Chemical characterization and antioxidant properties of *Lepista nuda* fruiting bodies and mycelia obtained by *in vitro* culture: effects of collection habitat and culture media

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ABSTRACT

Lepista nuda is an edible mushroom which presents important organoleptic qualities including a delicate flavor and good postharvest conservation. Its chemical and bioactive properties can be affected by habitat collection. Therefore, the main goal of the present work was to compare chemical composition and antioxidant potential of *Lepista nuda* samples from different habitats, and mycelia produced by *in vitro* culture, using different culture media. The commercial sample (cultivated) gave the highest levels of energy, polyunsaturated fatty acids (due to the contribution of linoleic acid) and phenolic compounds; the wild sample from oak forest gave the highest levels of organic acids. Mycelia samples showed higher levels of glucose, tocopherols and antioxidant activity. Particularly, PACH (Pachlewski medium) proved to be better for glucose production, PDA (Potato Dextrose Agar medium), PACH and FAD (Ferry & Das medium) for β- and γ-tocopherols, complete MMN (Melin-Norkans medium) for phenolic compounds and incomplete MMN for antioxidant properties. Overall, *in vitro* culture could be explored to obtain bioactive compounds from macrofungi for industrial applications, controlling environmental conditions to produce higher amounts of these compounds and to overcome the diversity in chemical composition observed in samples collected in different habitats.

Keywords: Lepista nuda; Mushroom; Mycelium; Chemical compounds; Antioxidant properties

1. Introduction

It is stated all over the world that mushrooms are rich in water, minerals, proteins, fibers and carbohydrates, and that they are low caloric foods due to low content in fat. There are available in literature several studies reporting nutrient analysis of different mushroom species from all over the world (Kalač, 2009; Ouzouni, Petridis, Koller, & Riganakos, 2009). In Portugal, our research group has been dedicated to mushrooms from Northeast Portugal, one of the European regions with higher biodiversity in wild mushrooms, most of them with a great gastronomic importance (Barros, Baptista, Correia, Casal, Oliveira, & Ferreira, 2007; Barros, Venturini, Baptista, Estevinho, & Ferreira, 2008; Heleno, Barros, Sousa, Martins, & Ferreira, 2009; Grangeia, Heleno, Barros, Martins, & Ferreira, 2011; Pereira, Barros, Martins, & Ferreira, 2012; Leal et al., 2013). Moreover, many studies have concluded that mushrooms possess bioactive compounds (e.g. antioxidants) such as phenolic compounds, tocopherols, ascorbic acid and carotenoids (Ferreira, Barros, & Abreu, 2009). Therefore, mushrooms have been recognized as functional foods and sources for the development of medicines and nutraceuticals. Not only fruiting bodies, but also mycelia accumulate several bioactive metabolites (Borchers, Keen, & Gershwin, 2004; Lindequist, Niedermeyer, & Jülich, 2005) that could be isolated for medicinal proposes.

In vitro culture methodology (for mycelia production) brings numerous advantages in the production of bioactive compounds. It facilitates compounds extraction and purification, increasing their economic value. From an ecological point of view, *in vitro* culture avoids the overexploitation of endangered or rare species, representing a promissory methodology that prioritizes sustainable conservation and rational use of biodiversity (Matkowski, 2008).

Lepista nuda (Bull. ex Fr). Cooke (Tricholomataceae) is an edible mushroom with several organoleptic qualities including a delicate flavor and good postharvest conservation, which are important economic aspects that influence sales and prices (Guinberteau, Olivier, & Bordaberry, 1989). The strong and subtle flavor, texture, and violet coloration present some originality that stimulates its consumption and even cultivation (Audouin, Vidal, & Richard, 1989; Noel-Suberville, Cruz, Guinberteau, & Montury, 1996). The fruiting body of *L. nuda* contains vitamin B1, triterponoids, sterols and orgonohalogens. Its infusion is used for preventing beriberi, while the decoction is used for the treatment of abscesses and wounds. The methanolic extract revealed antimicrobial (Dulger, Ergul, & Gucin, 2002; Barros et al., 2008) and antioxidant (Elmastas, Isildak, Turkekul, & Temur, 2007; Barros et al., 2008) properties. Nevertheless, chemical or bioactive properties can be affected by mushrooms habitat (Nikkarinen & Mertanen, 2004; Pereiraet al., 2012).

In this context, it was our aim to compare chemical composition and antioxidant potential of *Lepista nuda* samples from different habitats, and mycelia produced by *in vitro* culture, using different culture media.

