Research Article

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Pioppino mushroom in southern Italy: an undervalued source of nutrients and bioactive compounds

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Abstract

BACKGROUND: *Agrocybe aegerita* (V. Brig.) Singer, commonly known as Pioppino, is a popular edible mushroom, known in the Campania Region (Italy). Despite its habitual consumption, little nutritional and biochemical information is available. Thus, nutritional values, anti-radical properties and chemical composition of the wild Pioppino were compared to those of the cultivated *Agaricus bisporus* (J.E. Lange) Imbach (known as Champignon), equally analysed.

RESULTS: Macronutrient components (proteins, carbohydrates and lipids), free and protein amino acids and fatty acid content of poplar mushroom were achieved. Total phenol content of a defatted Pioppino alcoholic extract (PM) was determined, whereas DPPH and ABTS methods were applied to determine the radical scavenging capabilities of the extract. Ferricyanide and ORAC-fluorescein methods were also performed. Finally, LC-HRMS was used to identify and quantify the main metabolites in the extract. PM was mainly constituted of disaccharides, hexitol derivatives and malic acid. Coumaric acid isomers and C_6C_1 compounds were also detected.

CONCLUSION: All data revealed that wild Pioppino is an excellent functional food, by far exceeding that of the Champignon. Therefore, these data are useful to promote the consumption of this mushroom encouraging thus its biological cultivation, due to wild availability is strongly compromised by the extensive use of fungicides.

Keywords: *Agrocybe aegerita* (V. Brig.) Singer; amino acid content; nutritional value; anti-radical properties; metabolic profiling

INTRODUCTION

Fungi are unicellular or multicellular eukaryotic organisms, able to digest organic matter for life cycle, thus being heterotrophs as animals and protozoans.[1] The investigation on their peculiar ecological and biological features has been accompanied by the discovery of bioactive metabolites, whose presence in fungi could explain their use in traditional medicine.[2] Nowadays, growing evidence suggests that fungi constituents exert beneficial effects for health[3] and for the treatment of several human disorders, including cancer.[4]

In this context, edible mushrooms, both cultivated and wild, have gained great interest as source of nutrients and nutraceutical substances. Mushroom-induced human health promoting properties, such as the reported antimicrobial,[5] immunomodulatory,[6] anticoagulant[7] and antioxidant activities,[8] could be related to their abundance in carbohydrates, proteins with high content in essential amino acids, fatty acids, vitamins, fibres and minerals[9] and to the synergistic effects between nutrient and non-nutrient compounds.[10]

Basidiomycetes and Ascomycetes are the main mushrooms groups producing sporophores (fruiting bodies). The first ones produce their microscopic spores externally on the end of specialised cells called basidia, whereas Ascomycetes produce their spores internally, inside a sac called ascus.[11] Mostly, higher Basidiomycetes and some Ascomycetes are used in the form of extracts or powder for prevention, alleviation or healing of diseases, and/or in providing a balanced healthy diet, and are known as medicinal mushrooms but their use as drugs in Western countries is severely limited, partly due to their complex structure and lack of acceptable pharmacological purity.[12] Indeed, higher Basidiomycetes mushrooms, used in folk medicine throughout the world since ancient times, have been found to exert a broad array of beneficial activities and to markedly inhibit tumour cells growth.[13]

Agrocybe aegerita (V. Brig.) Singer, also known as black poplar or Pioppino mushroom (hereafter Pioppino), is an edible

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Basidiomycete belonging to the order Agaricales and to the genus *Agrocybe*. It is one of the constituents in the popular gourmet mix produced by mushroom cultivators and known for its anti-fungal and antitumour properties. Previous studies of the fruiting body of this species have reported the presence of palmitic acid, linoleic acid, ergosterol, mannitol and trehalose.[14,15] The genus *Agrocybe* is also reported to contain several bioactive metabolites, such as indole derivatives with free radical-scavenging ability,[16,17] polysaccharides with hypoglycaemic activity[16,18] and agrocybin, a peptide with anti-fungal activity.[19] Previous studies[20] demonstrated that the MeOH extract of Pioppino shows strong anti-inflammatory and antioxidant activities. Further analyses by the same authors were performed in order to investigate bioactive metabolites therein, allowing them to isolate ceramide that inhibits cyclooxygenase enzymes. Indeed, the chemical composition and nutritional value of Pioppino, a fairly adaptable saprophyte species that lends itself very well to the artificial cultivation or on broad-leaved tree, such as poplar and sambucus[21] has been poorly investigated.

Nevertheless, Pioppino is scientifically recognised as an important source of bioactive secondary metabolites[22,23] on the basis of which its health-beneficial qualities[20,24,25] were acclaimed, and of enzymes, which were proved to be promising biocatalysts in biotechnological applications.[26,27] Furthermore, the total phenol content of a Pioppino methanolic extract and its sub-fractions, as well as their antioxidant activity, were previously screened,[28] while it was reported that Pioppino was a rich source of carbohydrates, ash, proteins, as well as of tocopherol isoforms.[29] Recently, Pioppino as a functional food was also subjected to transcriptome and proteome analyses, for its anti-tumour, anti-oxidant, anti-fungal, hypocholesterolaemic and hypolipidaemic effects.[30] Furthermore, for the first time in this basidiomycete was found the first ribotoxin showing a high cytotoxicity and cell death promoting effects towards CNS model cell lines.[31]

Herein Pioppino was taken into consideration, in the course of a screening programme of edible wild species of the Campania Region (southern Italy). The wild species, collected on poplar stumps and highly appreciated for its taste and flavour by local consumers, was screened for its bio-chemical composition, nutritional values and antioxidant capability. Data obtained were compared to those from *Agaricus bisporus* (J.E. Lange) Imbach (hereafter Champignon), one of the most commonly and widely consumed mushrooms in the world. In order to obtain a comprehensive evaluation of metabolic profile of both the mushrooms, LC-HRMS-based analyses were also performed on their alcoholic extracts.

MATERIALS AND METHODS

Chemicals and reagents

Folin–Ciocalteu reagent, gallic acid, 2,2′-azo-bis(2-amidinopropane)-dihydrochloride (AAPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), *nor*-leucine (*nor*-Leu), fluorescein, salts and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®) were purchased from Sigma–Aldrich (Milan, Italy). Organic solvents were from Sigma–Aldrich. Chemicals and solvents for the Kjeldahl method were from Carlo Erba Reagents (Milan, Italy), whereas those for automated amino acid analysis were provided from Biochrom (Cambridge, UK). Fatty acid methyl esters (Supelco™ 37 component FAME mix) were obtained from Supelco (Park-Bellefonte, PA, USA).

Mushroom species

Three different samples (~0.5 kg) of mushrooms were collected. In particular, *A. aegerita* was collected on poplar stumps in rural Aversa sites (Aversa, Caserta, Campania Region in southern Italy), whereas *A. bisporus* was purchased in a local supermarket (Caserta, Italy). Edible parts (pileus, hymenium and stipe) of both mushrooms were freeze-dried using an Advantage LS 85 instrument (SP Scientific, Stone Ridge, NY, USA) reduced to fine dried powder and stored, protected from light and humidity, until further analyses.

Ash and moisture content

Ash content and moisture levels were determined according to the AOAC official method.[32]

Macronutrient content

Total protein content

Nitrogen concentration was obtained by the Kjeldahl method[32] and total protein content was estimated using a nitrogen factor of 4.38.[33] Triplicate aliquots (~0.2 g each) of freeze-dried powder of each mushroom were analysed using a Mineral Six digester and an Auto-Disteam semi-automatic distilling unit (International PBI, Milan, Italy).

Total lipid content

Triplicate aliquots of the investigated mushrooms (4.0 g) were extracted for 4 h through a Soxhlet apparatus using $CHCl_3$ as extracting solvent. The solutions obtained were dried using a rotary evaporator in order to provide the crude fat extracts. The lipid content was determined gravimetrically.

