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Analytical methods applied to the chemical characterization and antioxidant properties of three wild edible mushroom species from Northeastern Portugal

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Running title: Analytical methods applied to mushrooms chemical characterization

Abstract

The chemical composition and the antioxidant potential of three species of wild mushrooms from Northeastern Portugal, namely *Agaricus albertii*, *Agaricus urinascens* var. *excellens* and *Pleurotus eryngii* were compared. Standard procedures were followed in the nutritional value evaluation, while chromatographic procedures were used to analyse free sugars, fatty acids, tocopherols, phenolic compounds and organic acids. To assess the antioxidant potential, reducing power, radical-scavenging activity and lipid peroxidation inhibition were evaluated. *P. eryngii* revealed the highest levels of macronutrients, except proteins, as also the highest sugars, tocopherols and MUFA contents. *A. albertii* and *A. urinascens* var. *excellens* showed similar macronutrients composition. However, *A. albertii* revealed the highest content in PUFA and phenolic compounds. *P. eryngii* revealed the highest reducing power and radical-scavenging activity and *A. albertii* the highest lipid peroxidation inhibition. This study provides a detailed chemical characterization and antioxidant potential evaluation of three species of wild mushrooms from Portugal not yet previously reported. Thus, this work intended to contribute to the increase of information concerning species of edible mushrooms (directed to the scientific community and general population) as well as contribute to the conservation of these resources as sources of compounds of interest.

Keywords Wild mushrooms; Chemical characterization; Nutritional value; Antioxidant potential

Introduction

Mushrooms have been widely consumed as part of the normal diet, and also as a delicacy due to their highly desirable taste and aroma. Moreover, the nutritional, tonic and medicinal properties of mushrooms have been recognized for a long time (Mattila et al. 2000). Some mushroom species have been also described as having medicinal properties; antitumor, antiviral and hypolipidemic effects have been reported (Ferreira et al. 2010; Lindequist et al. 2005).

Agaricus albertii (Bon.) (also known as *Agaricus macrosporus* (F.H. Møller & Jul. Schff.) Pilát) is a good edible mushroom with a strong aniseed odour, in young and mature specimens (more rarely ammoniacal). It occurs in early summer to early autumn on soil amongst grass in pastures in circles, being a quite rare species. It has a hemispherical or campanulate creamy-white cap; the gills are white in the beginning, being brown/chocolate in mature specimens, and it has also a ring on stem (Jordan 2004; Rodríguez 2012). *Agaricus urinascens* var. *excellens* (F.H. Møller) Nauta is an edible mushroom that usually occurs in summer to autumn in trooping groups on soil in open broad-leaf and coniferous woodlands, give preference to spruce. It has a white or yellow-tinged cap, greyish pink gills and a ring on stem. The odor and taste are not distinctive but it is also a good edible (Jordan 2004). *Pleurotus eryngii* (D.C.:Fr.) Quélet is an excellent edible mushroom with a convex cap that after becomes flattened or depressed in the center. Its margin is very wound initially, but with maturity becomes more corrugated. It has very decurrent whitish gills and occurs in spring and autumn, generally associated with thistle roots (parasitic). Its smell is very pleasant and it has a sweet taste (Rodríguez et al. 2008; Rodríguez 2012).

The two *Agaricus* species described above are quite rare and so there is not much information about them. There are some studies reporting the heavy metals absorption

by *A. albertii* (Cocchi et al. 2006; Melgar et al. 2007) and its mycelium production (Fermor 1982). It can also be found some information about antimony contents in *A. urinascens* var. *excellens* from pollutant and clean areas (Borovička et al. 2006) and some work at a molecular/genetic level (Bunyard et al. 1996). Concerning *P. eryngii*, this species is more disperse and therefore we can find more information available. Nevertheless, majority of the studies regards cultivated mushrooms. There is some information about the nutritional quality of commercial *P. eryngii* from Italy and from Portugal (Manzi et al. 2004; Reis et al. 2012a). There are also some studies in polysaccharides and enzymes (Carbonero et al. 2006; Rodríguez et al. 2004) and about the antioxidant properties of this cultivated mushroom and its mycelium (Mishra et al. 2013; Reis et al. 2012b).