2. Material and methods

2.1. Samples

Lepista nuda commercial samples were obtained in a local supermarket in September 2011. The wild samples were obtained in Pine and Oak forests from Bragança (Northeast Portugal) and taxonomically identified following Moser (1983). Voucher specimens were deposited at herbarium of School of Agriculture of Polytechnic Institute of Bragança, Portugal. Mycelium was isolated from sporocarps of each sample (commercial, wild pine forest and wild oak fortes) on i) Complete solid Melin-Norkans

medium (cMMN) (Marx, 1969); ii) Incomplete solid MMN (iMMN) (Marx, 1969); iii) Potato Dextrose Agar medium (PDA) (Biolab); iv) Pachlewski (PACH) (adapted from Pachlewski, 1967); v) Ferry & Das (FAD) (Ferry & Das, 1968).

Mycelia were grown in Petri dishes (8 cm diameter) with 8 ml of solid media covered with cellophane (autoclaved three times), and placed at a 25 ºC in the dark. After 20 days, approximately, of incubation (when mycelium reached maximum radial growth) the mycelium was recovered from the medium.

All the samples were lyophilised (FreeZone 4.5 model 7750031, Labconco, Kansas, USA), quantified, reduced to a fine dried powder (20 mesh), and mixed to obtain homogenate samples for further analysis.

2.2. 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 and standards of sugars (D-(+)-glucose, D-(-)-fructose, D-(+)-mannitol, D-(+)-trehalose), tocopherols (α-, β- and γ-isoforms), organic acids (citric acid, fumaric acid, malic acid, oxalic acid and quinic acids), phenolic compounds (gallic, *p*-hydroxybenzoic and cinnamic acids), and trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic 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). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA).

2.3. Proximate composition

The samples were analysed for chemical composition (moisture, proteins, fat, carbohydrates and ash) using the AOAC 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).

2.4. Fatty Acids.

Fatty acids were determined after a transesterification procedure as described previously by the authors (Barros et al., 2007), using a gas chromatographer (DANI 1000) equipped with a split/splitless injector and a flame ionization detector (GC-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). The results were expressed in relative percentage of each fatty acid.

2.5. Sugars

Free sugars were determined by a High Performance Liquid Chromatography (HPLC) system consisted of an integrated system with a pump (Knauer, Smartline system 1000), degasser system (Smartline manager 5000) and auto-sampler (AS-2057 Jasco), coupled to a refraction index detector (RI detector Knauer Smartline 2300) as previously described by the authors (Heleno et al., 2009). 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 commercial standards of each compound. The results were expressed in g per 100 g of dry weight.

2.6. Organic acids

Organic acids were determined following a procedure previously optimized and described by the authors (Barros, Pereira, & Ferreira, 2012). Analysis was performed by ultra fast liquid chromatograph (UFLC) coupled to photodiode array detector (PDA), using a Shimadzu 20A series UFLC (Shimadzu Corporation). Detection was carried out in a PDA, using 215 nm and 245 as preferred wavelengths. The organic acids were quantified by 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.

2.7. Phenolic compounds. Phenolic compounds were determined by HPLC (Hewlett-Packard 1100, Agilent Technologies, Santa Clara, USA) as previously described by the authors (Barros, Dueñas, Ferreira, Baptista, & Santos-Buelga, 2009). Double online detection was carried out in the diode array detector (DAD) using 280 nm and 370 nm as preferred wavelengths and in a mass spectrometer (API 3200 Qtrap, Applied Biosystems, Darmstadt, Germany) connected to the HPLC system via the DAD cell outlet. The phenolic compounds were characterized according to their UV and mass spectra and retention times, and comparison with authentic standards when available. For quantitative analysis, calibration curves were prepared from different standard compounds. The results were expressed in g per 100 g of dry weight.

2.8. Tocopherols

Tocopherols were determined following a procedure previously optimized and described by the authors (Reis, Ferreira, Barros, & Martins, 2011). Analysis was performed by HPLC (equipment described in section 2.5.), 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 mg per 100 g of dry weight.

2.9. Antioxidant activity

2.9.1. General.

The lyophilized samples (~1 g) was stirred with methanol (40 ml) at 25 ºC at 150 rpm for 1 h and filtered through Whatman No. 4 paper. The residue was then extracted with an additional portion of methanol. The combined methanolic extracts were evaporated under reduced pressure (rotary evaporator Büchi R-210; Flawil, Switzerland), re-dissolved in methanol at 20 mg/ml (stock solution), and stored at 4 ºC for further use. Successive dilutions were made from the stock solution and submitted to *in vitro* assays already described by the authors (Pereira et al., 2012; Leal at al., 2013) to evaluate the antioxidant activity of the samples. The sample concentrations 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 (reducing power assay) against sample concentrations. Trolox was used as positive control.

