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ENZYMATIC AND ANTIOXIDANT ACTIVITIES OF SEVERAL EDIBLE MUSHROOMS SPECIES

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Abstract

Edible mushrooms are widely consumed for their nutritional value, bioactive compound content, and good taste. The objective of this study was to evaluate and compare the enzymatic and the antioxidant activities of six species commonly marketed in Romania (Pleurotus ostreatus, Agaricus bisporus white, Agaricus bisporus brown, Lentinula edodes (Shiitake), Shimeji white and Shimeji brown. The enzymatic activity of superoxide dismutase (SOD), catalase (CAT) and soluble peroxidase (POX) as well as the antioxidant activity against DPPH (2,2-diphenyl-1-picrylhydrazyl) radical and ABTS (2, 2'- azino -bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation were determined by colorimetric methods. The studied mushroom species have appreciable antioxidant enzymatic activities with an important role in human health. Pleurotus ostreatus is distinguished as having the highest antioxidant activity and high enzymatic activity. The results of this study recommend introducing the investigated mushrooms in diet as a source of exogenous antioxidants.

Key words*: antioxidant, enzymes activity, mushrooms, polyphenols.*

INTRODUCTION

Recently, the population's attention has been focused on diet, a key factor affecting health and the incidence of many diseases. Nutritional research has highlighted functional foods rich in bioactive compounds whose consumption is associated with well-being and healthy living. Mushrooms are widely consumed and appreciated as functional foods due to their organoleptic, nutritional and medicinal properties (Fogarasi et al., 2024; Yadav & Negi, 2021). Mushrooms are low in calories with low fat content and are rich in polyunsaturated fatty acids which are mainly in the form of linoleic, α-linolenic and oleic acids (Reiss et al., 2012; Fogarasi et al., 2020). Mushroom proteins, which constitute 19-35% of dry weight, contain an optimal qualitative and quantitative spectrum of the full range of essential amino acids, making them a good alternative to animal proteins (Ayimbila & Keawsompong, 2023).

Some proteins and peptides produced by mushrooms such as lectins, fungal immunomodulatory proteins, ribosome-inactivating proteins, antimicrobial proteins, ribonucleases, and laccases present important biological properties (Xu et al., 2011).

Mushrooms are a good source of minerals such as potassium, phosphorus, sodium, calcium, magnesium, and essential microelements required for human physiological functions such as copper, zinc, iron, molybdenum and selenium with antioxidant and anti-inflammatory properties (Senila et al., 2024). Mushrooms are a good source of vitamins, especially B vitamins, folic acid and ergosterol which can be easily converted into vitamin D2. Ultraviolet light exposure from sunlight can promote vitamin D production in mushrooms. Sun et al. (2022) report that ergosterol level varies between 2290 and 6200 μg/g in edible mushrooms *Agaricus bisporus* and *Pleurotus ostreatus* and after UV exposure vitamin D content range between 25.9-742 μg/g according to the UV irradiation method (Sun et al., 2022.)

Mushrooms are rich in dietary fiber (Flores et al., 2024) as well as polysaccharides, heteroglucans, peptidoglucans, and proteoglucans. Mushrooms are a complex source of numerous bioactive molecules and health promoting compounds that include: polyphenolic compounds, terpenes, alkaloids,

sterols, polysaccharides (Flores et al., 2024), peptides, proteins, lectins (Abdelshafy et al., 2022; Hamza et al., 2023; Yadav & Negi, 2021, Fogarasi et al., 2024).

Mushrooms contain numerous enzymes such as laccase, lipase, cellulase, peroxidase, amylase, xylanase, protease and pectinase (Sami et al., 2023; Slawinska and Kalbarczyk, 2011). Many of the enzymes extracted from mushrooms are used in the food industry in food processing or to increase the nutritional quality of foods.

In addition to their nutritional qualities, some edible mushrooms have multiple therapeutic properties, being rich in phytocompounds with pharmacological activity (Assemie & Abaya, 2022). Mushrooms are used to prevent and combat many diseases, having antioxidant, antitumor, antimicrobial, antiviral, antiobesity, antidiabetic, antinflammatory, detoxifying, immunemodulatory, hypoglycemic, cholesterol lowering, hepatoprotective, and analgesic properties (Hamza et al., 2024; Venturella et al., 2021). Many studies report positive effects as potential chemopreventive agents in various types of cancer (Ajith et al., 2007). Bioactive compounds from mushrooms have inhibited the growth of cancer cells and induced apoptosis (Sreedharan et al., 2025). Mushrooms contain phytocompounds with anti-inflammatory properties, managing with positive effects conditions such as arthritis, inflammatory liver and intestinal diseases (Chopra et al., 2021). The variation in phytochemicals content in mushrooms is dependent on many factors: genotype, stage of harvesting, growing conditions, substrate, storage conditions (Perez Montes et al., 2021).