Our research group has developed several works that intend to contribute to inventorying and documentation of the properties of wild mushrooms from Northeastern Portugal (nutritional and bioactive properties), providing better management and conservation of these natural resources and their *habitats* (Grangeia et al. 2011; Pereira et al. 2012; Reis et al. 2011). The present work aims contributing to achieve this goal, since it presents a detailed chemical characterization of the edible wild mushrooms described above, including evaluation of the nutritional value (*e.g.* macronutrients, and individual free sugars and fatty acids), bioactive compounds (*e.g.* tocopherols, phenolic compounds and organic acids), and antioxidant activity of their methanolic extracts (*e.g.* reducing power, radical-scavenging activity and inhibition of lipid peroxidation).

Material and Methods

Mushroom species

The wild mushroom species were collected in Bragança's outskirts (Northeastern Portugal), in the autumn of 2011. Information about these species is provided in Table 1. Taxonomic identification of sporocarps was made according to several authors (*e.g.* Courtecuisse and Duhem 2005; Kirk et al. 2001). All the samples were lyophilised (FreeZone 4.5 model 7750031, Labconco, Kansas, USA), reduced to a fine dried powder (20 mesh), mixed to obtain homogenous samples and stored in a desiccator, protected from light, until further analysis.

Standards and Reagents

Acetonitrile 99.9%, *n*-hexane 95% and ethyl acetate 99.8% were of HPLC grade from Fisher Scientific (Lisbon, Portugal). The fatty acids methyl ester (FAME) reference standard mixture 37 (standard 47885-U) was purchased from Sigma (St. Louis, MO, USA), as also other individual fatty acid isomers, sugars (D(-)-fructose, D(-)-mannitol, and D(+)-trehalose), tocopherols (α-, β-, γ-, and δ-isoforms) and all organic acids standards (oxalic acid; quinic acid; malic acid, citric acid and fumaric acid). Racemic tocol, 50 mg/mL, was purchased from Matreya (PA, USA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). Phenolic standards (*p*-hydroxybenzoic and *p*-coumaric acids), cinnamic acid and trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were purchased from Sigma (St. Louis, MO, USA). Methanol and all other chemicals and solvents were of analytical grade and purchased from common sources. Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA).

Chemical characterization of the mushroom species

Nutritional value. The samples were analysed for their chemical composition (moisture, proteins, fat, carbohydrates and ash) through standard procedures (AOAC 1995). The crude protein content (N × 4.38) of the samples was estimated by the macro-Kjeldahl method; the crude fat was determined by extracting a known weight of powdered sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at 600±15 ºC. Total carbohydrates were calculated by difference. Energy was calculated according to the following equation: Energy (kcal) = 4 × (g protein + g carbohydrate) + 9 × (g fat).

Sugars composition. Free sugars were determined by a high performance liquid chromatograph (HPLC) system consisted of an integrated system with a pump (Knauer, Smartline system1000), degasser system (Smart line manager 5000) and an auto-sampler (AS-2057 Jasco), coupled to a refraction index detector (RI detector Knauer Smartline 2300) as previously described by the authors (Reis et al. 2012a). Sugars identification was made by comparing the relative retention times of sample peaks with standards. Data were analyzed using Clarity 2.4 Software (DataApex). Quantification was based on the RI signal response of each standard, using the internal standard (IS, raffinose) method and by using calibration curves obtained from the commercial standards of each compound. The results were expressed in g per 100 g of dry weight.

Fatty acids composition. Fatty acids were determined after a transesterification procedure as described previously by the authors (Reis et al. 2012a). The fatty acid profile was analyzed with a DANI 1000 gas chromatographer (GC) equipped with a split/splitless injector and a flame ionization detector (FID). Fatty acid identification was made by comparing the relative retention times of FAME peaks from samples with

standards. The results were recorded and processed using CSW 1.7 software (DataApex 1.7) and expressed as a weight percentage of the crude fat.