2.9.2. Folin-Ciocalteu assay.

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 40°C for colour development. Absorbance was then measured at 765 nm (Analytikjena spectrophotometer; Jena, Germany). Gallic acid was used to obtain the standard curve (0.0094 – 0.15 mg/ml), and the reduction of *Folin-Ciocalteu* reagent by the samples was expressed as mg of gallic acid equivalents (GAE) per g of extract.

2.9.3. 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 50ºC 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, as also deionised 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, United States). The reducing power was obtained directly from the absorbances.

2.9.4. DPPH scavenging activity assay.

This methodology was performed using the Microplate Reader mentioned above. The reaction mixture on 96 wells plate consisted of a solution by 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 discolouration using the equation: % RSA = $[(A_{DPPH}-A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution containing the sample, and A_{DPPH} is the absorbance of the DPPH solution.

2.9.5. β-carotene/linoleate assay.

A solution of β-carotene was prepared by dissolving β-carotene (2 mg) in chloroform (10 ml). Two millilitres of this solution were pipetted into a round-bottom flask. The chloroform was removed at 40ºC 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 50ºC 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: (β-carotene content after 2h of assay/initial β-carotene content) × 100.

2.9.6. TBARS assay.

Porcine (*Sus scrofa*) brains were obtained from official slaughtering animals, dissected, and homogenized with a Polytron in 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 samples solutions (200 μL) in the presence of $FeSO_4$ (10 mM; 100 μL) and ascorbic acid (0.1 mM; 100 μL) at 37ºC for 1 h. The reaction was stopped by the addition of trichloro acetic acid (28% w/v, 500 μL), followed by thiobarbituric acid

(TBA,2%, w/v, 380 μL), and the mixture was then heated at 80ºC for 20 min. After centrifugation at 3000*g* for 10 min to remove the precipitated protein, the colour 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.

2.10. Statistical analysis

For each one of the specimens, 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 analyzed 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.

3. Results and Discussion

3.1. Proximate composition and fatty acids in Lepista nuda fruiting bodies

The results of the proximate composition of *Lepista nuda* fruiting bodies obtained from different habitats are shown in **Table 1**. The commercial sample (cultivated) gave the highest levels of moisture (91 g/100 g fw), proteins (12 g/100 g dw), carbohydrates (77 g/100 g dw) and energetic value (367 kcal/100 g dw). Otherwise, the wild samples from pine and oak forest showed the highest contents in ash (12 g/100 g dw) and fat (2 g/100 g dw), respectively. Carbohydrates were the most abundant macronutrients in all the samples, while fat were the less abundant one. Nevertheless, commercial fruiting body gave higher levels of proteins than ash, while the opposite was observed in both wild

samples. This might be due to the soil composition in which mushrooms have grown (Nikkarinen & Mertanen, 2004), with wild species absorbing higher levels of minerals.

The energetic value obtained for wild sample obtained in pine forest was similar to another sample from the same habitat (334 kcal/100 g dw) as reported previously by Barros et al. (2008), but lower than the value reported by Ouzoni et al. (2009) for a wild sample from Greece (392 kcal/100 g dw; calculated from data available).

The results of the main fatty acids found in the studied samples, as also their saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) percentages are also shown in **Table 1**. Up to twenty five fatty acids were detected in most of the samples (**Figure 1**). The major fatty acid found was linoleic acid (C18:2n6), which is in agreement with other reports (Noel-Suberville et al., 1996; Barros et al., 2008). Oleic acid (C18:1n9) and palmitic acid (C16:0) were also major fatty acids in different order depending on the sample. The commercial sample gave the highest PUFA levels (64%) due to the contribution of linoleic acid, wild sample from oak forest showed the highest MUFA content (37%) due to the higher levels of oleic acid, and wild sample from pine forest showed the highest SFA levels (34%) due to the higher contents in palmitic and stearic acids.

3.2. Chemical composition in Lepista nuda fruiting bodies and mycelia

Glucose was the main sugar in all mycelia samples, while trehalose predominated in fruiting bodies (**Table 2**). Mannitol was found in commercial fruiting body and in its mycelia grown in cMMN and PDA culture media, while fructose appeared only in the commercial and wild oak forest fruiting bodies. The commercial fruiting bodies gave the highest levels of fructose and mannitol, but the highest levels of glucose, trehalose and total sugars were found in mycelia cultured in PACH (21 g/100 g), iMMN (5 g/100

g dw) and PACH (24 g/100 g dw), respectively. The profile of commercial mushroom and mycelium cMMN samples can be observed in **Figure 2**A.