Researches were carried out focused on the identification and selection of species that ensure a high nutritional value for the consumer. In this context, the purpose of this study was to evaluate and compare the phenolic compounds content, the enzymatic activity of superoxide dismutase (SOD), catalase (CAT) and soluble peroxidase (POX) and the antioxidant activity of six species of cultivated mushrooms commonly marketed in Romania: *Pleurotus ostreatus, Agaricus bisporus* white; *Agaricus bisporus* brown, *Lentinula edodes (Shiitake), Shimeji* white and *Shimeji* brown.

MATERIALS AND METHODS

The biological material was represented by fruiting bodies of six cultivated mushroom species commonly marketed in Romania: *Pleurotus ostreatus, Agaricus bisporus* white, *Agaricus bisporus* brown, *Lentinula edodes (Shiitake, Shimeji* white and S*himeji* brown. The mushrooms were purchased from the local supermarket.

Reagents: Methanol used for the extraction was from Sigma-Aldrich. Gallic acid, ascorbic acid, 1.1-diphenyl-2-picrylhydrazyl, 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), 6-hydroxy-2,5,7,8-tetramethylchromon 2-carboxylic acid (Trolox), nitroblue tetrazolium chloride (NBT), were purchased from Sigma (Sigma-Aldrich, Sternheim, Germany). Folin-Ciocalteu reagent and guaiacol were purchased from Merck, (Darmstadt, Germany). All the other used chemicals were of analytical grade.

Sample preparation

For antioxidant enzymes extraction, fresh and mature whole fruiting bodies mushroom were homogenised with 0,1M phosphate buffer (pH 7.5) containing 0,01M EDTA. The homogenates were centrifuged for 5 minutes at 6000 g in a Hettich Rotina 380 centrifuge (Tuttlingen, Germany) and the supernatants were used for enzyme assays.

Methanolic extract: For the determination of antioxidant activity and total phenolic content the samples were extracted with 80% aqueous methanol (1:10 w:v) by sonicating for 60 min in a sonicate bath Fungilab (Madrid, Spain) equipped with a digital timer and a temperature controller at 24°C. The resulting slurries were centrifuged at 10000 g for 10 minutes and the supernatants were analyzed.

Biochemical analysis

The Superoxide dismutase (EC 1.15.1.1) activity (SOD) was assayed by measuring ability of sample extract to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). Superoxide dismutase activity IC50 % was expressed as the amount of sample (mg) which caused 50% inhibition of photochemical reduction of NBT (Soare et al., 2017).

Total soluble peroxidase (guaiacol-type E.C.1.11.1.7) activity (POX) was assayed by measuring the increase in absorbance at 470 nm (A470) due to guaiacol oxidation to tetraguaiacol on addition of H_2O_2 and their activity was expressed as ΔA/min/1g f.w. (Soare et al., 2017).

Catalase (E.C.1.11.1.6) activity (CAT) was assayed through the spectrophotometric method at 240 nm by recording the decrease of absorbance due to H_2O_2 consumption (Soare et al., 2017). The results were expressed as μM H_2O_2 /min/g f.w at 25°C.

The total phenolics content (TPC) was determined colorimetric by using the Folin-Ciocalteu method based on the oxidation of phenolic groups with phosphomolybdic and phosphotungstic acids. 2 mL Folin-Ciocalteu's phenol reagent (1:10) and 1.5 mL 7.5 % w/v Na_2CO_3 were added to 0.5 mL methanolic sample extract. The mixture was allowed to stand at room temperature in the dark for 60 min and then the absorbance was recorded at 765 nm (Dinu et al., 2018). The TPC was calculated using a standard curve prepared using gallic acid and expressed as mg GAE/1 g fw.

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay: The capacity of methanolic sample extract to reduce the radical 2,2-diphenyl-1-picrylhydrazyl has been evaluated colorimetrically at 517 nm (Dinu et al., 2018). The results values of percentage of DPPH free radical scavenging were compared with those obtained from two standard curves (Trolox and ascorbic acid). Antioxidant capacity values were expressed as μM TE/g fw and μM AsA/g fw respectively.

ABTS (2, 2'- azino -bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation scavenging activity was measured colorimetric at 734 nm using Trolox or ascorbic acid as standard. The final results were expressed as μM TE/g fw and μM AsA/g fw (Soare et al., 2017).