Tocopherols composition. Tocopherols were determined following a procedure previously optimized and described by the authors (Heleno et al. 2010). Analysis was performed by HPLC (equipment described above), and a fluorescence detector (FP-2020; Jasco) programmed for excitation at 290 nm and emission at 330 nm. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was based on the fluorescence signal response of each standard, using the IS (tocol) method and by using calibration curves obtained from commercial standards of each compound. The results were expressed in µg per100 g of dry weight.

Phenolic compounds composition. Phenolic acids determination was performed using a Shimadzu 20A series ultra fast liquid chromatograph (UFLC, Shimadzu Coperation, Kyoto, Japan) as previously described by Reis et al. (2012b). Detection was carried out in a photodiode array detector (PDA), using 280 nm as the preferred wavelength. The phenolic compounds were quantified by comparison of the area of their peaks recorded at 280 nm with calibration curves obtained from commercial standards of each compound. The results were expressed in µg per g of dry weight.

Organic acids composition. Organic acids were determined by UFLC coupled with a photodiode array detector (PDA; equipment described above) as previously optimized and described by the authors (Barros et al. 2013). The organic acids were quantified by the comparison of the area of their peaks recorded at 215 nm with calibration curves

obtained from commercial standards of each compound. The results were expressed in g per 100 g of dry weight.

Evaluation of the antioxidant potential of the mushroom species

Extracts preparation. The lyophilized powder (1.0-1.5 g) was extracted by stirring with 40 mL of methanol for 1 h and subsequently filtered through Whatman No. 4 paper. The residue was then extracted with 20 mL of methanol for 1 h. The combined methanolic extracts were evaporated at 40ºC (rotary evaporator Büchi R-210) to dryness re-dissolved in methanol at a concentration of 20 mg/mL, and stored at 4ºC until analysis. Successive dilutions were made from the stock solution and submitted to *in vitro* assays already described by Reis et al. (2012b) to evaluate the antioxidant activity of the samples. The sample concentrations (mg/mL) providing 50% of antioxidant activity or 0.5 of absorbance (EC_{50}) were calculated from the graphs of antioxidant activity percentages (DPPH, β-carotene/linoleate and TBARS assays) or absorbance at 690 nm (ferricyanide/Prussian blue assay) against sample concentrations. Trolox was used as a positive control.

Folin-Ciocalteu assay. Folin-Ciocalteu is a reagent that is known to react with all reducing species in a solution. It is a mixture of tungsten and molybdenum with a yellow colour and under alkaline conditions it reacts with all the antioxidants in solution. Therefore, lately it is considered an antioxidant activity evaluation assay rather than an assay to quantify total phenolics (Carocho and Ferreira, 2013).

One of the extract solutions (5 mg/mL; 1 mL) was mixed with *Folin-Ciocalteu* reagent (5 mL, previously diluted with water 1:10, v/v) and sodium carbonate (75 g/L, 4 mL). The tubes were vortex mixed for 15 s and allowed to stand for 30 min at 40ºC for color

development. Absorbance was then measured at 765 nm (Analytikjena spectrophotometer; Jena, Germany). Gallic acid was used to obtain the standard curve and the reduction of the *Folin-Ciocalteu* reagent by the samples was expressed as mg of gallic acid equivalents (GAE) per g of extract.

Ferricyanide/Prussian blue assay. The extract solutions with different concentrations (0.5 mL) were mixed with sodium phosphate buffer (200 mmol/L, pH 6.6, 0.5 mL) and potassium ferricyanide (1% w/v, 0.5 mL). The mixture was incubated at 50 °C for 20 min, and trichloroacetic acid (10% w/v, 0.5 mL) was added. The mixture (0.8 mL) was poured in the 48 wells plate, the same with deionized water (0.8 mL) and ferric chloride (0.1% w/v, 0.16 mL), and the absorbance was measured at 690 nm in ELX800 Microplate Reader (Bio-Tek Instruments, Inc; Winooski, USA).