Among organic acids, it was possible to quantify citric, fumaric, malic, oxalic and quinic acids in all the fruiting body samples (commercial or wild) (**Table 2**). The wild sample obtained in oak forest gave the highest levels of the last three mentioned compounds, as also of total organic acids (10 g/100 g dw). Most of the compounds disappeared in the mycelia samples obtained from different culture media; only oxalic acid remained but in lower amounts. The profile of commercial mushroom and mycelium cMMN samples can be observed in **Figure 2B**. Organic acids are involved in various fundamental metabolism pathways of carbohydrates, lipids and proteins as intermediate or end products, namely in Krebs cycle which is the central energy-yielding cycle of the cell (Voet, Voet, & Pratt, 2008). Therefore, it is logical that mycelia obtained under *in vitro* conditions consume these compounds along the growth.

p-Hydroxybenzoic acid and a related phenolic compound (cinnamic acid) were found in the studied samples (**Table 3**). Commercial mushroom revealed the highest content in *p*-hydroxybenzoic and cinnamic acids (0.29 and 0.08 mg/100 g dw, respectively). Among mycelia, the one grown in cMMN revealed the highest concentration of both compounds. Our research group reported previously the presence of other phenolic compounds, such as protocatechuic and *p*-coumaric acids, and the absence of cinnamic acid in *L. nuda* samples also collected in Bragança (Northeast Portugal) but in 2007 (Barros et al., 2009).

Regarding tocopherols, β- and γ-tocopherols were found in all the samples (**Figure 2C**), while α-tocopherol was only found in wild fruiting bodies from oak forest and mycelium grown in cMMN culture medium, but in very low amounts (**Table 2**). The α-tocopherol was also the less abundant isoform in wild sample from pine forest

previously studied (Barros et al., 2008), and δ-tocopherol was also absent in other *Lepista nuda* samples studied by different authors (Elmastas et al., 2007; Barros et al., 2008). PDA, PACH and FAD showed to be the best culture media for tocopherols production (6-7 mg/100 g dw). In a previous work we also observed an increase in γ-tocopherol levels in ectomycorrhizal fungi mycelia produced *in vitro* when compared to their fruiting bodies (Reis et al., 2011), and herein the same was observed also for the β-isoform.

3.3. Antioxidant activity of Lepista nuda fruiting bodies and mycelia

To study the antioxidant activity of the samples, five methodologies were performed: *Folin-Ciocalteu* and Ferricyanide/Prussian blue assays that evaluate reducing power, DPPH assay to evaluate free radicals scavenging activity and β-carotene/linoleate and TBARS assays to evaluate lipid peroxidation inhibition. The results are presented in **Table 3** and show that iMMN culture media is the most indicate to increase *Lepista nuda* antioxidant activity. Mycelium grown in this medium gave the highest antioxidant activity in all the assays: the highest values in *Folin-Ciocalteu* assay, expressed in gallic acid equivalents, and the lowest EC_{50} values in the other assays, that means highest reducing power or antioxidant potential.

The wild samples studied herein gave lower DPPH scavenging activity and β-carotene bleaching inhibition, but higher reducing power and TBARS inhibition than the ones reported in other works (Elmastas et al., 2007; Barros et al., 2008).

Overall, commercial sample of *Lepista nuda* fruiting bodies gave the highest levels of energy, PUFA (due to the contribution of linoleic acid) and phenolic compounds; the wild sample from oak forest gave the highest levels of organic acids. Mycelia samples

showed higher levels of glucose, tocopherols and antioxidant activity. Particularly, PACH medium proved to be better for glucose production, PDA, PACH and FAD for β- and γ-tocopherols, cMMN for phenolic compounds and iMMN for antioxidant properties. This study proves that: i) the chemical composition of fruiting bodies is highly dependent on the habitat in which they grow; ii) cultivated samples reveal increased levels of macronutrients but also secondary metabolites such as phenolic compounds; iii) the levels of organic acids drastically decrease in mycelia samples, while tocopherols increase.

In vitro culture could be explored to obtain bioactive compounds from macrofungi for industrial applications, controlling environmental conditions to produce higher amounts of these compounds and to overcome the diversity in chemical composition observed in samples collected in different habitats.