Total carbohydrate content

Carbohydrates were estimated by subtracting (moisture + crude protein + crude fat + ash) from 100.[34]

Free sugars, ascorbic acid, and cation content

For the analysis of fructose and glucose composition, three aliquots of about 40 mg of freeze-dried mushroom were dissolved with cold ethanol. Thus, the supernatants obtained after centrifugation were assayed by using an enzymatic method, as previously reported.[35] Oxidised and reduced forms of ascorbate content were determined, as previously reported.[36] Briefly, ascorbate was measured spectrometrically. This assay measures the A_{265nm} that is specifically removable by ascorbate oxidase (AO), which converts reduced ascorbate (ASC) to non-absorbing oxidised forms. ASC is measured without pre-treatment of extracts; ASC and the relatively stable oxidised form, dehydroascorbate (DHA), are measured together as 'total ascorbate' after conversion of DHA to ASC by incubation with thiols such as dithiothreitol (DTT). The analysis of the cation composition (ammonium, calcium, magnesium, potassium and sodium) was obtained by using a Bio-LC® (Dionex Corp., Sunnyvale, CA, USA) equipped with a IonPac® CS12A column (2 × 250 mm; Dionex Milan, Italy). Protocols were employed as suggested by the manufacturer (Application Brief 117 by Dionex Corp.).

Amino acid composition

For the analysis of free amino acid composition, three aliquots of about 100 mg of freeze-dried mushrooms were precipitated with 80% cold ethanol (2.0 mL) in the presence of *nor*-Leu (200.0 nmol)

as internal standard, homogenised with a Teflon pestle and centrifuged at 18 000 × *g* at 4 °C. The supernatants were lyophilised, treated with 3% sulfosalicylic acid (500 µL) to precipitate any protein fraction still present, and centrifuged again.[37] Thus, the supernatants obtained were analysed. For the analysis of total (free and protein) amino acids, about 10 mg of freeze-dried mushrooms were hydrolysed with 0.5 mL of 6 mol L^{-1} HCl containing 0.05% thioglycolic acid and *nor*-Leu as an internal standard at 110 °C for 20 h.[38] Following hydrolysis, HCl was removed under vacuum and samples were re-suspended in 0.5 mL of 0.2 mol L^{-1} lithium citrate buffer (pH 2.2). Aliquots of hydrolysed and non-hydrolysed samples were directly analysed on a Biochrom 30 amino acid analyser (Biochrom, Cambridge, UK), equipped with a post-column ninhydrin derivatisation system.

Gas chromatographic analysis of fatty acid methyl esters

The analyses of fatty acid methyl ester contents were performed as previously reported. [39] Briefly, each $CHCl_3$ crude extract (1.0 mg; see the 'Total lipid content' section) was dissolved in 2.0 mL of 2 mol L^{-1} KOH in MeOH. The solution was stirred for 30 min at room temperature. Then, heptane (0.8 mL) was added, and the solution was vortexed and centrifuged at 4200 × *g* for 10 min. An aliquot of the organic upper phase (1.0 µL) was analysed by GC (100 m × 0.25 mm i.d., 0.2 µm SP2380, fused silica capillary column; Supelco, Sigma–Aldrich). The fatty acid methyl esters were identified by comparing their retention times with those of the standard fatty acid methyl esters (Grain Fatty Acid Methyl Ester mix 47801, Supelco).

Evaluation of the antioxidant capability of mushrooms

The total phenol content (TPC) and antioxidant capability of Champignon (CM) and Pioppino (PM) mushrooms were measured on methanol extracts previously obtained through ultrasound-assisted maceration. After a first extraction with hexane, three replicate samples (2.0 g each) of each mushroom underwent ultrasound-assisted extraction using an ultrasonic bath (Branson M3800, Carouge, Switzerland) at 40 kHz frequency. The defatted materials were re-extracted using pure methanol. Four sonication cycles were performed (30 min each) in order to achieve the maximum recovery of the mushrooms' metabolic content. At the end of each sonication cycle, samples were centrifuged at 2044 × *g* for 10 min in a Beckman GS-15R centrifuge (Beckman Coulter, Milan, Italy) fitted with the S4180 rotor. The obtained supernatants were dried under vacuum using a rotary evaporator (Heidolph Hei-VAP Advantage, Saffron Walden, UK) to yield crude extracts, which were stored at −20 °C until use.

Folin–Ciocalteu, ABTS, DPPH, and ORAC assays were carried out performing three replicate measurements for three samples ($n = 3$) of each extract. Recorded activities were compared to a blank. Results are the means ± SD values. Student's *t*-test was applied in order to determine statistical significance (the significance level was set at $P < 0.05$).

Total phenol content determination

Total phenol content of CM and PM extracts was determined according to the Folin–Ciocalteu procedure on aliquots of extracts in methanol as previously reported.[40] Briefly, analysed samples (1.0 mg mL^{-1} in DMSO) were mixed with 0.500 mL of Folin–Ciocalteu reagent (FCR) and 4.0 mL of Na_2CO_3 (7.5% w/v). After stirring reaction mixture at room temperature for 3 h, the absorbance was read at 765 nm using a Shimadzu UV-1700 spectrophotometer (Shimadzu, Salerno, Italy).The content of total phenols (TPC value) of the samples was expressed as mg of gallic acid equivalents (GAE) per gram of seed flour.

Determination of ABTS radical cation scavenging capacity

The determination of $ABTS^{\bullet+}$ solution scavenging capacity was performed as previously reported.[41] The results were expressed in terms of TEAC values (µmol of Trolox® equivalents per gram of extract).

Determination of DPPH radical scavenging capacity

The determination of DPPH• (2,2-diphenyl-1-picrylhydrazyl) scavenging capability was performed as previously reported.[41] The results were expressed in terms of TEAC values (µmol of Trolox® equivalents per gram of extract).

Oxygen radical absorbance capacity assay

The ORAC assay was performed as follows: each extract (20 µL; 0.781, 1.56, 3.12, 6.25, 12.5 and 25.0 µg mL^{-1}, final concentration levels) and fluorescein (FL; 120.0 µL; 70 nmol L^{-1}, final concentration) were pre-incubated for 15 min at 37 °C in 75 mmol L^{-1} phosphate buffer (pH 7.4). Then 2,2′-azo-bis(2-amidinopropane)-dihydrochloride (AAPH) solution (60.0 µL; 36 mmol L^{-1}, final concentration) was rapidly added. In parallel with the test samples, a blank (FL + AAPH) and solutions of the reference antioxidant Trolox® (1–4 µmol L^{-1}, final concentration levels) were properly prepared in PBS. The fluorescence decay ($\lambda_{ex} = 485$ nm, $\lambda_{em} = 525$ nm) was recorded every minute for 120 min using a Tecan SpectraFluor fluorescence and absorbance reader. Antioxidant curves (fluorescence vs. time) were normalised to the curve of the blank. From the normalised curves, the area under the fluorescence decay curve (AUC) was calculated. Linear regression equations between net AUC ($AUC_{antioxidant} - AUC_{blank}$) and antioxidant concentration were calculated for all the samples. The antioxidant activity (ORAC value) was calculated by using the Trolox® calibration curve. The ORAC values were expressed as Trolox® equivalents (µmol L^{-1}).

LC and LC-MS/MS analyses

In order to provide a preliminary phytochemical overview of Pioppino and Champignon mushrooms, LC-ESI-MS/MS analyses were applied to their methanolic extracts. A Shimadzu NEXERA UHPLC system was used with an XTerra MS C18 column (100 × 4.6 mm; 3.5 µm particle size). Separation was achieved with a gradient of water (A) and methanol (B), both with 0.02% formic acid. Starting with 95% A, a linear gradient was followed to 80% A in 5 min, then decreasing to 25% A at 10 min, and to 10% A at 14 min, then returning to the starting conditions and allowing to re-equilibrate for 2 min. The total analysis time was 16 min, the flow rate was 0.5 mL min^{-1}, and the injection volume was 5.0 µL.

MS analysis was performed using the AB SCIEX TripleTOF 4600 system with a DuoSpray™ ion source operated in electrospray ionisation. Data were collected by information dependent acquisition (IDA) using a TOF-MS survey scan 50–1000 Da (100 ms) and three dependent TOF-MS/MS scans 20–800 Da (100 ms) using collision energy (CE) of 35 V with collision energy spread (CES) of ± 15 V. Each sample was analysed in negative ion mode to maximise coverage of the metabolome. Qualitative data processing were performed using PeakView® Software with XIC Manager and MultiQuant™ Software version 2.1.

