Study and characterization of selected nutrients in wild mushrooms from Portugal by gas chromatography and high performance liquid chromatography

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A B S T R A C T

The present work aims at contributing to the documentation of the nutritional composition of wild mushrooms. Fatty acid and sugar profiles of 10 different Portuguese wild mushrooms were obtained by gas chromatography coupled to a flame ionization detector (GC/FID) and high performance liquid chromatography coupled to a refraction index detector (HPLC/RID), respectively, the latter methodology being then completely validated. The macronutrient profile in general revealed that the wild mushrooms were rich sources of protein (24.32–76.63 g/100 g) and carbohydrates (10.35–55.48 g/100 g), and had low amounts of fat (0.36–2.63 g/100 g). The highest energetic contribution was guaranteed by *Hygrophoropsis aurantiaca*. The analysis of fatty acid composition allowed the quantification of 25 fatty acids. Unsaturated fatty acids and, in particular, oleic and linoleic acids, were predominant (17–61% and 20–54%, respectively). In the analysis of free sugars, all the compounds were separated in a period of time of 10 min; the method used proved to be sensitive, reproducible and accurate. Arabinose (1.53–7.66 g/100 g), mannitol (0.38–18.41 g/100 g) and trehalose (0.21–18.66 g/100 g) were the most abundant sugars.

1. Introduction

Since the dawn of human civilization, fruiting bodies of fungi (mushrooms) are appreciated not only for texture and flavour but also for their chemical and nutritional properties [1]. More than 140,000 species of mushrooms exist in nature, but less than 25 species (*Agaricus bisporus*, *Pleurotus* spp., *Lentinus edodes*, *Volvariella volvacea*, *Auricularia* spp. etc.) are widely accepted as food and only a few have attained the level of an item of commerce [2]. However, wild edible mushrooms have been used as food and food flavouring material in soups and sauces for centuries, due to their unique and delicate flavour and they have also been traditionally eaten seasonally by specific groups of people (local people, enthusiasts and gourmets) providing a source of minerals and vitamins when fresh vegetables were not available. Wild edible mushrooms are rich in trace minerals, and have high water, protein, fibre, and carbohydrate contents, and low fat/energy levels making them an excellent food for use in low caloric diets [3–12]. Edible mushrooms species are highly nutritious and may compare favourably with meat, eggs and milk. Some investigations have even contended that the amino acid compositions of mushrooms are comparable to animal proteins [13,14], which is particularly important considering the cost of those proteins and the outbreak of diseases connected with animal meat. The nutritional potential and implications of this gradual replacement of meat with mushroom require careful examination involving detailed chemical and biological studies [5].

Our research group has been interested in the nutritional characterization of wild mushrooms, and in the last years we studied 16 different mushroom species from the Northeast of Portugal, one of the European regions with higher wild mushrooms diversity: *Agaricus arvensis*, *Lactarius deliciosus*, *Leucopaxillus giganteus*, *Sarcodon imbricatus*, *Tricholoma portentosum* [15], *L. deliciosus*, *Macrolepiota mastoidea*, *Macrolepiota procera* [16], *A. bisporus*, *Agaricus silvaticus*, *Agaricus silvicola* [17], *Cantharellus cibarius*, *Lepista nuda*, *Lycoperdon molle*, *Lycoperdon perlatum* and *Ramaria botrytis* [18]. Assuming that the proportion of mushrooms used is only 5% of an estimated total including undiscovered and unexamined species, there are still thousands to characterize. Even among the already known species the proportion of well investigated mushrooms is very low. Therefore, we intend to go on in the study of this matrix, documenting the nutritional composition of all these unique species, and making the information available for a better management and conservation of this natural resource and related habitats. In particular, data on *Cortinarius glaucopus*, *Hygrophoropsis aurantiaca*, *Hypholoma capnoides*, *Laccaria laccata*, *Lactarius salmonicolor*, *Lepista inversa*, *Suillus mediterraneensis* and *Tricholoma imbricatum* have not been reported yet. Nutritional composition of *Russula delica* and *Fistulina hepatica* from Greece was studied [19] but there are no studies on their individual composition in sugars and fatty acids.

In this work, we report the chemical composition of 10 different Portuguese wild mushrooms, with reference to the contents of moisture,

proteins, fat, carbohydrate and ash. On the basis of the samples composition, an estimation of the mushrooms nutritional role was also performed. Among the individual components, fatty acid and sugar profiles were obtained by GC/FID and HPLC/RID, respectively, the latter methodology being completely validated.