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Table 1. Proximate composition and main fatty acids in *Lepista nuda* fruiting bodies from different habitats.

	Commercial	Wild Pine forest	Wild Oak forest
Moisture (g/100 g fw)	90.61± 0.96	85.52± 1.11	89.06± 0.45
Ash (g/100 g dw)	9.56 ± 0.06^{c}	26.15 ± 2.42^{a}	13.20 ± 0.85^{b}
Proteins (g/100 g dw)	12.18 ± 0.22^{a}	8.11 ± 0.58^{b}	11.59 ± 0.61^{a}
Fat (g/100 g dw)	1.14 ± 0.00^{b}	1.27 ± 0.05^{b}	2.04 ± 0.21^{a}
Carbohydrates (g/100 g dw)	77.12 ± 0.20^{a}	64.47 ± 1.54^{c}	73.17 ± 1.17^{b}
Energy(kcal/100 g dw)	367.46 ± 0.16^{a}	334.32 ± 62.30^{c}	357.40 ± 1.65^{b}
C16:0 (relative percentage)	14.86 ± 0.12^{c}	26.97 ± 0.78^{a}	20.70 ± 0.39^{b}
C18:0 (relative percentage)	0.88 ± 0.06^{c}	2.77 ± 0.08^{a}	1.55 ± 0.26^{b}
C18:1n9c (relative percentage)	16.05 ± 0.40^{c}	25.02 ± 1.20^{b}	35.74 ± 1.02^{a}
C18:2n6c (relative percentage)	63.88 ± 0.44^{a}	37.63 ± 0.19^{b}	37.71 ± 1.20^{b}
SFA (relative percentage)	18.90 ± 0.16^{c}	34.43 ± 1.05^{a}	24.80 ± 0.01^{b}
MUFA (relative percentage)	16.69 ± 0.27^{c}	27.21 ± 1.22^{b}	36.74 ± 1.07^{a}
PUFA (relative percentage)	64.41 ± 0.43^{a}	38.36 ± 0.17^{b}	38.46 ± 1.08^{b}

In each row different letters mean significant differences between samples ($p<0.05$). Palmitic acid (C16:0); Stearic acid (C18:0); Oleic acid (C18:1n9c); Linoleic acid (C18:2n6c). Twenty one more fatty acids were also identified and quantified (data not shown).

SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids.

Table 2. Sugars, organic acids, phenolic compounds and tocopherols in *Lepista nuda* fruiting bodies from different habitats and in mycelia obtained in different *in vitro* culture media.