Table 1. Nutritional data of Pioppino (*A. aegerita*) and Champignon (*A. bisporus*) mushrooms. Values are means (±SD) of triplicate analyses ($n = 3$) and are expressed in 1 kg fresh weight (FW) basis

Parameter	Pioppino (g kg^{-1})	Champignon (g kg^{-1})
Proteins	20.5 ± 0.6	13.4 ± 0.7
Lipids	5.1 ± 0.2	4.1 ± 0.4
Moisture	905 ± 1.4	925 ± 1.8
Ash	8.7 ± 0.2	8.7 ± 0.3
Total carbohydrates	60.70	48.80

Table 2. Glucose, fructose and ascorbic acid from Pioppino (*A. aegerita*) and Champignon (*A. bisporus*) mushrooms. Values are means (±SD) of triplicate analyses ($n = 3$) and are expressed in 1 kg fresh weight (FW) basis

Parameter	Pioppino (g kg^{-1})	Champignon (g kg^{-1})
Glucose	0.203 ± 0.034	0.135 ± 0.006
Fructose	0.114 ± 0.039	0.096 ± 0.013
Ascorbic acid	0.082 ± 0.013	0.008 ± 0.002
(dehydroascorbic acid)	(0.006 ± 0.0)	(0.003 ± 0.0)

RESULTS AND DISCUSSION

Edible mushrooms are sources of food, easily digestible, whose wide intake derives not only from their characteristic texture and flavour, but also from their chemical and nutritional features. Mushrooms are rich in proteins showing an important content of essential amino acids, and are low in fats and carbohydrates.[42] The presence of phenols and other bioactive secondary metabolites enhances the medicinal and nutraceutical value of edible mushrooms. In this context, in the course of a screening program on wild edible species from the Campania Region (Italy), *A. aegerita*, known as Pioppino, was of interest. They are also called 'delicious mushrooms', they commonly grow on old poplars, which in some regions of the southern Italy, and peculiarly in the Campania Region, represent a support for grapevines. This agronomic technique is known as 'vite maritata' (literally, married grapevine).[43]

Nutritional values

Nutritional values of Pioppino, as well as of those of Champignon are reported in Table 1. Pioppino showed a moisture content slightly lower than Champignon (about 900 vs. 920 g kg^{-1}), whereas its protein (20.5 g kg^{-1}) and lipid (5.1 g kg^{-1}) contents were higher than those exhibited by Champignon, about 1.5- and 1.2-fold, respectively. Ash content, total carbohydrates were also similar.

Evaluating both free fructose and glucose it was found that the investigated mushrooms differed only in their glucose content, which was about 1.5-fold higher for Pioppino (Table 2). Calculated glucose/fructose ratios were about 1.8 and 1.4 for Pioppino and Champignon, respectively. Ascorbic acid was about 10.3-fold higher in Pioppino than in Champignon (0.082 g and 0.008 g, respectively, per 1 kg of fresh product).

Considering the dietary significance of ammonium, calcium, magnesium, potassium and sodium cations, their content was also determined in both the mushrooms (Table 3). In particular, magnesium (required for many enzyme systems in human metabolism) was higher in Pioppino (about 2.1-fold) than in Champignon, whereas both mushrooms had similar content

Table 3. Cation content from Pioppino (*A. aegerita*) and Champignon (*A. bisporus*) mushrooms. Values are means (±SD) of triplicate analyses ($n = 3$) and are expressed in 1 kg fresh weight (FW) basis

Cation	Pioppino (g kg^{-1})	Champignon (g kg^{-1})
Ammonium	0.196 ± 0.010	0.223 ± 0.015
Calcium	0.098 ± 0.011	0.121 ± 0.023
Magnesium	0.563 ± 0.072	0.269 ± 0.048
Potassium	3.359 ± 0.239	3.287 ± 0.045
Sodium	0.004 ± 0.001	0.017 ± 0.001

of other cations determined. Muthu and Shanmugasundaram, studying the mineral composition of cultivated Pioppino, showed that they were good sources of nutritionally important mineral elements.[44] On the basis of their results, Na (0.29 g kg^{-1}) was present in higher amount than K (0.085 g kg^{-1}), whereas Italian wild Pioppino showed a precious content in K (3.359 g kg^{-1} of fresh product) suggesting that the intake of the Pioppino could help to maintain normal blood pressure levels.[45] Furthermore, potassium helps to maintain normal heart rhythm, fluid balance, muscle, and nerve function. K/Na ratio was 4.34-fold higher in wild Pioppino than in cultivated Champignon, although both the mushrooms equally appeared to be rich in potassium.

Amino acid content

The content of free and total (free plus protein) amino acids is reported in Table 4. Comparing the free amino acid content of Pioppino with that of Champignon, quali-quantitative differences were found. In fact, the free amino acid content was 0.69-fold lower in Pioppino that in Champignon (1.15 and 1.67 g, respectively, per kg of fresh product). Glutamic acid was the most abundant among free amino acids, 0.249 g kg^{-1} for Pioppino (~22% of the total free amino acid content) vs. 0.283 g kg^{-1} for Champignon (~17% of the total free amino acid content). The second most abundant free amino acid was alanine, 0.114 g kg^{-1} for Pioppino vs. 0.140 g kg^{-1} for Champignon, whereas asparagine was higher in Pioppino (0.114 g kg^{-1}) than in Champignon (0.066 g kg^{-1}). Taking into account only the protein amino acids (which can be calculated subtracting the free from the total amino acid content in Table 4), all of them were higher in Pioppino. Both samples showed the presence of non-protein amino acids. The total amount of them in Champignon was 2.2 times higher than in Pioppino (0.221 g kg^{-1} compared to 0.101 g kg^{-1}). In particular, γ-amino-*n*-butyric acid (GABA) and L-ornithine (Orn) were the most abundant in Champignon (0.051 and 0.057 g kg^{-1}, respectively). Instead, L-ornithine (Orn) and cystathionine (Cysth) were the most abundant in Pioppino (0.016 and 0.014 g kg^{-1}, respectively), whereas γ-amino-*n*-butyric acid (GABA) was present in a very small amount. The amount of each of the other non-protein amino acids did not exceed 0.03 g kg^{-1} of the fresh product. Finally, urea (0.045 g kg^{-1}) was 2.3-fold higher in Champignon than in Pioppino (0.020 g kg^{-1}), whereas taurine, previously detected in different edible mushrooms,[46] was 8.0-fold higher in Pioppino (0.024 g kg^{-1}) than in Champignon (0.003 g kg^{-1}).

Total amino acids content was about 1.8-fold in Pioppino than in Champignon (14.20 and 7.92 g kg^{-1}, respectively). Pioppino showed Glx (glutamic acid + glutamine) as the most abundant among total amino acids (2.230 g kg^{-1}, about 16% of total), followed by alanine (1.820 g kg^{-1}), Asx [(aspartic acid + asparagine)

Table 4. Free and total amino acid composition of Pioppino (*A. aegerita*) and Champignon (*A. bisporus*) mushrooms. Protein amino acids are reported in bold. Values are means (± SD) of triplicate analyses ($n = 3$) and are expressed in 1 kg fresh weight (FW) basis