2. Materials and methods

2.1. Mushroom species

Samples of *C. glaucopus* (Schaeff), *F. hepatica* (Schaeff.: Fr.), *H. aurantiaca* (Wulf.: Fr.) Mre., *H. capnoides* (Fr.) Quel., *L. laccata* (scop.: Fr.) Berk. & Broome, *L. salmonicolor* (Heim y Leclair), *L. inversa* (Scop.: Fr.) Pat., *R. delica* (Fr.), *S. mediterraneensis* (Jacquetant & Blum) Redeuilh, *T. imbricatum* (Fr.) P. Kumm. were collected under *Quercus pyrenaica* Willd. and mixed stands of *Quercus* sp. and *Pinus sylvestris* Ait., in Bragança (Northeast of Portugal), during the autumn of 2008. *Mycena rosea* (Schumach.) *Gramberg* and *Tricholoma sulphureum* (Bull.) P. Kumm. are not edible species and *C. glaucopus* (SchSff.: Fr.) S.F. Gray is a species of unknown edibility. Taxonomic identification of sporocarps was made according to several authors [20–25], and online keys (http://www.mycokey.com/), and representative voucher specimens were deposited at the herbarium of Escola Superior Agrária of Instituto Politécnico de Bragança. All the samples were lyophilised (Ly-8-FM-ULE, Snijders) and reduced to a fine dried powder (20 mesh).

2.2. Standards and reagents

Acetonitrile 99.9% pure, of HPLC grade was purchased from Lab-Scan (Lisbon, Portugal). All the other reagents were of analytical grade purity: methanol and diethyl ether were supplied by Lab-Scan (Lisbon, Portugal); toluene from Riedel-de-Haën; sulphuric acid from Fluka (St. Gallen, Switzerland). The fatty acids methyl ester (FAME) reference standard mixture 37 (fatty acids C4 to C24; standard 47885-U) was from Supelco (Bellefonte, PA, USA) and purchased from Sigma (St. Louis, MO, USA), as well as other individual fatty acid isomers and the sugar standards. All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Water was treated in a Mili-Q water purification system (TGI Pure Water Systems, USA).

2.3. Determination of the nutritional value

Samples of mushrooms were analysed for chemical composition (protein, fat, carbohydrates and ash) using the AOAC procedures [26]. The crude protein content ($N \times 4.38$) of the samples was estimated by the macro-Kjeldahl method; the crude fat was determined by extracting a known weight of powdered mushroom sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at (600 ± 15) °C; reducing sugars were determined by DNS (dinitrosalicylic acid) method. Total carbohydrates were calculated by difference: Total carbohydrates = 100 − (moisture + protein + fat + ash), where moisture, protein, fat and ash, stand for their masses respectively, expressed in units of 1 g. Total energy was calculated according to the following equations: Energy (Kcal) = 4 × (protein + carbohydrate) + 9 × (lipid), where protein and carbohydrate stand for their masses, respectively, expressed in units of 1 g.

2.4. Determination of sugars by HPLC

2.4.1. Preparation of standard solutions

Individual solutions (~10 mg/ml) of L(+)-arabinose, D(−)-fructose, L-fucose, D(+)-galactose, D(+)-glucose anhydrous, lactose 1-hydrate, maltose 1-hydrate, maltulose monohydrate, D(+)-mannitol, D(+)-mannose, D(+)-melezitose, D(+)-melibiose monohydrate, D(+)- raffinose pentahydrate, L(+)-rhamnose monohydrate, D(+)-sucrose, D(+)-trehalose, D(+)-turanose and D(+)-xylose were prepared in water and stored at −20 °C. A stock standard mixture with arabinose, mannitol and trehalose was prepared in water with the final concentration of 100 mg/ml. Fructose was used as internal standard (IS), being prepared a stock solution at 25 mg/ml in water, kept at −20 °C.

2.4.2. Extraction procedure

Dried sample powder (1.0 g) was spiked with the IS (5 mg/ml), and was extracted with 40 ml of 80% aqueous ethanol at 80 °C for 30 min. The resulting suspension was centrifuged at 15,000 g_n for 10 min. The supernatant was concentrated at 60 °C under reduced pressure and defatted three times with 10 ml of ethyl ether, successively. After concentration at 40 °C, the solid residues were dissolved in water to a final volume of 5 ml, filtered through a 0.22 μm disposable LC filter disk, transferred into an injection vial and analysed by HPLC.