	Commercial sample of fruiting body and *in vitro* cultured mycelia						Wild samples of fruiting body	
	Fruiting body	cMMN	iMMN	PDA	PACH	FAD	Pine forest	Oak forest
Fructose	0.17 ± 0.01^{a}	nd	nd	nd	nd	nd	nd	0.13 ± 0.02^{b}
Glucose	nd	6.12 ± 0.17^{c}	6.94 ± 0.13^{c}	3.73 ± 0.44^{d}	21.15 ± 0.54^{a}	11.88 ± 0.59^{b}	1.18 ± 0.02^{e}	1.12 ± 0.02^{e}
Manittol	1.63 ± 0.02^{a}	0.27 ± 0.01^{c}	nd	0.45 ± 0.19^{b}	nd	nd	nd	nd
Trehalose	1.85 ± 0.00^{f}	4.79 ± 0.10^{b}	5.35 ± 0.09^{a}	3.02 ± 0.30^{d}	3.17 ± 0.05^{d}	2.25 ± 0.03^{e}	3.97 ± 0.11^{c}	2.25 ± 0.02^{e}
Total (g/100 g dw)	3.65 ± 0.01^{d}	13.60 ± 0.16^{b}	12.30 ± 0.04^{cb}	11.20 ± 2.03^{c}	24.32 ± 0.59^{a}	14.13 ± 0.62^{b}	5.16 ± 0.09^{d}	3.50 ± 0.05^{d}
Citric acid	0.57 ± 0.04^{a}	nd	nd	nd	nd	tr	0.25 ± 0.01^{c}	0.35 ± 0.04^{b}
Fumaric acid	0.02 ± 0.00^{c}	tr	tr	tr	tr	tr	0.07 ± 0.00^{a}	0.06 ± 0.00^{b}
Malic acid	1.75 ± 0.19^{a}	nd	nd	nd	nd	tr	1.39 ± 0.05^{b}	1.79 ± 0.02^{a}
Oxalic acid	2.41 ± 0.31^{c}	0.88 ± 0.06^{d}	1.02 ± 0.04^{d}	0.05 ± 0.01^{e}	0.07 ± 0.00^{e}	tr	3.09 ± 0.02^{b}	3.49 ± 0.00^{a}
Quinic acid	3.08 ± 0.18^{b}	nd	nd	nd	nd	tr	2.43 ± 0.01^{c}	3.89 ± 0.00^{a}
Total (g/100 g dw)	7.83 ± 0.11^{b}	0.88 ± 0.06^{d}	1.02 ± 0.04^{d}	0.05 ± 0.01^{e}	0.07 ± 0.00^{e}	tr	7.23 ± 0.01^{c}	9.59 ± 0.28^{a}
p-Hydroxybenzoic acid	0.29 ± 0.03^{a}	0.13 ± 0.01^{cb}	0.04 ± 0.00^{d}	tr	tr	nd	0.10 ± 0.01^{c}	0.15 ± 0.02^{b}
Cinnamic acid (mg/100 g dw)	0.08 ± 0.01^{a}	0.01 ± 0.00^{b}	nd	nd	nd	nd	tr	0.01 ± 0.00^{b}
α-Tocopherol	tr	0.03 ± 0.00^{a}	nd	nd	nd	nd	tr	0.01 ± 0.00^{b}
β-Tocopherol	0.01 ± 0.00^{b}	0.26 ± 0.00^{b}	0.05 ± 0.00^{b}	6.41 ± 0.22^{a}	6.32 ± 0.22^{a}	6.40 ± 0.22^{a}	0.02 ± 0.00^{b}	0.07 ± 0.00^{b}
γ-Tocopherol	0.03 ± 0.01^{d}	0.08 ± 0.02^{cb}	0.11 ± 0.00^{b}	0.20 ± 0.02^{a}	0.06 ± 0.00^{cd}	0.20 ± 0.02^{a}	0.04 ± 0.00^{de}	0.05 ± 0.00^{cde}
Total (mg/100 g dw)	0.03 ± 0.02^{b}	0.37 ± 0.02^{b}	0.15 ± 0.01^{b}	6.61 ± 0.21^{a}	6.38 ± 0.23^{a}	6.38 ± 0.23^{a}	0.15 ± 0.01^{b}	0.38 ± 0.02^{b}

In each row different letters mean significant differences between samples ($p<0.05$). nd-not detected; tr- traces.

Table 3. Antioxidant activity *Lepista nuda* fruiting bodies from different habitats and in mycelia obtained in different *in vitro* culture media.

		Commercial sample of fruiting body and *in vitro* cultured mycelia						Wild samples of fruiting body	
		Fruiting body	cMMN	iMMN	PDA	PACH	FAD	Pine forest	Oak forest
Reducing power	Folin-Ciocalteu assay (mg GAE/g extract)	20.54 ± 0.31^{d}	30.27 ± 3.30^{b}	33.57 ± 2.25^{a}	17.01 ± 1.01^{e}	24.30 ± 1.39^{c}	9.19 ± 1.06^{f}	15.98 ± 1.23^{e}	16.57 ± 0.44^{e}
	Ferricyanide/Prussian blue assay (EC_{50}; mg/ml)	1.50 ± 0.01^{c}	0.53 ± 0.03^{f}	0.51 ± 0.00^{f}	1.52 ± 0.04^{c}	1.02 ± 0.02^{e}	1.95 ± 0.02^{b}	2.08 ± 0.00^{a}	1.44 ± 0.02^{d}
Scavenging activity	DPPH scavenging activity assay (EC_{50}; mg/ml)	8.73 ± 0.48^{cb}	5.72 ± 0.49^{cb}	3.67 ± 0.05^{c}	6.78 ± 0.07^{cb}	9.55 ± 0.95^{cb}	102.75 ± 23.03^{a}	16.05 ± 0.18^{b}	15.48 ± 0.23^{cb}
Lipid peroxidation inhibition	β-carotene/linoleate assay (EC_{50}; mg/ml)	9.48 ± 1.02^{d}	16.67 ± 0.98^{ba}	8.02 ± 1.35^{e}	10.15 ± 0.89^{d}	15.50 ± 0.43^{b}	17.50 ± 0.70^{a}	12.24 ± 0.57^{c}	11.68 ± 0.61^{c}
	TBARS assay (EC_{50}; mg/ml)	1.75 ± 0.16^{bc}	3.52 ± 0.31^{b}	0.87 ± 0.04^{c}	3.35 ± 0.66^{b}	2.21 ± 0.46^{bc}	5.44 ± 0.43^{ba}	3.76 ± 0.53^{b}	8.17 ± 1.75^{a}

In each row different letters mean significant differences between samples ($p<0.05$).
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.
EC_{50} values for trolox (positive control): 0.03, 0.04, 0.003 and 0.004 mg/ml for Ferricyanide/Prussian blue, DPPH scavenging activity, β-carotene/linoleate and TBARS assays, respectively.