DPPH radical-scavenging activity. This methodology was performed using the Microplate Reader mentioned above. The reaction mixture on 96 well plate consisted of a solution by the well of the extract solutions with different concentrations (30 μL) and methanolic solution (270 μL) containing DPPH radicals (6×10^{-5} mol/L). The mixture was left to stand for 30 min in the dark, and the absorption was measured at 515 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation: $\%RSA=[(A_{DPPH}-A_S)/A_{DPPH}]\times100$, where A_S is the absorbance of the solution containing the sample, and A_{DPPH} is the absorbance of the DPPH solution.

Inhibition of β-carotene bleaching or β-carotene/linoleate assay. A solution of β-carotene was prepared by dissolving β-carotene (2 mg) in chloroform (10 mL). Two milliliters of this solution were pipetted into a round-bottom flask. The chloroform was

removed at 40 °C under vacuum and linoleic acid (40 mg), Tween 80 emulsifier (400 mg), and distilled water (100 mL) were added to the flask with vigorous shaking. Aliquots (4.8 mL) of this emulsion were transferred into test tubes containing extract solutions with different concentrations (0.2 mL). The tubes were shaken and incubated at 50 °C in a water bath. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm. β-Carotene bleaching inhibition was calculated using the following equation: (Absorbance after 2 h of assay/ initial absorbance) × 100.

Thiobarbituric acid reactive substances (TBARS) assay. Porcine (*Sus scrofa*) brains were obtained from official slaughtered animals, dissected, and homogenized with Polytron in an ice cold Tris-HCl buffer (20 mM, pH 7.4) to produce a 1:2 w/v brain tissue homogenate which was centrifuged at 3000 *g* for10 min. An aliquot (100 µL) of the supernatant was incubated with the different concentrations of the sample solutions (200 µL) in the presence of $FeSO_4$ (10 mM; 100 µL) and ascorbic acid (0.1 mM; 100 µL) at 37 °C for 1 h. The reaction was stopped by the addition of trichloroacetic acid (28% w/v, 500 µL), followed by thiobarbituric acid (TBA, 2%, w/v, 380 µL), and the mixture was then heated at 80 °C for 20 min. After centrifugation at 3000 g for 10 min to remove the precipitated protein, the color intensity of the malondialdehyde (MDA)-TBA complex in the supernatant was measured by its absorbance at 532 nm. The inhibition ratio (%) was calculated using the following formula: Inhibition ratio (%) = [(A−B)/A] × 100%, where A and B were the absorbance of the control and the sample solution, respectively.

Statistical analysis

For each one of the mushroom species three samples were used and all the assays were carried out in triplicate. The results are expressed as mean values and standard deviation (SD). The results were analysed using one-way analysis of variance (ANOVA) followed by Tukey's HSD Test with $\alpha = 0.05$. This analysis was carried out using SPSS v. 18.0 program.

Results and discussion

The results of the nutritional evaluation of the three studied mushrooms are displayed in Table 2. *A. albertii*, the species with the highest moisture value (90.73 g/100 g fw), revealed the highest protein content (19.83 g/100 g dw). However, it was *P. eryngii* that revealed the highest amounts of fat and carbohydrates (4.36 and 78.60 g/100 g dw, respectively), being the species with the highest energy contribution (362.00 kcal/100 g dw).

Concerning the free sugars analysis, fructose was found in both *Agaricus* species (Table 3); mannitol and trehalose were quantified in all the studied samples. *P. eryngii* was the species with the highest content in free sugars (15.62 g/100 g dw) due to the higher concentration of trehalose (14.21 g/100 g dw).

The main fatty acids found and quantified in the studied species are presented in Table 4. Palmitic, stearic, oleic and linoleic acids were the main fatty acids present in the studied species, the latter being present in higher concentrations in *Agaricus* species (51.21% and 75.75%). Polyunsaturated fatty acids predominated over saturated fatty acids. The highest amounts of PUFA were determined in *Agaricus* species (mainly in *A. albertii*; 76.47%) and the highest content of MUFA were determined in *Pleurotus* species (49.05%). With regard to tocopherols, the four vitamers (α-, β-, γ- and δ-tocopherol) were only found in *P. eryngii* (Table 4), with a higher concentration of β-

tocopherol (48.24 μg/100 g dw), this could be due to the fact that this species has a higher content in total fat, than *Agaricus* species. Since vitamin E is quite sensitive and easily oxidized (Fernandes et al., 2012; Dionísio et al., 2009), it probably suffered degradation during the time of collection/storage.