	Pioppino		Champignon	
Amino acids[a]	Total amino acids (g kg^{-1})	Free amino acids (g kg^{-1})	Total amino acids (g kg^{-1})	Free amino acids (g kg^{-1})
Essential amino acids				
His	0.312 ± 0.046	0.018 ± 0.001	0.169 ± 0.017	0.021 ± 0.001
Ile	0.542 ± 0.038	0.021 ± 0.003	0.367 ± 0.042	0.079 ± 0.008
Leu	1.070 ± 0.088	0.040 ± 0.003	0.638 ± 0.022	0.113 ± 0.006
Lys	0.934 ± 0.088	0.020 ± 0.0	0.456 ± 0.005	0.031 ± 0.003
Met	0.246 ± 0.021	0.003 ± 0.0	0.113 ± 0.003	0.002 ± 0.0
Phe	0.673 ± 0.064	0.043 ± 0.003	0.113 ± 0.003	0.112 ± 0.002
Thr	0.829 ± 0.052	0.042 ± 0.004	0.563 ± 0.026	0.083 ± 0.006
Trp	ND	0.004 ± 0.0	ND	0.006 ± 0.001
Val	0.675 ± 0.043	0.040 ± 0.004	0.451 ± 0.039	0.086 ± 0.006
Non essential amino acids				
AAAA	–	0.010 ± 0.0	–	0.024 ± 0.001
Aaba	–	0.001 ± 0.0	–	0.013 ± 0.001
Ala	1.820 ± 0.134	0.114 ± 0.008	1.810 ± 0.015	0.140 ± 0.004
Arg	0.917 ± 0.090	0.061 ± 0.002	0.351 ± 0.013	0.010 ± 0.0
Asn	–	0.114 ± 0.008	–	0.066 ± 0.009
Asp	–	0.072 ± 0.001	–	0.062 ± 0.003
Asx	1.130 ± 0.104	–	0.489 ± 0.014	–
Baiba	–	–	–	0.001 ± 0.0
β-ala	–	0.003 ± 0.0	–	0.004 ± 0.0
Citr	–	0.002 ± 0.0	–	0.004 ± 0.0
Cys	0.057 ± 0.046	0.011 ± 0.001	0.023 ± 0.025	0.042 ± 0.004
Cysth	–	0.014 ± 0.001	–	0.027 ± 0.003
Ethan	–	0.007 ± 0.0	–	0.007 ± 0.001
GABA	–	0.005 ± 0.004	–	0.051 ± 0.006
Gln	–	0.055 ± 0.033	–	0.068 ± 0.003
Glu	–	0.249 ± 0.007	–	0.283 ± 0.010
Glx	2.230 ± 0.240	–	0.841 ± 0.023	–
Gly	0.697 ± 0.066	0.013 ± 0.0	0.386 ± 0.014	0.030 ± 0.004
Hylys	–	–	–	0.009 ± 0.002
Hypro	–	–	–	0.001 ± 0.0
Homocys	–	0.002 ± 0.0	–	0.001 ± 0.0
1-Mhis	–	0.001 ± 0.0	–	0.001 ± 0.0
3-Mhis	–	0.002 ± 0.001	–	0.001 ± 0.0
Orn	–	0.016 ± 0.002	–	0.057 ± 0.004
Pea	–	0.002 ± 0.0	–	0.002 ± 0.001
Phser	–	0.010 ± 0.001	–	0.014 ± 0.001
Pro	0.671 ± 0.090	0.023 ± 0.006	0.306 ± 0.067	0.056 ± 0.004
Sarc	–	0.002 ± 0.001	–	0.001 ± 0.0
Ser	0.904 ± 0.090	0.044 ± 0.002	0.512 ± 0.0	0.063 ± 0.005
Taur	–	0.024 ± 0.002	–	0.003 ± 0.0
Tyr	0.493 ± 0.046	0.039 ± 0.004	0.328 ± 0.010	0.047 ± 0.004
Urea		0.020 ± 0.003		0.045 ± 0.008
Total	14.20	1.15	7.92	1.67

[a] Free and protein amino acids. Three-letter code has been used.

1.130 g kg^{-1}], leucine (1.070 g kg^{-1}), lysine (0.934 g kg^{-1}), arginine (0.917 g kg^{-1}) and serine (0.904 g kg^{-1}), overall accounting for about by 63% of the total. Alanine was the most abundant among the total amino acids (1.810 g kg^{-1}) of Champignon, other abundant amino acids were, in decreasing order, Glx (glutamic acid + glutamine; 0.841 g kg^{-1}), leucine (0.638 g kg^{-1}), threonine (0.563 g kg^{-1}), serine (0.512 g kg^{-1}) and Asx (aspartic acid + asparagine; 0.489 g kg^{-1}), overall accounting for about 61% of the total. Then, as regards sulfur amino acids (methionine and cysteine), it did not exceed 2% of total amino acids for both the species.

In addition, the amount of essential amino acids [His, Ile, Leu, Lys, Met, Phe, Thr, Val; Trp (tryptophan was not included as it was not determined in the total hydrolysed samples; see Table 4)] was 5.3 g kg^{-1} in Pioppino vs. 2.9 g kg^{-1} in Champignon, representing about 37% of the total for both the species. These data show

Table 5. Fatty acid composition before extraction from total lipids of Pioppini (*A. aegerita*) and Champignon (*A. bisporus*) mushrooms. Values are means (±SD) of triplicate analyses ($n = 3$) and are expressed in 1 kg fresh weight (FW) basis

Fatty acid	Pioppino (g kg^{-1})	Champignon (g kg^{-1})	Types of omega (ω)
Saturated			–
Palmitic (C16:0)	0.107 ± 0.008	0.103 ± 0.0	–
stearic (C18:0)	0.046 ± 0.005	0.054 ± 0.012	–
Total saturated	0.153	0.157	
Unsaturated			
Palmitoleic (C16:1)	Traces	0.004 ± 0.002	*n*-7
Oleic (18:1)	0.019 ± 0.004	0.007 ± 0.0	*n*-9
Linoleic (C18:2)	0.618 ± 0.043	0.858 ± 0.203	*n*-6
γ-Linolenic (C18:3)	0.003 ± 0.001	0.243 ± 0.007	*n*-6
Arachidonic (C20:4)	0.009 ± 0.0	Traces	*n*-6
Eicosapentaenoic (C20:5)	0.010 ± 0.0	0.006 ± 0.002	*n*-3
Total unsaturated	0.659	1.118	–
Total fatty acids	0.81	1.28	–

that the amount of free essential amino acids were 0.23 g and 0.53 g kg^{-1} in Pioppino and Champignon, respectively.

Fatty acid composition analysis

GC-MS analysis showed that Pioppino and Champignon have relatively low fatty acid levels (Table 5). In particular, the fatty acid composition of Pioppino was richer in unsaturated acid content (0.659 g kg^{-1}; about 81% of total) than in those saturated (0.153 g kg^{-1}; 18.9%; Table 5), whereas in Champignon the unsaturated acid content was 87% (1.118 g kg^{-1}). The most abundant unsaturated acid in Pioppino was linolenic (C18:2; 0.618 g kg^{-1}) which represented 76% of total fatty acids; whereas traces of palmitoleic acid (C16:1) were detected. Instead, the most abundant unsaturated acids in Champignon were linolenic (C18:2; 0.858 g kg^{-1}) and γ-linoleic (C18:3; 0.243 g kg^{-1}), which represented 67% and 19% of total, respectively; traces of arachidonic acid were detected. Considering the saturated fatty acid detected no substantial variations have been found for both the mushrooms. The results of the fatty acids showed that the major fatty acid is linoleic acid (C18:2), contributing to the prevalence of polyunsaturated fatty acids (PUFA), while palmitic acid (C16:0) was the major of saturated fatty acids (SFA).

Antioxidant activity of methanolic extracts of the investigated mushrooms

In order to determine the total polyphenol content (TPC) of wild Pioppino harvested in the South of Italy, samples of the poplar mushrooms and Champignon were extracted through ultrasound-assisted maceration. The utilised extractive procedure consisted in a first defatting step, carried out using hexane as extractant. The materials, deprived of solvent vapours, were re-extracted with pure methanol obtaining alcoholic extracts, which underwent TPC estimation. Folin–Ciocalteu assay is a simple method based on a redox reaction, commonly used for the measurement of phenolic content in natural products. Indeed, it is also able to react with any reducing substance, including nitrogen-containing compounds.[47] Thus, the results obtained by this method cannot be considered an absolute measure of the amount of phenolics, but they provide a useful information of the reducing properties of a substrate.[40] By interpolation on a calibration curve of gallic acid, it was possible to estimate the effective reducing capacity of the two extracts investigated. Data acquired allowed us to observe that Pioppino (PM) showed a TPC value equal to 39.6 ± 1.29 mg GAE per g of extract, whereas in Champignon (CM) TPC is equal to 21.3 ± 0.05 mg GAE per g (Fig. 1). The calculated values for PM were higher than 17.36 mg GAE per g and 15.3 mg GAE per g as reported by Petrović *et al.*[29] and Lo and Cheung,[28] respectively. This result could be due to the use of a defatting solvent that could enhance the reducing power of alcoholic extracts from mushrooms, decreasing the antagonist role of constituents, which are not able to react with Folin–Ciocalteu reagent. In this context, Pioppino also showed a TPC value higher than that reported by Dubost *et al.*, who measured a TP content of 8 mg GAE per g DW in the whole white button mushrooms when extracted with ethanol.[48]