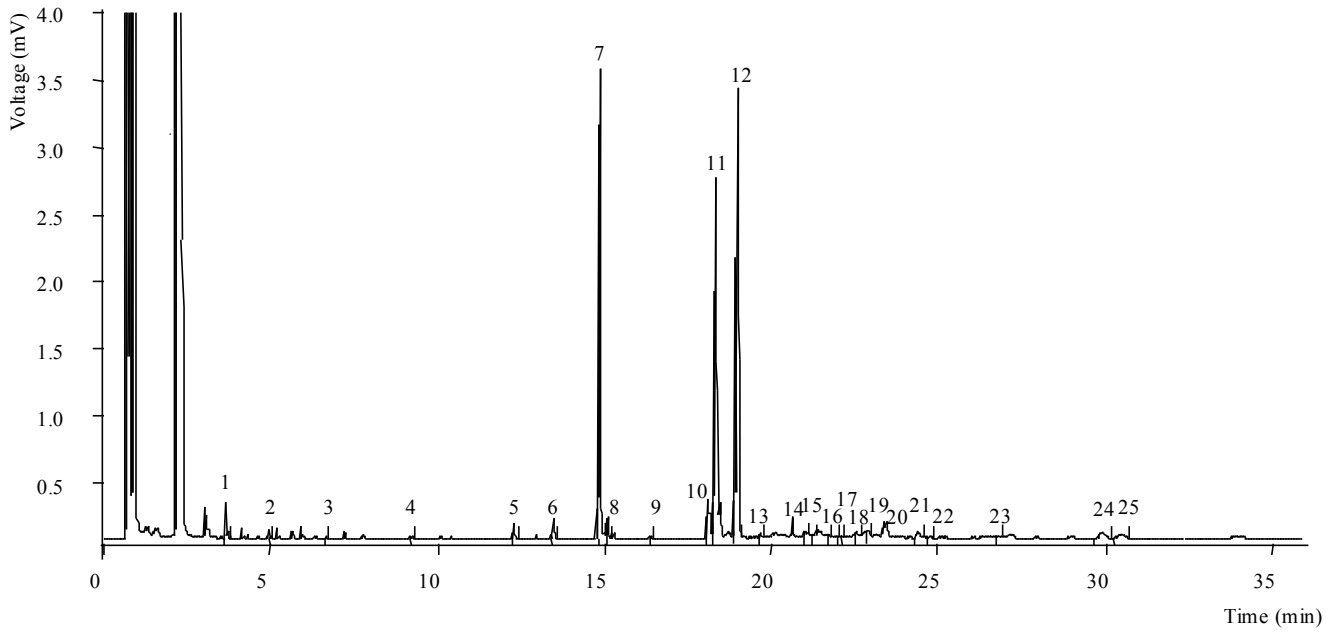

Figure 1. Fatty acid profile of *Lepista nuda* fruiting body wild oak forest. 1-Caproic acid (C6:0); 2-Caprylic acid (C8:0); 3-Capric acid (C10:0); 4-Lauric acid (C12:0); 5-Myristic acid (C14:0); 6-Pentadecanoic acid (C15:0); 7-Palmitic acid (C16:0); 8-Palmitoleic acid (C16:1); 9-Heptadecanoic acid (C17:0); 10-Stearic acid (C18:0); 11-Oleic acid (C18:1n9c); 12-Linoleic acid (C18:2n6c); 13-α-Linolenic acid (C18:3n3); 14-Arachidic acid (C20:0); 15-cis-11-Eicosenoic acid (C20:1c); 16-cis-11,14-Eicosadienoic acid (C20:2c); 17- cis-11,14,17-Eicosatrienoic acid and Heneicosanoic acid (C20:3n3+C21:0); 18- Arachidonic acid (C20:4n6); 19- cis-5,8,11,14,17-Eicosapentaenoic acid (C20:5n3); 20-Behenic acid (C22:0); 21- Erucic acid (C22:1n9); 22- cis- 13,16- Docosadianoic acid (C22:2) 23-Tricosanoic acid (C23:0); 24-Lignoceric acid (C24:0); 25-Nervonic acid (C24:1).