Regarding phenolic compounds, *p*-coumaric acid was detected only in *Agaricus* species (Table 5). *p*-Hydroxybenzoic acid was the main phenolic acid detected, and it was present in higher amounts in *A. albertii* (8.23 mg/100 g dw; Figure 1). This was the species with the highest content of phenolic acids (11.76 mg/100 g dw) and also with the highest amount of cinnamic acid (2.51 mg/100 g dw), that was also quantified in all the studied species. Concerning organic acids, malic acid was the most abundant organic acid detected in the studied mushrooms (Table 5). The highest amounts of organic acids were quantified in *P. eryngii* (7.37 g/100 g dw).

Analyzing the results of the antioxidant potential of the studied mushrooms (Table 6) we can conclude that *P. eryngii* was the species with the highest reducing power, revealing the highest content of total phenolics (18.43 mg GAE/g extract) and the lowest EC_{50} value for the ferricyanide/Prussian blue assay (2.17 mg/mL). This species also revealed the highest DPPH radical-scavenging activity (9.21 mg/mL). Considering the results of the lipid peroxidation inhibition, evaluated through the β-carotene/linoleate and TBARS assays, we can conclude that *A. albertii* (that displayed a total phenolics concentration similar to *P. eryngii*; 18.36 mg GAE/g extract) revealed the lowest EC_{50} values, proving that it may be a mushroom with antioxidant potential. Noted that this species showed the highest content in phenolic acids, what can be related to these results, while in *P. eryngii* we verified a higher content of organic acids and tocopherols that could also be contributors of antioxidant potential.

As referred above, there is a very little information available about the *Agaricus* species studied in this work and, concerning *P. eryngii*, most of the information found refers to commercial species. Thus, this work emerges as a new source of information about these wild edible mushroom species.

The studied wild mushrooms proved to be a rich source of macronutrients (high contents in carbohydrates and protein, lowest contents in fat with the valuable contribution of unsaturated fatty acids), which is in agreement with the literature (Kalač 2009; Reis et al. 2011) and makes them excellent foods that can be used in low caloric diets.

Sugars provide the bulk of dietary energy, and glucose and fatty acids are the major metabolic fuels used for energy production (Flatt 1988). Fructose is a good power source; however, numerous studies show that this sugar increases the incidence of obesity, dyslipidemia, insulin resistance, and hypertension (Akram and Hamid 2012). Mannitol is an energy store as well as an osmoticum in fungi (Hammond and Nichols 1976) and because of their mannitol contents mushrooms are useful for diabetic foods (Hamano 1997). Trehalose may function as a reserve which is metabolized when the sporocarps are maturing (Koide et al. 2000). It is also a good option since it shows good sweetness like sucrose, being one of the most stable saccharides. Thus, *P. eryngii* emerges as a good source of sugars, since it was not detected fructose in this species and, even then, it revealed the highest content in total sugars (healthier).

Linoleic acid was among the fatty acids quantified, whose consumption, as an omega-6 PUFA, reduces the risk of Coronary Heart Disease (CHD) (Harris et al. 2009). Given the biological activities of this fatty acid found in the studied mushroom species, we can consider them a source of molecules with health benefits.

The mushrooms investigated also proved to be a good source of bioactive compounds that could contribute for their antioxidant potential as these molecules are considered non-enzymatic antioxidants (Carocho and Ferreira 2013). Phenolic acids have antioxidant activity mainly as free radical scavengers. Although these compounds (phenolic acids) have been found mainly in the *A. albertii* and act as radical scavengers, *P. eryngii* was the mushroom species with the highest radical-scavenging activity (evaluated through DPPH radical assay). Thus, we can infer that phenolic acids are not the main contributors for the antioxidant potential of *A. albetii*.