The greater responsiveness of the Pioppino alcoholic extract was confirmed also in the evaluation of the scavenging capacity toward two radical species target, ABTS$^{\bullet+}$ and DPPH$^{\bullet}$. In both cases the observed activities were dose-dependent (Fig. 1; Table 6), even if the one vs. ABTS$^{\bullet+}$ was more pronounced. In fact, expressing the scavenging activity of mushroom extracts in terms of EC_{50}, where a lower value indicates a more potent radical-scavenging effect, it was observed that the extract dose, able to reduce the cation radical probe by 50%, was equal to 34.2 µg mL^{-1} for Pioppino, about twice lower than that of Champignon (72.9 µg mL^{-1}). The ABTS$^{\bullet+}$ scavenging capacity was also expressed in term of TEAC value. Furthermore, the reductive ability of each extract was also measured through an electron transfer reaction ($Fe^{3+} \rightarrow Fe^{2+}$) using a ferric salt as oxidant agent. Pioppino was markedly more effective than the other one to reduce Fe^{3+}, providing a TEAC value 11.3-fold higher (Table 6). The ORAC assay allowed us to evaluate the decrease in the fluorescence of a fluorescent probe (fluorescein) due to the action of peroxyl radicals generated by the AAPH thermal decomposition. The linearity between the net AUC and the dose was checked for both the extracts, which were tested at lower doses than those adopted in the colorimetric tests based on the high sensitivity of the ORAC method. The decay of the fluorescence curve was observed at time intervals strongly dependent on the tested dose (Fig. 2). ORAC values were estimated equal to 1.25 and 1.51 Trolox® Equivalents (µmol L^{-1}) for PM and CM extracts, respectively.

Tentative identification of constituents from Pioppino and Champignon alcoholic extracts

The alcoholic extracts of investigated mushrooms (PM and CM extracts) were analysed by liquid chromatography–high resolution mass spectrometry (LC-HRMS) (Table 7) in full scan mode. The complexity of the extract was only partially resolved by data dependent acquisition method using dynamic exclusion, which enabled the collection of HRMS/MS spectra for resolved peaks, and by literature data and MS/MS-based data resource and database ReSpect (RIK EN tandem mass spectral database).[49]

Compound **1**, common to both the investigated fungi extract, was a hexitol. Its molecular ion was 181.0718, indicating the formula of $C_6H_{14}O_6$ (+0.7 ppm in PM, + 3.6 in CM). Its main product ions were at *m/z* 163, 131, 119, 101 and 89. The compound was tentatively identified as mannitol, a polyol widely distributed in filamentous fungi, which can be stored in fungal hyphae, or further metabolised in order to store reducing power or constitute a reserve carbon source.[50] The developing fruiting bodies of Champignon are reported to accumulate mannitol,

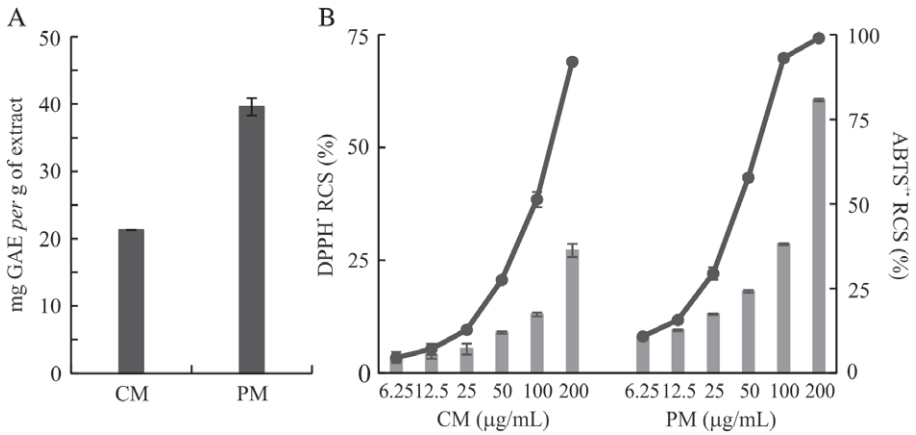

Figure 1. (A) Total phenol content of defatted alcoholic extracts from Pioppino (PM) and Champignon (CM) mushrooms expressed as mg gallic acid equivalents *per* g of each extract (mg GAE g^{-1}) ± SD; (B) radical scavenging capacity (RSC, %) of different dose levels of PM and CM extracts towards DPPH (■) and ABTS cation (●) radicals. Values are reported as mean ± SD of measurements carried out on three samples ($n = 3$).

Table 6. Antioxidant activity of PM and CM extracts expressed as EC_{50} (μg mL^{-1}) vs $ABTS^{\cdot+}$ and $DPPH^{\cdot}$ and TEAC values (Trolox® Equivalents Antioxidant Capacity, $EC_{50\ Trolox}/EC_{50\ sample} \times 100$). TEAC values from Fe^{3+} RP (Reducing Power) data are also shown

Parameter	EC_{50} $DPPH^{\cdot}$	EC_{50} $ABTS^{\cdot+}$	TEAC $DPPH^{\cdot}$	TEAC $ABTS^{\cdot+}$	TEAC Fe^{3+} RP
PM	>200	34.2	6.46	27.1	25.0
CM	>200	72.9	0.075	12.7	2.21

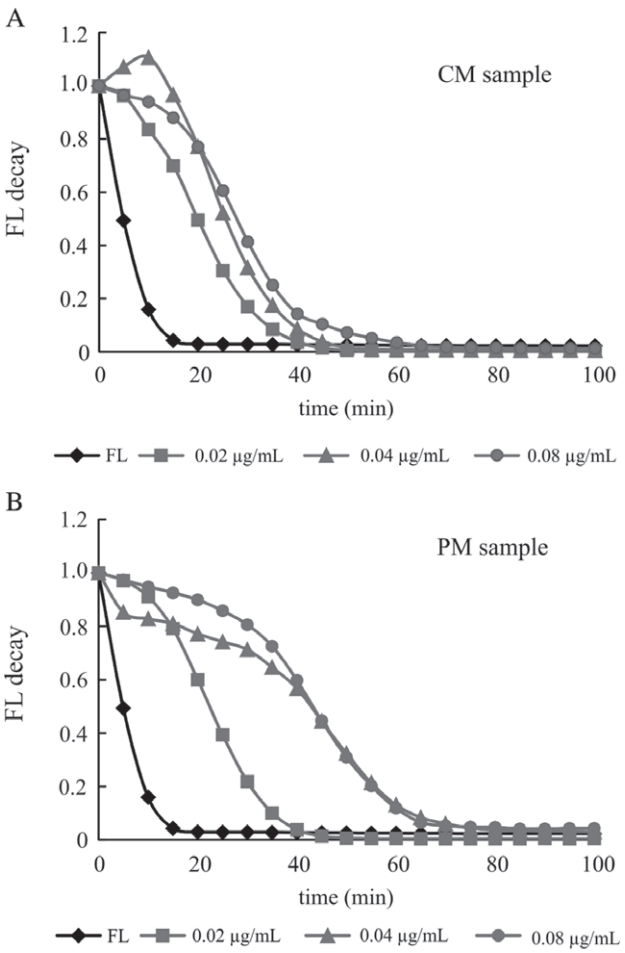

Figure 2. Fluorescence decay curves of fluorescein induced by AAPH in the presence of defatted alcoholic extracts from Pioppino (PM) and Champignon (CM) mushrooms.

which was supposed to be an osmoticum during growth of the mushroom.[51]

Compound **2** was identified as saccharopine, an unusual amino acid, whose occurrence in Champignon mushrooms was previously reported.[52] Compound **3**, whose molecular ion was at *m/z* 341.1089 (−0.3 ppm), was tentatively identified as trehalose, a disaccharide composed of two glucose molecules bound by an α,α-1,1 linkage. Mushrooms are reported to contain up to 10–25% of this not reducing sugar by dry weight.[53] Using a fragment search of ReSpect with MS/MS query data, high confidence levels were found.[49] The high presence of trehalose in the mycelium of Champignon allowed to suppose that the disaccharide functioned in translocation of carbon from mycelium to fruit bodies, at the onset of fructification,[54] whereas Petrović *et al.*[29] showed that trehalose was the dominant sugar in Pioppino. Compounds **4–6** were also disaccharides. The ion at *m/z* 377.0856 (+2.6 ppm) for compound **4** was in accordance with the presence of a disaccharide chloride adduct. MS/MS spectra highlighted the chloride adduct, which was the base peak, decomposed via loss of neutral HCl providing the $[M-H]^-$ ion at *m/z* 341, together with the fragment ions at *m/z* 179, 119 and 89. This fragmentation pattern was in accordance with the presence of a reducing disaccharide.[55] The disaccharide **5** ionised as formylated molecule. In fact, the MS/MS spectrum of the ion at *m/z* 387.1144 (+3.6 ppm in PM, −0.2 in CM) provided fragment ions whose *m/z* ratio was strictly close to those of previous identified compounds. The fragmentation of the dimer ion at *m/z* 683.2251 yielded for compound **6** the deprotonated ion $[M-H]^-$ at *m/z* 341 arising from the neutral loss of one disaccharide molecule.[56] The finding of this high variability of saccharide compounds, together with the estimated carbohydrates content (Table 1), are in line with literature data stating that carbohydrate content of mushrooms represents the bulk of their fruiting bodies.[57,58]