The spectrophotometric measurements were performed with a Thermo Scientific Evolution 600 UV-Vis spectrophotometer with VISION PRO software. All determinations were performed in triplicate.

Statistical Analysis

The data obtained were analyzed, and all results were expressed as means. The statistical significance of differences between variants was determined with the analysis of variance (ANOVA: single factor), and the means were compared using Duncan's multiple range test ($p \leq 0.05$).

RESULTS AND DISCUSSIONS

In this paper, the enzymatic activity of catalase, peroxidase and superoxide dismutase, enzymes that are the primary enzymatic defenses against free radicals, was determined. SOD, CAT and POX are important active free-radical scavenging enzymes. SOD is an enzyme localized in cytosol, chloroplast, mitochondria and peroxisomes and catalyses the dismutation of the superoxide anion into oxygen and hydrogen peroxide and CAT was responsible for eliminating H_2O_2. Catalase, absent in chloroplast is localized in mitochondria and peroxizomes. This enzyme catalyses the reduction of hydrogen peroxide to H_2O and molecular oxygen. Catalase and peroxidases are among the most studied enzymes in fruits and vegetables. Catalase and peroxidase have different affinities for reactive oxygen species and appear to have different cellular roles in hydrogen peroxide scavenging. Catalase does not need a reductant to scavenge H_2O_2 whereas peroxidase needs a reductant. Catalase has a lower affinity for hydrogen peroxide (mM range) than peroxidase (μM range) (Cruz de Carvahlo, 2008). Peroxidase may function as a fine regulator of intracellular reactive oxygen species whereas catalase might function as a bulk remover of excess reactive oxygen species production under stress conditions (Cruz de Carvahlo, 2008). Hydroxyl radical and hydrogen peroxide are the most toxic species because they can nonspecifically oxidize all classes of biological macromolecules and their elimination from the cell through the action of these enzymes is very important.

In this paper the obtained results show that the activity of antioxidant enzymes varies with the investigated mushroom species (Table 1).

The results obtained for superoxide dismutase activity, expressed as IC 50% (mg), vary between 0.882 mg (*Agaricus bisporus* brown) and 3.33 mg (*Shimeji* brown) as follows: *Shimeji* brown > *Lentinula edodes* > *Shimeji* white > *Agaricus bisporus* white > *Pleurotus ostreatus* > *Agaricus bisporus* brown (Table 1).

In the case of catalase, the enzymatic activity ranges from 0.949 mM H_2O_2/min/g fw (*Shimeji*) white) to 3.248 mM H_2O_2/min/g fw (*Pleurotus ostreatus*).

Table 1. Enzymatic activity of investigated mushrooms

	SOD IC50 (mg)	CAT mM H_2O_2/min /g fw	POX ΔA/min/ 1g f.w
Pleurotus ostreatus	0.926d	3.248a	0.98a
Agaricus bisporus white	0,95d	1.583c	0.84b
Agaricus bisporus brown	0.882d	2.317b	0.78b
Lentinula edodes (Shiitake)	2.5b	2.415b	0.88ab
Shimeji white	1.612c	0.949d	0.8b
Shimeji brown	3.33a	1.418c	0.75b
LSD 5%	0.182	0.299	0.136

Mean values with different letters significantly differ, and statistical significance was set at $p < 0.05$.

The literature is poor in data regarding catalase and peroxidase from mushrooms. In a study that investigate several mushrooms from Dambovita, Romania, in terms of enzymatic and non-enzymatic antioxidants, *Agaricus bisporus* brown was shown to have the highest catalase and peroxidase activity among cultivated mushrooms (Radulescu et al., 2019). Susmitha et al. (2013) show that mushrooms are a good source for the extraction and purification of catalase which can be used efficiently as a therapeutic treatment **(**Susmitha et al., 2013**)**. The medicinal properties of mushroom catalases have been attributed to inhibition of platelet aggregation reduction of blood cholesterol and glucose concentrations, prevention or alleviation of heart disease also prevention or alleviation of infections caused by bacterial, viral, fungal and parasitic pathogens (Susmitha et al., 2013)

The results obtained for peroxidase activity are shown in Table 1. In the case of peroxidase, the enzymatic activity ranges from 0.75 ΔA/min/1 g f.w (*Shimeji* brown) to 0.98 ΔA/min/1 g f.w (*Pleurotus ostreatus*). Peroxidase (POX) is an heme iron protein that is mostly located in the cell wall. This enzyme has been recognized to be involved in the control of development, lignification, pathogen defense and the catabolism of growth regulators (Van Huystee, 2003). Among the investigated mushroom species *Pleurotus ostreatus* stands out with the highest antioxidant enzymes activities while Shimeji brown has the lowest POX and SOD activity. The lowest CAT activity is found in Shimeji white.