2.4.3. HPLC analysis

The HPLC equipment consisted of an integrated system with a Smartline pump 1000, a degasser system Smartline manager 5000, a Smartline 2300 RI detector (Knauer, Germany), and an AS-2057 autosampler (Jasco, Japan). Data were analysed using Clarity 2.4 Software (DataApex). The chromatographic separation was achieved with an Eurospher 100-5 NH_2 column (4.6 mm × 250 mm, 5 mm, Knauer) operating at 35 °C (7971R Grace oven). The mobile phase used was acetonitrile/deionized water, 7:3 (v/v) at a flow rate of 1 ml/min, and the injection volume was 20 μl. The compounds were identified by chromatographic comparisons with authentic standards. The results are expressed in g/100 g of dry weight, calculated by internal normalization of the chromatographic peak area.

The linearity and sensitivity of the HPLC analysis was determined and the method was validated by the instrumental precision, repeatability and accuracy, using *T. imbricatum*.

2.5. Determination of fatty acids by GC

Fatty acids were determined by gas–liquid chromatography with flame ionization detection (GC/FID)/capillary column as described previously by the authors [16], and after the following trans-esterification procedure: fatty acids were methylated with 5 ml of methanol: sulphuric acid:toluene 2:1:1 (v:v), during at least 12 h in a bath at 50 °C and 160 rpm; then 3 ml of deionized water were added, to obtain phase separation; the FAME were recovered with 3 ml of diethyl ether by shaking in vortex, and the upper phase was passed through a micro-column of sodium sulphate anhydrous, in order to eliminate the water; the sample was recovered in a vial with Teflon, and before injection the sample was filtered with 0.2 μm nylon filter from Milipore. The fatty acid profile was analyzed with a DANI model GC 1000 instrument equipped with a split/splitless injector, a flame ionization detector (FID) and a Macherey–Nagel column (30 m × 0.32 mm ID × 0.25 μm d_f). The oven temperature program was as follows: the initial temperature of the column was 50 °C, held for 2 min, then a 10 °C/min ramp to 240 °C and held for 11 min. The carrier gas (hydrogen) flow-rate was 4.0 ml/min (0.61 bar), measured at 50 °C. Split injection (1:40) was carried out at 250 °C. For each analysis 1 μl of the sample was injected in GC. Fatty acid identification was made by comparing the relative retention times from samples with FAME peaks (standards). The results were recorded and processed using CSW 1.7 software (DataApex 1.7) and expressed in relative percentage of each fatty acid.

2.6. Statistical analysis

For each mushroom species three samples were analysed and also all the assays were carried out in triplicate. The results are expressed as mean values and standard deviation (SD) or standard errors (SE).

Table 1
Macronutrients composition (g/100 g) and energetic value (Kcal/100 g) of wild mushrooms in a dry weight basis (mean ± SD; $n = 3$).

Samples	Moisture	Total fat	Crude protein	Ash	Carbohydrates	Reducing sugars	Energy
Cortinarius glaucopus	91.67 ± 0.31^{a}	1.89 ± 0.15^{c}	50.09 ± 0.65^{c}	16.40 ± 0.12^{c}	31.62 ± 0.73^{e}	2.22 ± 0.17^{de}	343.84 ± 0.05^{d}
Fistulina hepatica	87.51 ± 0.98^{f}	2.63 ± 0.49^{cb}	63.69 ± 1.16^{b}	11.30 ± 0.53^{d}	22.98 ± 0.43^{f}	2.77 ± 0.06^{c}	364.98 ± 2.16^{c}
Hygrophoropsis aurantiaca	84.59 ± 1.27^{h}	2.20 ± 0.11^{cb}	36.40 ± 0.60^{d}	5.92 ± 0.06^{e}	55.48 ± 0.52^{a}	1.77 ± 0.30^{e}	387.32 ± 0.25^{a}
Hypholoma capnoides	83.57 ± 2.08^{i}	0.36 ± 0.05^{d}	36.36 ± 0.21^{d}	28.29 ± 2.51^{a}	34.99 ± 2.42^{ed}	2.06 ± 0.13^{e}	288.64 ± 10.05^{f}
Laccaria laccata	88.25 ± 1.86^{c}	3.76 ± 0.58^{a}	62.78 ± 1.07^{b}	20.69 ± 1.50^{b}	12.77 ± 0.78^{g}	2.75 ± 0.36^{c}	336.08 ± 6.88^{d}
Lactarius salmonicolor	87.72 ± 0.94^{e}	2.03 ± 0.36^{cb}	37.28 ± 0.11^{d}	23.28 ± 1.41^{b}	37.41 ± 1.42^{d}	2.72 ± 0.34^{c}	317.05 ± 5.85^{e}
Lepista inversa	87.73 ± 1.01^{d}	2.48 ± 0.21^{cb}	76.63 ± 0.46^{a}	10.54 ± 0.07^{d}	10.35 ± 0.45^{g}	3.39 ± 0.11^{b}	370.24 ± 0.77^{bc}
Russula delica	86.69 ± 0.73^{g}	0.91 ± 0.16^{d}	50.59 ± 1.02^{c}	22.93 ± 2.16^{b}	25.57 ± 1.32^{f}	4.44 ± 0.48^{a}	312.81 ± 9.15^{e}
Suillus mediterraneensis	91.20 ± 1.85^{b}	2.61 ± 0.49^{b}	24.32 ± 0.35^{e}	27.64 ± 0.80^{a}	45.42 ± 1.34^{b}	3.27 ± 0.43^{b}	302.48 ± 1.49^{fe}
Tricholoma imbricatum	82.42 ± 1.15^{j}	1.88 ± 0.11^{c}	50.45 ± 0.83^{c}	6.45 ± 0.27^{e}	41.21 ± 0.56^{c}	2.64 ± 0.20^{dc}	383.61 ± 1.44^{ba}