The $[M-H]^-$ ion at *m/z* 266.1146 for compound **7** was in accordance with the formula $C_{12}H_{17}N_3O_4$. This compound was tentatively identified as agaritine, a phenylhydrazine which is

Table 7. LC HRMS data of defatted alcoholic extracts from Pioppino (PM) and Champignon (CM) mushrooms

						PM sample			CM sample		
	Compound	Formula	Mass (Da)	Adduct	Extraction mass (Da)	Found at mass (Da)	Error (ppm)	%[a]	Found at mass (Da)	Error (ppm)	%[a]
1	Hexitol	$C_6H_{14}O_6$	182.07904	$[M-H]^-$	181.07176	181.0719	0.7	31.02	181.07241	3.6	66.65
2	Saccharopine	$C_{11}H_{20}N_2O_6$	276.13214	$[M-H]^-$	275.12486	275.12465	−0.7	22.0	275.12497	0.4	2.91
3	Disaccharide 1	$C_{12}H_{22}O_{12}$	342.11621	$[M-H]^-$	341.10894	341.10882	−0.3	3.11	ND		
4	Disaccharide 2		378.0864	$[M+Cl]^-$	377.0856	377.0884	2.6	0.09	ND		
5	Disaccharide 3		388.12169	$[M+HCOO]^-$	387.11441	387.11581	3.6	0.2	387.11432	−0.2	0.53
6	Disaccharide 4		684.23242	$[2M-H]$	683.22515	683.22407	−1.6	0.23	ND		
7	Agaritine	$C_{12}H_{17}N_3O_4$	267.12191	$[M-H]^-$	266.11463	266.1144	−0.9	traces	266.11439	−0.9	2.78
8	Pentosylhexitol	$C_{11}H_{22}O_{10}$	314.1213	$[M-H]^-$	313.11402	313.11441	1.2	0.39	313.11404	0.1	1.52
9	Ergothioneine	$C_9H_{15}N_3O_2S$	229.0885	$[M-H]^-$	228.08122	228.08126	0.1	0.54	228.08109	−0.6	0.56
10	γ-Glutaminyl-4-hydroxybenzene	$C_{11}H_{14}N_2O_4$	238.09536	$[M-H]^-$	237.08808	237.08804	−0.2	0.05	237.08784	−1	8.39
11	Pentosyl xanthosine	$C_{15}H_{28}O_{13}$	416.15299	$[M-H]^-$	415.14571	415.14605	0.8	0.09	ND		
12	Homogentisic acid	$C_8H_8O_4$	168.04226	$[M-H]^-$	167.03498	167.03445	−3.2	0.25	ND		
13	Malic acid	$C_4H_6O_5$	134.02152	$[M-H]^-$	133.01425	133.01441	1.2	35.10	133.01474	3.7	12.99
14	Pentos-2-ulose	$C_5H_8O_5$	148.03717	$[M-H]^-$	147.0299	147.03019	2	0.77	ND		
15	Fumaric acid	$C_4H_4O_4$	116.01096	$[M-H]^-$	115.00368	115.004	2.8	2.21	115.00412	3.8	1.80
16	Veratric acid	$C_9H_{10}O_4$	182.05791	$[M-H]^-$	181.05063	181.05078	0.8	0.15	ND		
17	*p*-Cumaric acid	$C_9H_8O_3$	164.04734	$[M-H]^-$	163.04007	163.04011	0.2	2.20	ND		
18	*o*-Cumaric acid	$C_9H_8O_3$	164.04734	$[M-H]^-$	163.04007	163.04023	1	1.20	ND		
19	Ferulic acid	$C_{10}H_{10}O_4$	194.05791	$[M-H]^-$	193.05063	ND			193.05035	−1.5	0.60
20	Sinapic acid	$C_{11}H_{12}O_5$	224.06847	$[M-H]^-$	223.0612	ND			223.0614	0.9	0.72
21	Cinnamic acid	$C_9H_8O_2$	148.05243	$[M-H]^-$	147.04515	ND			147.04559	3	0.53
22	δ-Tocopherol	$C_{27}H_{46}O_2$	402.34978	$[M-H]^-$	401.3425	401.3431	1.5	0.10	ND		
23	γ-Tocopherol	$C_{28}H_{48}O_2$	416.36543	$[M-H]^-$	415.35815	415.3593	2.8	0.30	ND		

[a] Percentage of each identified compound based on peak areas

contained in large amount in cultivated Champignon.[59] MS^2 experiments showed that the fragmentation pattern was in accordance with that reported by Janák *et al.*[60] Compound **8** showed the $[M-H]^-$ ion at *m/z* 313.1140 (+1.2 ppm in PM, +0.1 in CM) and MS/MS fragment ions at *m/z* 181, 163, 149, 119, 113, 101 and 89. Thus, the neutral loss of 132 Da from $[M-H]^-$ ion gave a mannitol-like fragmentation pattern allowing us to hypothesise the presence of a pentosylhexitol.

The $[M-H]^-$ ion at *m/z* 228.0812 (+0.1 ppm in PM, −0.6 in CM), together with its MS/MS fragment ions, were in accordance for metabolite **9** with ergothioneine. Mushrooms were discovered to be the primary source of ergothioneine. This naturally occurring thiol containing amino acid is reported to exert antioxidant properties through multiple mechanisms.[61] The metabolite **10** was γ-glutaminyl-4-hydroxybenzene (GHB), which was reported to exhibit a precursor role in melanogenesis.[62] The fructifying mycelium of Champignon appeared as a site of intense synthesis of agaritine and GHB.[63]

Compound **11** showed the $[M-H]^-$ ion at *m/z* 415.1457 (+0.8 ppm) and MS/MS fragment ions at *m/z* 415, 283, 151, according to the presence of a pentosyl xanthosine.

The metabolite **12** showed the $[M-H]^-$ ion at *m/z* 167.0350 (−3.2 ppm), indicating the formula $C_8H_8O_4$, according to vanillic acid or homogentisic acid. The only loss of a CO_2 moiety, together with the lack of fragment ion due to the neutral loss of $CH_3^{\bullet}$ allowed us to identify it tentatively as homogentisic acid. Homogentisic acid was found as the free phenolic acid significantly present in different edible mushrooms.[64]

Compounds **13** and **15** were malic acid and fumaric acid, respectively. The comparison of their retention times and MS/MS spectra with those of pure reference compounds allowed us to unequivocally identify the metabolites. Compound **14**, exhibiting the $[M-H]^-$ ion at *m/z* 147.0299 (+2.0 ppm) and MS^2 fragment ions at *m/z* 129, and 117, was tentatively identified as a pentos-2-ulose.

Compound **16** showed the $[M-H]^-$ ion at *m/z* 181.0506 (+0.8 ppm), indicating the formula $C_9H_{10}O_4$, according to veratric acid, a benzoic acid derivative, which was reported to have anti-inflammatory and anti-oxidant activities.[65]

Metabolites **17–21** were identified as C_6C_3 phenols by comparison of their relative retention times and MS/MS spectra with those of reference pure compounds. In particular, compounds **17** and **18** were identified as *o*-coumaric acid and *p*-coumaric acid, respectively, whereas compounds **19**, **20** and **21**, whose presence was detected only in CM sample, were ferulic acid, sinapic acid and cinnamic acid, respectively.