The content of total phenolic compounds (TPC) varies depending on the analyzed mushroom species (Table 2). TPC varies between 0.6085 mg GAE/1 g fw and 1.538 mg GAE/1 g fw in the order: *Pleurotus ostreatus*>*Agaricus bisporus* brown> *Agaricus bisporus* white> *Shimeji* brown > *Lentinula edodes* > *Shimeji* white. The highest TPC was determined in the *Pleurotus ostreatus* being 2.53 times higher than in *Shimeji* white mushrooms.

The data reported in the literature for the content of phenolic compounds vary because the content of bioactive compounds in mushrooms may vary depending on many factors and the extraction method (Yim et al., 2009; Perez Montes et al., 2021).

The results obtained in our study are higher than those presented in other papers investigating the same species: in five selected wild edible mushrooms from Romania TPC varies between 408.57 mg GAE/100 g DW and 806.58 mg GAE/100 g DW (Fogarasi et al., 2020); in eight types of edible mushrooms TPC varies between 1 and 6 mg of phenolics per gram of dried mushroom, depending on the species (Palacios et al., 2011). In 43 commonly consumed mushrooms in China TPC varies between 0.8 mg GAE/1 g dw and 11.93 mg GAE/1 g dw (Islam et al. 2016).

For *Agaricus bisporus*, TPC was found to be 4020 mg GAE/kg DW (Keles et al., 2011), and 2.04 mg GAE/g Dw (Robaszkiewicz, 2010); and for *Pleurotus ostreatus* 2686 mg GAE/kg DW (Keles et al., 2011) and 1.44 mg GAE/g DW (Robaszkiewicz, 2010);

Other authors present similar results to our study: TPC in the range of 4.2 to 10.6mgGAE/g dw (Dubost et al., 2007); TPC concentration for the five commercial mushrooms ranged from 5.66 to 13.16 mg GAE/g dm. (Bach et al., 2019) but in other studies the reported data are higher than our results (Radulescu et al., 2019, Boonsong et al, 2016). In a study exploring sixteen of the most popular edible wild mushroom species as potential sources of antioxidants, the highest total polyphenol content, exceeding 100 mg/100 g fresh mass, was found in five mushrooms (Witkowska et al., 2011).

Table 2. Total phenolic content and antioxidant activity in investigated mushrooms species

Mushrooms species	TPC (mg GAE/ g fw)	Antiox. activity ABTS (µM TE/ g fw)	Antiox. activity ABTS (µM AsA E/g fw)	Antiox. activity DPPH (µM TE/g fw))	Antiox. activity DPPH (µM AsA E/ g fw)
Pleurotus ostreatus	1.538 a	1.995 a	1.815 a	2.711 a	1.961 a
Agaricus bisporus white	0.984 c	1.113 d	0.908 d	2.173 b	1.612 b
Agaricus bisporus brown	1.108 b	1.525 b	1.336 b	1,779 d	1.350 c
Lentinula edodes (Shiitake)	0.638 d	0.928 e	0.720 c	0.828 f	0.718 e
Shimeji white	0.609 d	1.121 d	0.927 d	0.881 f	0.754 e
S*himeji* brown	0.673 d	1.216 c	1.022 c	1.270 c	1.012 d
LSD 5%	0.099	0.093	0.066	0.116	0.113

Mean values with different superscript letters significantly differ, and statistical significance was set at $p < 0.05$.

Numerous studies show the presence of phenolic compounds in the composition of mushrooms, the most common being: gallic acid, gallic, protocatechuic, p-hydroxybenzoic, pcoumaric, cinnamic, salicylic, syringic, ferulic, chlorogenic and caffeic acids and a wide spectrum of flavonoid compounds (Yim et al., 2009; Islam et al., 2016; Reis et al., 2012). It has been proven that the phenolic compounds from mushrooms present several biological activities such as antioxidant, antitumor, antimicrobial, hypoglycemic, and anti-inflammatory activities, explaining many of the beneficial effects of mushrooms. The antioxidant character of phenols is determined by their redox properties. Polyphenols protect the cell against oxidative damage caused by free radicals. It is known that free radicals in excess are involved in the occurrence and development of many diseases including cancer, diabetes, cataracts, rheumatoid arthritis, neurodegenerative diseases, cardiovascular problems and degenerative processes associated with aging (Cruz de Carvalho, 2008).

In this study antioxidant activity of the investigated mushroom species was determined by the ability of extracts to scavenge the DPPH and ABTS cation radical which are the most accepted and used methods of evaluating antioxidant activity. The results were calculated using two standards: Trolox and ascobic acid.