Organic acids play an important role in the human body being part of the Krebs cycle. For example, malic acid is a member of the C4-dicarboxylic acid family and an intermediate of the tricarboxylic acid (TCA) cycle. In addition, some organic acids could also have biological effects, like fumaric acid that possesses anti-inflammatory, neuroprotective and chemopreventive activities (Baati et al. 2011). Citric and ascorbic acid are also organic acids known for their antioxidant activity. For example, ascorbic acid is effective in scavenging the superoxide radical anion, hydrogen peroxide, hydroxyl radical, singlet oxygen and reactive nitrogen oxide (Barros et al. 2011). *P. eryngii* was the species that revealed the highest reducing power and DPPH radical-scavenging activity. Since it was the species with the highest content in organic acids, maybe these molecules and the verified activity were related.

Vitamin E, constituted by eight isoforms, with four tocopherols (α-tocopherol, β-tocopherol, γ-tocopherol and δ-tocopherol) and four tocotrienols (α -tocotrienol, β -tocotrienol, γ -tocotrienol and δ-tocotrienol) is the only major lipid-soluble, chain breaking antioxidant found in plasma, red cells and tissues, allowing it to protect the integrity of lipid structures. This vitamin acts by halts lipid peroxidation (Burton and

Traber 1990). Therefore, tocopherols present in *P. eryngii* species, can also be related with the antioxidant potential of this mushroom.

Overall, *P. eryngii* seems to be the most interesting species, as it was the mushroom with the highest levels of macronutrients (unless proteins), organic acids and tocopherols and the species with the highest reducing power and radical-scavenging activity. However, also *A. albertii* revealed interesting results, once it presented the highest contents in PUFA, phenolic compounds and demonstrated the highest lipid peroxidation inhibition potential.

The results stated in this work contributes for one of the purposes of our research group, realize or contribute for mycological inventories, doing biochemical assays in species from the Northeast of Portugal, an area with a huge mycological biodiversity. These studies attest the interest of mushrooms as food and ensure the presence of compounds with biological activity. The research undertaken aims not only to increase the scientific knowledge at this level, but also seeks to help to the information, current and secure, reaches the society in general (consumers of these products).

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

Conflict of Interest

Filipa S. Reis declares that she has no conflict of interest.

Lillian Barros declares that she has no conflict of interest.

Maria João Sousa declares that she has no conflict of interest.

Anabela Martins declares that she has no conflict of interest.

Isabel C.F.R. Ferreira declares that she has no conflict of interest.

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Table 1. Information about the wild mushroom species studied.

Scientific name	*Agaricus albertii* (Bon.)	*Agaricus urinascens* var. *excellens* (F.H. Møller) Nauta	*Pleurotus eryngii* (D.C.:Fr.) Quélet
English name	Macro mushroom	Unknown	King oyster mushroom
Edibility	Edible	Edible	Edible
Ecology	Saprotrophic	Saprotrophic	Parasitic/Saprotrophic
Date of collection	Autumn 2011	Autumn 2011	Autumn 2011

Table 2. Nutritional value of the studied wild mushrooms (mean ± SD).

	Agaricus albertii	*Agaricus urinascens* var. *excellens*	*Pleurotus eryngii*
Moisture (g/100 g fw)	90.73 ± 0.24^a	87.72 ± 0.26^b	82.59 ± 0.36^c
Ash (g/100 g dw)	22.13 ± 0.71^b	29.64 ± 1.82^a	14.95 ± 0.91^b
Proteins (g/100 g dw)	19.83 ± 0.03^a	14.47 ± 0.61^b	2.09 ± 0.01^c
Fat (g/100 g dw)	1.38 ± 0.06^b	1.37 ± 0.23^b	4.36 ± 0.14^a
Carbohydrates* (g/100 g dw)	56.66 ± 0.54^b	54.52 ± 1.83^b	78.60 ± 0.75^a
Energy(kcal/100 g dw)	318.36 ± 1.78^b	288.29 ± 4.36^c	362.00 ± 2.06^a

*Determined by difference; fw- fresh weight; dw- dry weight. In each row different letters mean significant differences ($p<0.05$).

Table 3. Composition in free sugars of the studied wild mushrooms (mean ± SD).