Compounds **22** and **23** were β- and γ-tocopherols, respectively. The presence of these constituents in Pioppino was previously reported by Petrović *et al.*,[29] who found γ-tocopherol as the dominant vitamer, followed by β-tocopherol, δ-tocopherol and α-tocopherol. No traces of α-tocopherol were found in PM sample. The presence of these latter compounds, which were detected only in trace amounts, together with that of Coumaric acid isomers, which constitute the extract by 3.2% and homogentisic acid, could explain the anti-radical and reducing efficacies of the PM extract. However δ- and γ-tocopherol are not detected in CM

samples, while are retrieved in *Agaricus bisporus* from Poland.[66] In fact, although CM extract also contained phenol compounds, they accounted for only 1.3%. It was not of minor importance the high content of malic acid (~35%) and of carbohydrates in PM extract, which encourages further research for optimising the nutraceutical use of the prepared mushroom complex. Indeed, malic acid, a Krebs cycle intermediate metabolite and a component of the malate–aspartate shuttle, was already investigated for benefits derived from a malate–oligosaccharide solution. It was reported that this supplementation was able to regulate the level of energy metabolism, improve cardiac function, alleviate sports fatigue, and promote regeneration.[67] In particular, malate–oligosaccharide solution was shown to affect positively the antioxidant capacity of athletes' endurance.

CONCLUSIONS

Agrocybe aegerita, commonly known as Pioppino, is one of the most delicious, fragrant and popular edible mushrooms, whose wild availability is strongly compromised by the extensive use of fungicides. The comparison of nutritional values between Pioppino and cultivated Champignons clearly indicates that wild Pioppino, collected in a rural area of the Campania Region, is a rich source of nutrients, especially amino acids, malic acid and sugars. The interesting free radical scavenging properties of the Pioppino defatted alcoholic extract, despite its low concentration of phenolic compounds, encourages further studies aimed at an in-depth evaluation of its nutraceutical value.

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REFERENCES

1 Alexopoulos CJ, Mims CW and Blackwell MM, *Introductory Mycology*. Wiley, Chichester (1996).
2 Barros L, Cruz T, Baptista P, Estevinho LM and Ferreira IC, Wild and commercial mushrooms as source of nutrients and nutraceuticals. *Food Chem Toxicol* **46**:2742–2747 (2008).
3 Chang ST and Miles PG, Medicinal value, in *Mushrooms*. CRC Press, Boca Raton, pp. 39–51 (2004).
4 Ivanova TS, Krupodorova TA, Barshteyn VY, Artamonova AB and Shlyakhovenko VA, Anticancer substances of mushroom origin. *Exp Oncol* **36**:58–66 (2014).
5 Smolskait L, Venskutonis PR and Talou T, Comprehensive evaluation of antioxidant and antimicrobial properties of different mushroom species. *LWT – Food Sci Technol* **60**:462–471 (2015).
6 Kozarski M, Klaus A, Niksic M, Jakovljevic D, Helsper JPFG and Van Griensven LJLD, Antioxidative and immunomodulating activities of polysaccharide extracts of the medicinal mushrooms *Agaricus bisporus*, *Agaricus brasiliensis, Ganoderma lucidum* and *Phellinus linteus*. *Food Chem* **129**:1667–1675 (2011).
7 Doljak B, Stegnar M, Urleb U, Kreft S, Umek A, Ciglarič M *et al.*, Screening for selective thrombin inhibitors in mushrooms. *Blood Coagul Fibrinol* **12**:123–128 (2001).
8 Khatua S, Paul S and Acharya K, Mushroom as the potential source of new generation of antioxidant: A review. *Res J Pharm Technol* **6**:496–505 (2013).
9 Kalaè P, A review of chemical composition and nutritional value of wild-growing and cultivated mushrooms. *J Sci Food Agric* **93**:209–218 (2013).
10 Heleno SA, Barros L, Martins A, Morales P, Fernández-Ruiz V, Glamoclija J *et al.*, Nutritional value, bioactive compounds, antimicrobial activity and bioaccessibility studies with wild edible mushrooms. *LWT – Food Sci Technol* **63**:799–806 (2015).
11 Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, Eriksson OE *et al.*, A higher-level phylogenetic classification of the Fungi. *LWT – Food Sci Technol* **111**:509–547 (2007).
12 Wasser SP, Medicinal mushroom science: Current perspectives, advances, evidences, and challenges. *Biomed J* **37**:345–356 (2014).
13 Wasser SP and Weis AL, Medicinal properties of substances occurring in higher basidiomycetes mushrooms: current perspectives (review). *Int J Med Mushrooms* **1**:31–62 (1999).
14 Seo SY, Ahn MS, Choi SR, Song EJ, Choi MK and Kim YS, Analysis of nutritional compositions and biological activity of *Agrocybe aegerita*. *J Mushroom Sci Prod* **9**:116–122 (2011).
15 Zhang Y, Mills GL and Nair MG, Cyclooxygenase inhibitory and antioxidant compounds from the fruiting body of an edible mushroom, *Agrocybe aegerita*. *Phytomedicine* **10**:386–390 (2003).
16 Wu T and Xu B, Antidiabetic and antioxidant activities of eight medicinal mushroom species from China. *Int J Med Mushrooms* **17**:129–140 (2015).
17 Kim W-G, Lee I-K, Kim J-P, Ryoo I-J, Koshino H and Yoo I-D, New indole derivatives with free radical scavenging activity from *Agrocybe cylindracea*. *J Nat Prod* **60**:721–723 (1997).
18 Kiho T, Sobue S and Ukai S, Structural features and hypoglycemic activities of two polysaccharides from a hot-water extract of *Agrocybe cylindracea*. *Carbohyd Res* **251**:81–87 (1994).
19 Ngai PH, Zhao Z and Ng TB, Agrocybin, an antifungal peptide from the edible mushroom *Agrocybe cylindracea*. *Peptides* **26**:191–196 (2005).
20 Diyabalanage T, Mulabagal V, Mills G, DeWitt DL and Nair MG, Health-beneficial qualities of the edible mushroom, *Agrocybe aegerita. Food Chem* **108**:97–102 (2008).
21 Rollán MG, *Cultivo de setas y trufas*. Mundi-Prensa, Madrid, Spain (2007).
22 Zhong JJ and Xiao JH, Secondary metabolites from higher fungi: discovery, bioactivity, and bioproduction, in *Biotechnology in China I*, ed. by Zhong JJ, Bai FW and Zhang W. Springer-Verlag, Berlin Heidelberg, pp. 79–150 (2009).
23 Muthu N and Shanmugasundaram K, Comparative study of phytochemicals in aqueous and silver nanoparticles extracts of *Agrocybe aegerita*, (V. Brig.) singer black poplar mushroom. *Int J Pharma Bio Sci* **6**:B190–B197 (2015).
24 Liang Y, Guo LX and Sun H, In vivo antitumor activity of the active protein components from *Agrocybe aegerita* on tumor-bearing mouse model. *Modern Food Sci Technol* **30**:1–5 (2014).
25 Yang N, Li DF, Feng L, Xiang Y, Liu W, Sun H *et al.*, Structural basis for the tumor cell apoptosis-inducing activity of an antitumor lectin from the edible mushroom *Agrocybe aegerita*. *J Mol Biol* **387**:694–705 (2009).
26 Pecyna MJ, Ullrich R, Bittner B, Clemens A, Scheibner K, Schubert R *et al.*, Molecular characterization of aromatic peroxygenase from *Agrocybe aegerita*. *App Microbiol Biot* **84**:885–897 (2009).
27 Yang N, Tong X, Xiang Y, Zhang Y, Liang Y, Sun H *et al.*, Molecular character of the recombinant antitumor lectin from the edible mushroom *Agrocybe aegerita*. *J Biochem* **138**:145–150 (2005).
28 Lo KM and Cheung PCK, Antioxidant activity of extracts from the fruiting bodies of *Agrocybe aegerita* var. *alba*. *Food Chem* **89**:533–539 (2005).
29 Petrović J, Glamočlija J, Stojković D, Ćirić A, Barros L, Ferreira ICFR *et al.*, Nutritional value, chemical composition, antioxidant activity and enrichment of cream cheese with chestnut mushroom *Agrocybe aegerita* (Brig.) Sing. *J Food Sci Technol* **52**:6711–6718 (2015).
30 Wang M, Gu B, Huang J, Jiang S, Chen Y, Yin Y *et al.*, Transcriptome and proteome exploration to provide a resource for the study of *Agrocybe aegerita*. *PLoS One* **8**:e56686 (2013).
31 Landi N, Pacifico S, Ragucci S, Iglesias R, Piccolella S, Amici A *et al.*, Purification, characterization and cytotoxicity assessment of Ageritin: The first ribotoxin from the basidiomycete mushroom *Agrocybe aegerita*. *Biochim Biophys Acta* **1861**:1113–1121 (2017).
32 Association of Official Analytical Chemists (AOAC), *Official Methods of Analysis*, 17th edn. AOAC, Arlington, VA (2000).
33 Reis FS, Barros L, Martins A and Ferreira IC, Chemical composition and nutritional value of the most widely appreciated cultivated mushrooms: an inter-species comparative study. *Food Chem Toxicol* **50**:191–197 (2012).
34 FAO, *Food Energy – Methods of Analysis and Conversion Factors. Report of a Technical Workshop, 3–6 December 2002, FAO Food and Nutrition Paper No. 77*. FAO, Rome (2003).
35 Carillo P, Mastrolonardo G, Nacca F and Fuggi A, Nitrate reductase in durum wheat seedlings as affected by nitrate nutrition and salinity. *Funct Plant Biol* **32**:209–219 (2005).