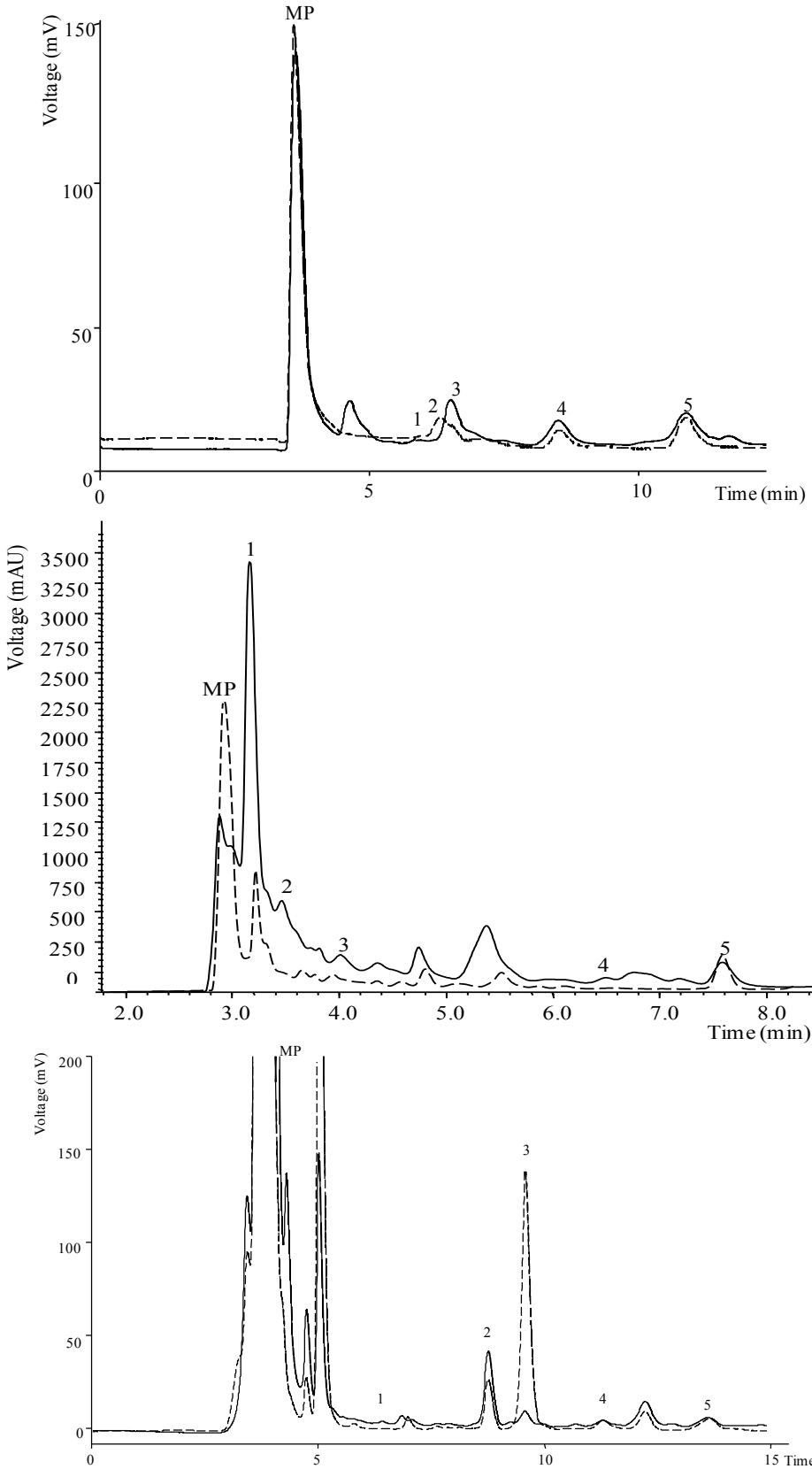

Figure 2. (A) Sugars profile of (——) commercial sample and (----) cMMN mycelium: MP- mobile phase; 1- fructose; 2- glucose; 3-mannitol; 4- trehalose; 5-raffinose (IS); **(B)** Organic acids profile of (——) commercial sample ---- and cMMN mycelium: MP- mobile phase; 1- oxalic acid; 2-quinic acid; 3- malic acid; 4- citric acid; 5- fumaric acid; **(C)** Tocopherols profile of (——) oak forest sample and (----) FAD mycelium sample: MP- mobile phase; 1- α-tocopherol; 2-BHT; 3- β-tocopherol; 4- γ-tocopherol; 5-tocol (IS).