	Agaricus albertii	*Agaricus urinascens* var. *excellens*	*Pleurotus eryngii*
Fructose (g/100g dw)	0.48 ± 0.03[b]	0.60 ± 0.01[a]	nd
Mannitol (g/100g dw)	4.78 ± 0.02[a]	0.77 ± 0.01[c]	1.40 ± 0.09[b]
Trehalose (g/100g dw)	0.70 ± 0.04[c]	0.14 ± 0.02[b]	14.21 ± 0.23[a]
Sum of soluble sugars (g/100g dw)	5.97 ± 0.03[b]	1.50 ± 0.01[c]	15.62 ± 0.31[a]

dw- dry weight. In each row different letters mean significant differences ($p<0.05$).

Table 4. Composition in major fatty acids and tocopherols of the studied wild mushrooms (mean ± SD).

	Agaricus albertii	*Agaricus urinascens* var. *excellens*	*Pleurotus eryngii*
C16:0	11.14 ± 0.07[c]	14.88 ± 0.31[b]	17.44 ± 0.21[a]
C18:0	3.12 ± 0.04[c]	3.57 ± 0.02[b]	4.77 ± 0.08[a]
C18:1n9	2.05 ± 0.60[c]	5.47 ± 0.55[b]	47.52 ± 0.07[a]
C18:2n6	75.75 ± 0.51[a]	51.21 ± 1.09[b]	24.71 ± 0.28[c]
Total SFA (% of total FA)	21.14 ± 0.06[c]	28.79 ± 0.13[a]	25.79 ± 0.18[b]
Total MUFA (% of total FA)	2.39 ± 0.61[c]	19.16 ± 0.97[b]	49.05 ± 0.24[a]
Total PUFA (% of total FA)	76.47 ± 0.55[a]	52.05 ± 1.10[b]	25.17 ± 0.24[c]
α-Tocopherol (μg/100g dw)	nd	nd	6.79 ± 1.80
β-Tocopherol (μg/100g dw)	nd	nd	48.24 ± 9.53
γ-Tocopherol (μg/100g dw)	nd	nd	31.55 ± 7.56
Sum of tocopherols (μg/100g dw)	nd	nd	86.58 ± 3.77

C16:0- Palmitic acid; C18:0- Stearic acid; C18:1n9- Oleic acid; C18:2n6- Linoleic acid. SFA- Saturated fatty acids; MUFA- Monounsaturated fatty acids; PUFA- Polyunsaturated fatty acids. More 20 FA were quantified (data not shown) in trace/lower amounts.
dw- dry weight; nd- not detected. In each row different letters mean significant differences ($p<0.05$).

Table 5. Composition in phenolic and organic acids of the studied wild mushrooms (mean ± SD).

	Agaricus albertii	*Agaricus urinascens* var. *excellens*	*Pleurotus eryngii*
p-Hydroxybenzoic acid (mg/100 g dw)	8.23 ± 0.56[a]	3.27 ± 0.49[b]	3.81 ± 0.41[b]
p-Coumaric acid (mg/100 g dw)	3.53 ± 0.70[a]	1.33 ± 0.24[b]	nd
Sum of phenolic acids (mg/100 g dw)	11.76 ± 1.26[a]	4.60 ± 0.26[b]	3.81 ± 0.41[c]
Cinnamic acid (mg/100 g dw)	2.51 ± 0.08[a]	1.75 ± 0.25[b]	0.85 ± 0.01[c]
Oxalic acid (g/100 g dw)	0.62 ± 0.01[b]	0.87 ± 0.00[a]	0.25 ± 0.01[c]
Quinic acid (g/100 g dw)	0.65 ± 0.05	nd	nd
Malic acid (g/100 g dw)	1.39 ± 0.03[b]	0.76 ± 0.05[c]	6.15 ± 0.23[a]
Citric acid (g/100 g dw)	0.71 ± 0.05[a]	0.50 ± 0.00[b]	0.43 ± 0.00[c]
Fumaric acid (g/100 g dw)	0.16 ± 0.00[b]	0.10 ± 0.00[c]	0.55 ± 0.00[a]
Sum of organic acids (g/100 g dw)	3.53 ± 0.04[b]	2.22 ± 0.04[c]	7.37 ± 0.23[a]

dw- dry weight; nd- not detected. In each row different letters mean significant differences ($p<0.05$).