In each column and for each species, different letters mean significant differences ($p < 0.05$).

The results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD Test with $\alpha = 0.05$. This treatment was carried out using SPSS v. 16.0 software.

3. Results and discussion

3.1. Macronutrients profile

The macronutrients profile and estimated energetic value obtained for the wild mushroom species are shown in Table 1. Moisture ranged from 82.42 g/100 g of fresh material in *T. imbricatum* and 91.67 g/100 g in *C. glaucopus*. Protein was found in high levels and varied between 24.32 g/100 g in *S. mediterraneensis* and 76.63 g/100 g in *L. inversa*. Albumins (24.8%), globulins (11.5%), glutelin-like material (7.4%), glutelins (11.5%), prolamins (5.7%) and prolamine-like material (5.3%) are the mainly proteins present in mushrooms [12]. Several authors referred mushrooms as a good source of essential amino acids such as: leucine, valine, threonine, lysine, methionine and tryptophan. Leucine and valine were found to be the most abundant essential amino acids, comprising 25–40% of the total amino acid content [27,28]. Wild mushroom proteins also contain considerable amounts of non-essential amino acids such as: alanine, arginine, glycine, glutamic acid, aspartic acid, proline and serine. They are important in providing structure to cells, tissues and organs and therefore essential for growth and repair [29].

Fat ranged from 0.36 g/100 g in *H. capnoides* and 3.76 g/100 g in *L. laccata*. Mushrooms are recognized as an excellent choice for low energy diets, as they have high water and low fat content (average of 2–8% of dry weight). Fat in mushrooms contains all classes of lipid compounds including free fatty acids, mono-, di-, and triglycerids, sterols, sterol esters and phospholipids [1].

Carbohydrates, calculated by difference, were also an abundant macronutrient and ranged from 10.35 g/100 g in *L. inversa* and 55.48 g/100 g in *H. aurantiaca*. These results are in agreement with other authors who reported carbohydrates contents between 3 and 65% of dry weight [1]. Reducing sugars are only a small part of carbohydrates content since wild edible mushrooms are rich in non-starch polysaccharides (dietary fiber, 3–32% of dry weight), such as glycogen (animal and fungi reserve polysaccharide), β-glucan and chitin (structural polymers) [12].

Table 2
Analytical characteristics of the sugars analysis method.

	R_t (retention time)		Correlation coefficient (r^2)	Linearity range (mg/ml)	Limit	
	min	CV, %($n = 10$)			LOD (mg/ml)	LOQ (mg/ml)
Fructose (IS)	6.041	0.13	–	–	–	–
Mannitol	6.470	0.31	0.9997	0.3–80.0	0.07	0.22
Trehalose	8.672	0.58	0.9997	0.3–80.0	0.07	0.24

The wide variety and abundance of minerals are the most characteristic features of fungi, being higher than in agricultural plants, vegetables and fruits [30]. Ash content varied between 5.92 g/100 g in *H. aurantiaca* and 28.29 g/100 g in *H. capnoides*.

Among all the studied species, only *F. hepatica* and *R. delica* chemical composition had already been described [19], but from a different country. Despite being the same species our samples presented higher levels of proteins and lower concentration of carbohydrates and fat; it is known that the chemical composition of mushrooms are affected by a number of factors, namely mushroom strain/type, composition of growth media (for in vitro cultured species), time of harvest, management techniques, handling conditions, and preparation of the substrates (in case of cultivated species) [30] and soil/substrate composition or host associated species in case of wild species either saprotrophic or mycorrhizal.