The values of DPPH radical scavenging activity ranged from 0.8278 µM TE/g fw to 2.711 µM TE/g fw in terms of Trolox equivalents and from 0.7184 µM AsA/g fw to 1.961 µM AsA/g fw in terms of Ascorbic acids equivalents in order: *Lentinula edodes (Shiitake)*< *Shimeji* white < *Shimeji* brown < *Agaricus bisporus* brown < *Agaricus bisporus* white< *Pleurotus ostreatus* (Table 2). The antioxidant activity was also measured and compared for free radical scavenging activities against ABTS radical cation (Table 2). All results showed significant ABTS radical cation scavenging activity which ranged from 0.9279 µM TE/g fw to 1.9954 µM TE/g fw in terms of Trolox equivalents and from 0.72 µM AsA/g fw to 1.815 µM AsA/g fw in terms of Ascorbic acids equivalents. *Agaricus bisporus* white has ABTS cation radical scavenging activity value (1.1128 µM TE/g fw and 0.908 µM AsAE/g fw) close to the values determined for Shimeji white (1.1205 µM TE/g fw; 0.927 µM AsAE/g fw) while DPPH radical scavenging activity (2.173 µM TE/g fw; 1.6118 µM AsAE/g fw) is 2.46 times higher than the value for Shimeji white in terms of Trolox equivalent (0.8814 µM TE/g fw) and 2.13 times higher in terms of Ascorbic acid equivalent (0.7539 µM AsAE/g fw). The different results determined by the two methods are explained by the kinetics of the reactions between phenols and the types of free radicals, the spectrum and concentration range of phenols (Henriquez et al., 2004).

Numerous bioactive compounds in the composition of mushrooms are responsible for their antioxidant properties (Fogarasi et al., 2024, Gan et al., 2013). The concentration of these compounds varies with different factors and we find the same influence on antioxidant activity. Mushrooms can be used as an exogenous source of antioxidants to help the body defend against the harmful effects of excess free radicals.

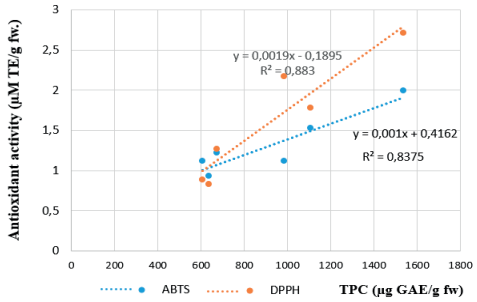

Figure 1. Corelation between antioxidant activity expressed as Trolox equivalent and total phenolic content

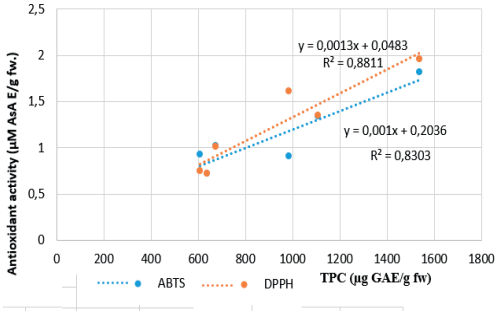

Figure 2. Corelation between antioxidant activity expressed as Ascorbic acid equivalent and total phenolic content

A significant positive correlation was observed between DPPH or ABTS radicals scavenging capacity and TPC suggesting that the antioxidant activity is due to the content of phenolic compounds (Figure 1 and Figure 2). Although mushrooms also have antioxidant enzymatic activity, this probably does not represent a major contribution to the antioxidant activity. The positive correlation between antioxidant activity and TPC was also reported in other studies (Gan et al., 2013; Fogarasi et al., 2020; Puttaraju et al., 2006; Dubost et al., 2007) and for other plant species (Dinu et al., 2018; Dinu et al., 2021).

CONCLUSIONS

The obtained results show that studied biochemical indices vary depending on the analyzed species, the studied mushrooms having high phenolic content and high antioxidant activity.

Among the investigated mushroom species *Pleurotus ostreatus* stands out with the highest antioxidant enzymes activities and antioxidant activity. *Lentinula edodes* (Shiitake) has the lowest antioxidant activity and a low phenolic content. The obtained results show that Shimeji brown has the lowest POX and SOD activity while the lowest CAT activity is found in Shimeji white.

Despite the fact that the enzymatic activity analyzed is not very high, mushrooms can be recommended as beneficial in protecting against oxidative stress.

The studied mushrooms can be used as functional foods being a rich source of bioactive compounds with high nutritional value and health promoting properties.

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