Table 6. Antioxidant properties of the studied wild mushrooms (mean ± SD).

	Agaricus albertii	*Agaricus urinascens* var. *excellens*	*Pleurotus eryngii*
Folin-Ciocalteu assay (mg GAE/g extract)	18.36± 0.10[a]	15.44 ± 0.28[b]	18.43 ± 4.94[a]
Ferricyanide/Prussian blue assay (EC_{50}; mg/mL)	3.04 ± 0.02[b]	3.71 ± 0.03[a]	2.17 ± 0.05[c]
DPPH scavenging activity assay(EC_{50}; mg/mL)	10.17 ± 0.23[b]	13.80 ± 0.25[a]	9.21 ± 0.06[c]
β-carotene/linoleate assay (EC_{50}; mg/mL)	3.78 ± 0.37[b]	4.30 ± 0.27[b]	14.90 ± 1.50[a]
TBARS assay (EC_{50}; mg/mL)	0.61 ± 0.26[c]	6.60 ± 0.92[a]	1.59 ± 0.02[b]

Concerning the *Folin-Ciocalteu* assay, higher values mean higher reducing power; for the other assays, the results are presented in EC_{50} values, what means that higher values correspond to lower reducing power or antioxidant potential. EC_{50}: Extract concentration corresponding to 50% of antioxidant activity or 0.5 of absorbance for the Ferricyanide/Prussian blue assay. In each row different letters mean significant differences between species (p<0.05).

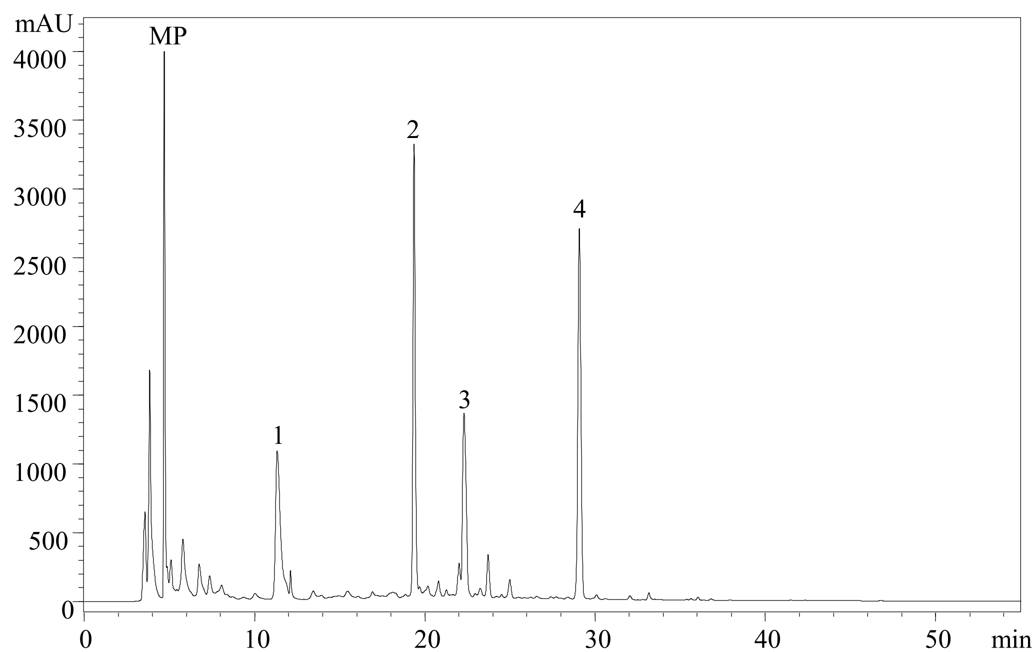

Figure 1. Individual chromatograms of *Agaricus albertii* phenolic compounds recorded at 280 nm. 1-*p*-Hydroxybenzoic acid; 2-*p*-Coumaric acid; 3- not identified; 4-Cinnamic acid. MP– mobile phase.