36 Queval G and Noctor G, A plate reader method for the measurement of NAD, NADP, glutathione, and ascorbate in tissue extracts: Application to redox profiling during *Arabidopsis rosette* development. *Anal Biochem* **363**:58–69 (2007).
37 Iriti M, Di Maro A, Bernasconi S, Burlini N, Simonetti P, Picchi V *et al.*, Nutritional traits of bean (*Phaseolus vulgaris*) seeds from plants chronically exposed to ozone pollution. *J Agric Food Chem* **57**: 201–208 (2009).
38 Landi N, Pacifico S, Piccolella S, Di Giuseppe AM, Mezzacapo MC, Ragucci S *et al.*, Valle Agricola lentil, an unknown lentil (*Lens culinaris* Medik.) seed from southern Italy as a novel antioxidant and prebiotic source. *Food Funct* **6**:3155–3164 (2015).
39 Ferrara L, Dosi R, Di Maro A, Guida V, Cefarelli G, Pacifico S *et al.*, Nutritional values, metabolic profile and radical scavenging capacities of wild asparagus (*A. acutifolius* L.). *J Food Comp Anal* **24**:326–333 (2011).
40 Pacifico S, Galasso S, Piccolella S, Kretschmer N, Pan SP, Marciano S *et al.*, Seasonal variation in phenolic composition and antioxidant and anti-inflammatory activities of *Calamintha nepeta* (L.) Savi. *Food Res Int* **69**:121–132 (2015).
41 Di Maro A, Pacifico S, Fiorentino A, Galasso S, Gallicchio M, Guida V *et al.*, Raviscanina wild asparagus *(Asparagus acutifolius* L.): A nutritionally valuable crop with antioxidant and antiproliferative properties. *Food Res Int* **53**:180–188 (2013).
42 Mattila P, Konko K, Eurola M, Pihlava JM, Astola J, Vahteristo L *et al.*, Contents of vitamins, mineral elements, and some phenolic compounds in cultivated mushrooms. *J Agric Food Chem* **49**:2343–2348 (2001).
43 Buono R and Vallariello G, La vite maritata in Campania. *Delpinoa* **44**:53–63 (2002).
44 Muthu N and Shanmugasundaram K, Proximate and mineral compositions of edible mushroom *Agrocybe aegerita*. *J Pharmacogn Phytochem* **5**:116–119 (2016).
45 Perez V and Chang ET, Sodium-to-potassium ratio and blood pressure, hypertension, and related factors. *Adv Nutr (Bethesda, Md)* **5**:712–741 (2014).
46 Shim MJ, Park T, Park J and Kim BK, Detection of taurine in Basidiomycetes. *J Anhui Agricul Col* **26**:302–305 (1999).
47 Ikawa M, Schaper TD, Dollard CA and Sasner JJ, Utilization of Folin–Ciocalteu phenol reagent for the detection of certain nitrogen compounds. *J Agric Food Chem* **51**:1811–1815 (2003).
48 Dubost NJ, Ou B and Beelman RB, Quantification of polyphenols and ergothioneine in cultivated mushrooms and correlation to total antioxidant capacity. *Food Chem* **105**:727–735 (2007).
49 Sawada Y, Nakabayashi R, Yamada Y, Suzuki M, Sato M, Sakata A *et al.*, RIKEN tandem mass spectral database (ReSpect) for phytochemicals: a plant-specific MS/MS-based data resource and database. *Phytochemistry* **82**:38–45 (2012).
50 Jobic C, Boisson AM, Gout E, Rascle C, Fevre M, Cotton P *et al.*, Metabolic processes and carbon nutrient exchanges between host and pathogen sustain the disease development during sunflower infection by *Sclerotinia sclerotiorum*. *Planta* **226**:251–265 (2007).
51 Kues U and Liu Y, Fruiting body production in Basidiomycetes. *Appl Microbiol Biotechnol* **54**:141–152 (2000).
52 Oka Y, Ogawa T and Sasoka K, Occurrence of L-saccharopine and gamma-L-glutamylglycine in the mushroom, *Agaricus bisporus*. *Agric Biol Chem* **43**:1995–1996 (1979).
53 Higashiyama T, Novel functions and applications of trehalose. *Pure Applied Chem* **74**:1263–1269 (2002).
54 Wannet WJ, Hermans JH, van Der Drift C and Op Den Camp HJ, HPLC detection of soluble carbohydrates involved in mannitol and trehalose metabolism in the edible mushroom *Agaricus bisporus*. *J Agric Food Chem* **48**:287–291 (2000).
55 Jiang Y and Cole RB, Oligosaccharide analysis using anion attachment in negative mode electrospray mass spectrometry. *J Am Soc Mass Spectr* **16**:60–70 (2005).
56 Wan D, Yang H, Yan C, Song F, Liu Z and Liu S, Differentiation of glucose-containing disaccharides isomers by fragmentation of the deprotonated non-covalent dimers using negative electrospray ionization tandem mass spectrometry. *Talanta* **115**:870–875 (2013).
57 Wani BA, Bodha RH and Wani AH, Nutritional and medicinal importance of mushrooms. *J Med Plants Res* **4**:2598–2604 (2010).
58 Singh NB and Singh P, Biochemical composition of *Agaricus bisporus*. *J Indian Bot Soc* **81**:235–237 (2002).
59 Kondo K, Watanabe A, Iwanaga Y, Abe I, Tanaka H, Nagaoka MH *et al.*, Analysis of agaritine in mushrooms and in agaritine-administered mice using liquid chromatography–tandem mass spectrometry. *J Chromatogr B, Analyt Technol Biomed Life Sci* **834**:55–61 (2006).
60 Janák K, Størmer FC and Koller GEB, The content of agaritine in spores from *Agaricus bisporus*. *Food Chem* **99**:521–524 (2006).
61 Weigand-Heller AJ, Kris-Etherton PM and Beelman RB, The bioavailability of ergothioneine from mushrooms (*Agaricus bisporus*) and the acute effects on antioxidant capacity and biomarkers of inflammation. *Prev Med* **54**(Suppl):S75–78 (2012).
62 Stüssi H and Rast DM, The biosynthesis and possible function of γ-glutaminyl-4-hydroxybenzene in *Agaricus bisporus*. *Phytochemistry* **20**:2347–2352 (1981).
63 Soulier L, Foret V and Arpin N, Occurrence of agaritine and gamma-glutaminyl-4-hydroxybenzene (GHB) in the fructifying mycelium of *Agaricus bisporus*. *Mycol Res* **97**:529–532 (1993).
64 Palacios I, Lozano M, Moro C, D'Arrigo M, Rostagno MA, Martínez JA *et al.*, Antioxidant properties of phenolic compounds occurring in edible mushrooms. *Food Chem* **128**:674–678 (2011).
65 Shin SW, Jung E, Kim S, Lee KE, Youm JK and Park D, Antagonist effects of veratric acid against UVB-induced cell damages. *Molecules (Basel, Switzerland)* **18**:5405–5419 (2013).
66 Ferreira IC, Barros L and Abreu RM, Antioxidants in wild mushrooms. *Curr Med Chem* **16**:1543–1560 (2009).
67 Qiang F, Effect of malate-oligosaccharide solution on antioxidant capacity of endurance athletes. *Open Biomed Eng J* **9**:326–329 (2015).

