelude to successful cultivation of *Hericium* in the Philippines: understanding its mycelial growth response on different culture media and its antibacterial activity. International Journal of Pharmaceutical Research and Allied Sciences 7:1−7. https://doi.org/10.4489/MYCO.2008.36.2.088

Kovács D, Vetter J (2015) Chemical composition of the mushroom *Laetiporus sulphureus* (Bull.) Murill. Acta Alimentaria 44:104−110. https://doi.org/10.1556/AAlim.44.2015.1.10

Kozarski M, Klaus A, Jakovljevic D, Todorovic N, Vunduk J, Petrović P, Niksic M, Vrvic MM, van Griensven L (2015) Antioxidants of edible mushrooms. Molecules 20(10):19489−19525. https://doi.org/10.3390/molecules201019489

Kozarski M, Klaus A, Niksic M, Jakovljevic D, Helsper JPFG, van Griensven LJLD (2011) Antioxidative and immunomodulating activities of polysaccharide extracts of the medicinal mushrooms *Agaricus bisporus, Agaricus brasiliensis, Ganoderma lucidum* and *Phellinus linteus*. Food Chemistry 129:1667–1675. https://doi.org/10.1016/j.foodchem.2011.06.029

Kumari D, Reddy MS, Upadhyay RC (2011) Antioxidant activity of three species of wild mushroom genus *Cantharellus* collected from North-Western Himalaya, India. International Journal of Agriculture and Biology 13:415–418.

Lee YK, Park JH (2001) December 08. Extraction of pharmacologically active polysaccharide (beta-glucan) fraction from *Polyporus umbellatus*. South Korea patent application KR2001108709A. 2001.12.08.

Lin L, Xu J. (2020) Fungal pigments and their roles associated with human health. Journal of Fungi 6(4):280. https://doi.org/10.3390/jof6040280

Lu W, Adachi I, Kano K, Yasuta A, Toriizuka K, Ueno M, Horikoshi I (1985) Platelet aggregation potentiators from Cho-Rei. Chemical and Pharmaceutical Bulletin 33:5083−5087. https://doi.org/10.1248/cpb.33.5083

Mata-Gómez L, Montañez JC, Méndez-Zavala A, Aguilar CN (2014) Biotechnological production of carotenoids by yeasts: An overview. Microbial Cell Factories 13:12. https://doi.org/10.1186/1475-2859-13-12

Ohsawa T, Yukawa M, Takao C, Murayama M, Bando H (1992) Studies on constituents of fruit body of *Polyporus umbellatus* and their cytotoxic activity. Chemical and Pharmaceutical Bulletin 40:143−147. https://doi.org/10.1248/cpb.40.143

Palacios I, Lozano M, Moro C, D'Arrigo M, Rostagno M, Martínez, JA, García-Lafuente A, Guillamón E, Villares A (2011) Antioxidant properties of phenolic compounds occurring in edible mushroom. Food Chemistry 128:674−678. https://doi.org/10.1016/j.foodchem.2011.03.085

Pan X, Qin P, Liu R, Li J, Zhang F (2016) Molecular mechanism on two fluoroquinolones-induced oxidative stress: evidences from Copper/zinc Superoxide Dismutase. RSC Advances – The Royal Society of Chemistry 6:91141–91149. https://doi.org/10.1021/acs.jafc.8b02197

Pasailiuk MV (2019) Biological peculiarities of a rare mushroom *Sparassis nemecii* (Sparassidaceae, Polyporales) on plant substrates in pure culture. Ukrainian Journal of Botany 76:493−498. https://doi.org/10.15407/ukrbotj76.06.493

Pasailiuk MV (2020) Growing of *Polyporus umbellatus*. Current Research in Environmental & Applied Mycology (Journal of Fungal Biology) 10(1):457−465. https://doi.org/10.5943/cream/10/1/35

Piyarach K, Nipawan K, Chadapon C, Daluwan S, Kunjana R (2020) Effect of drying on β-carotene, α-carotene, lutein and zeaxanthin content in vegetables and its application for vegetable seasoning. E3S Web of Conferences 141:6. https://doi.org/10.1051/e3sconf/202014102007

Pleszczyńska M, Wiater A, Siwulski M, Szczodrak J (2012) Successful large-scale production of fruiting bodies of *Laetiporus sulphureus* (Bull.: Fr.) Murrill on an artificial substrate. World Journal of Microbiology & Biotechnology 29(4):753−758. https://doi.org/10.1007/s11274-012-1230-z

Podkowa A, Kryczyk-Poprawa A, Opoka W, Muszynska B (2021) Culinary–medicinal mushrooms: a review of organic compounds and bioelements with antioxidant activity. European Food Reserch and Technology 247:513–533. https://doi.org/10.1007/s00217-020-03646-1

Rahman MM, Islam MB, Biswas M, Alam AHMK (2015) *In vitro* antioxidant and free radical scavenging activity of different parts of *Tabebuia pallida* growing in Bangladesh. BMC Research Notes 8:621. https://doi.org/10.1186/s13104-015-1618-6

Ribeiro B, Pinho PG, Andrade PB, Oliveira C, César A, Ferreira S, Baptista P, Valentão P (2011) Do bioactive carotenoids contribute to the color of edible mushrooms? The Open Chemical and Biomedical Methods Journal 4:14-18. https://doi.org/10.2174/1875038901104010014

Robaszkiewicz A, Bartosz G, Lawrynowicz M, Soszyński M (2010) The role of Polyphenols, β-Carotene, and Lycopene in the antioxidative action of the extracts of dried, edible mushrooms. Journal of Nutrition and Metabolism 2010:173274. https://doi.org/10.1155/2010/173274

Scalbert A, Johnson IT, Saltmarsh M (2005) Polyphenols: antioxidants and beyond. The American Journal of Clinical Nutrition 81:215S–217S. https://doi.org/10.1093/ajcn/81.1.215S

Sułkowska-Ziaja K, Muszyńska B, Gawalska A, Sałaciak K (2018) *Laetiporus sulphureus* – chemical composition and medicinal value. Acta Scientarium Polonorum Hortorum Cultus 17(1):89–98. https://doi.org/10.24326/asphc.2018.1.8

https://doi.org/10.6092/issn.2531-7342/16457

Sun L, Liu Q, Bao C, Fan J (2017) Comparison of free total amino acid compositions and their functional classifications in 13 wild edible mushrooms. Molecules 24;22(3):350. https://doi.org/10.3390/molecules22030350

Turfan N, Ayan S, Pekşen A, Selin Akın S (2020) Antioxidant enzyme activities of some wild and cultivated edible mushrooms in Turkey. International Journal of Agriculture and Wildlife Science 6(2):202–209 https://doi.org/10.24180/ijaws.749512

Valadon LRG (1976) Carotenoids as additional taxonomic characters in fungi: a review. Transactions of the British Mycological Society. 67(1):1-15.

Wang Z, Binder M, Dai Y-C, Hibbett DS (2004) Phylogenetic rela-tionships of Sparassis inferred from nuclear and mitochondrialribosomal DNA and RNA polymerase sequences. Mycologia 96:1015–1029. https://doi.org/10.1016/S0007-1536(76)80001-0

Wang Y, Wu B, Shao J, Jia J, Tian Y, Shu X, Ren X, Guan Y (2018) Extraction, purification and physico chemical properties of a novel lectin from *Laetiporus sulphureus* mushroom. Lebensmittel-Wissenschaft & Technologie 91:151−159. https://doi.org/10.1016/j.lwt.2018.01.032

Xing YM, Zhang LC, Liang HQ, Lv J, Song C, Guo SX, Wang CL, Lee TS, Lee MW (2013) Sclerotial formation of *Polyporus umbellatus* by low temperature treatment under artificial conditions. PLoS ONE 8:e56190. https://doi.org/10.1371/journal.pone.0056190

Yosioka I, Yamamoto T (1964) The constituents of chuling. 2-Hydroxytetracosanoic acid. Yakugaku Zasshi 84:742−744.

Yuwa-Amornpitak T, Butkhup L, Yeunyaw PN (2020) Amino acids and antioxidant activities of extracts from wild edible mushrooms from a community forest in the Nasrinual District, Maha Sarakham, Thailand. Food Science Technology (Campinas) 40(3):712–720. https://doi.org/10.1590/fst.18519

Zhang Y, Lu X, Fu Z, Wang Z, Zhang J (2011) Sulphated modification of a polysaccharide obtained from fresh persimmon (*Diospyros kaki* L.) fruit and antioxidant activities of the sulphated derivatives. Food Chemistry 127(3):1084−1090. https://doi.org/10.1016/j.foodchem.2011.01.100

Zhang CC, Yin X, Cao CY, Wei J, Zhang Q, Gao JM (2015) Chemical constituents from *Hericium erinaceus* and their ability to stimulate NGF-mediated neurite outgrowth on PC12 cells. Bioorganic & Medicinal Chemistry Letters 25(22):5078−5082. https://doi.org/10.1016/j.bmcl.2015.10.016

Zhang CC, Cao CY, Kubo MH, Kenichi YXT, Fukuyama Y, Gao JM (2017) Chemical constituents from *Hericium erinaceus* promote neuronal survival and potentiate neurite outgrowth via the TrkA/Erk1/2 pathway. International Journal of Molecular Sciences 18:1659. https://doi.org/10.3390/ijms18081659

Zhang J, Wang F, Liu K, Liu Q, Yang Y, Dong C (2018) Heat and light stresses affect metabolite production in the fruit bodyof the medicinal mushroom *Cordyceps militaris*. Applied Microbiology and Biotechnology 102:4523–4533. https://doi.org/10.1007/s00253-018-8899-3

Zhao JD, Zhang XQ (1992) The polypores of China. J. Cramer, Berlin – Stuttgart, Germany.

Zhou D, Ruan J, Cai Y, Xiong Z, Fu W, Wei A (2010) Antioxidant and hepatoprotective activity of ethanol extract of *Arachniodes exilis* (Hance) Ching. Journal of Ethnopharmacology 010(129):232–237. https://doi.org/10.1016/j.jep.2010.03.016

Zhuang YL, Sun LP, Zhao X, Wang JF, Hou H, Li BF (2009) Antioxidant and melanogenesis-inhibitory activities of collagen peptide from Jellyfish (*Rhopilema esculentum*). Journal of the Science of Food and Agriculture 89:1722–1727. https://doi.org/10.1002/jsfa.3645