On the basis of the proximate analysis, it was calculated the energetic contribution of the different species; the highest values are guaranteed by *H. aurantiaca*, while *H. capnoides* give the lowest energy contribution (Table 1).

3.2. Sugars profile by HPLC

The analytical characteristics of the method for sugars analysis included evaluation of linearity and determination of limits of detection and

Table 3
Method validation parameters obtained using *T. imbricatum*.

	Precision	Repeatability	Accuracy
	CV, % ($n = 6$)	CV, % ($n = 6$)	(Recovery, %)
Mannitol	0.82	1.02	92.11 ± 4.86
Trehalose	1.47	2.09	91.04 ± 2.24

Table 4
Sugar composition (g/100 g of dry weight) of the wild mushrooms (mean ± SD; $n = 3$).

Mushroom species	Arabinose	Mannitol	Trehalose	Total sugars
Cortinarius glaucopus	nd	1.06 ± 0.16^{ef}	18.66 ± 0.37^{a}	19.72 ± 0.35^{a}
Fistulina hepatica	7.76 ± 0.63^{a}	2.12 ± 0.22^{ef}	2.95 ± 0.22^{e}	12.82 ± 0.93^{c}
Hygrophoropsis aurantiaca	1.53 ± 0.38^{c}	4.31 ± 0.68^{d}	7.56 ± 1.01^{b}	13.40 ± 0.96^{c}
Hypholoma capnoides	nd	0.38 ± 0.04^{f}	1.58 ± 0.40^{f}	1.96 ± 0.44^{e}
Laccaria laccata	nd	0.64 ± 0.05^{f}	5.81 ± 0.33^{c}	6.45 ± 0.34^{d}
Lactarius salmonicolor	nd	13.48 ± 1.95^{b}	0.35 ± 0.05^{g}	13.83 ± 1.98^{c}
Lepista inversa	nd	1.86 ± 0.08^{ef}	4.32 ± 0.27^{d}	6.18 ± 0.35^{d}
Russula delica	nd	18.41 ± 0.38^{a}	0.21 ± 0.03^{g}	18.62 ± 0.35^{ab}
Suillus mediterraneensis	4.03 ± 0.85^{b}	2.89 ± 0.31^{ed}	1.18 ± 0.19^{gf}	8.10 ± 1.11^{d}
Tricholoma imbricatum	nd	10.53 ± 0.28^{c}	6.56 ± 0.22^{cb}	17.09 ± 0.48^{b}

nd—not detected.
The results are expressed as mean ± SD ($n = 3$). In each column and for each species, different letters mean significant differences ($p < 0.05$).

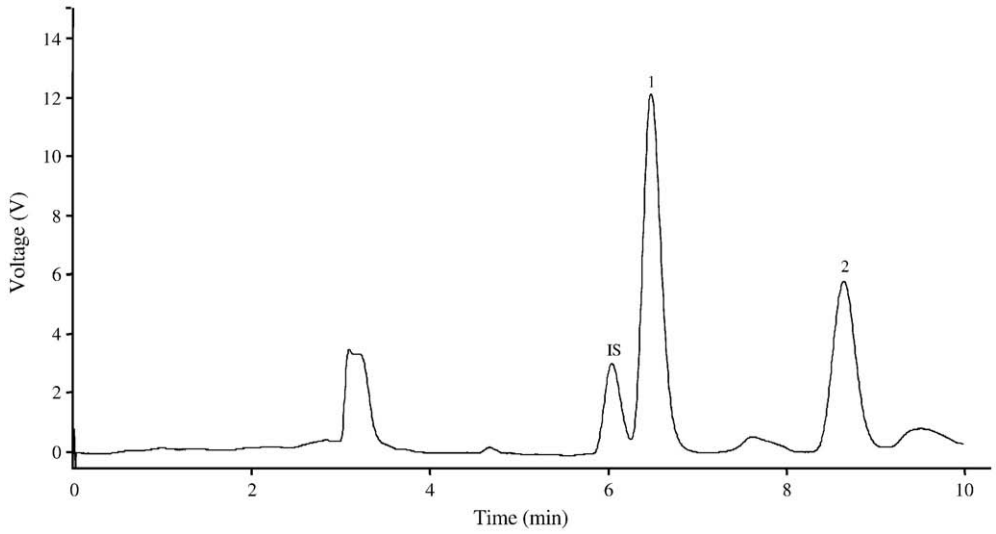

Fig. 1. Individual sugar chromatogram of *T. imbricatum*. IS—internal standard (fructose); 1-mannitol; 2-trehalose.

quantification (Table 2). For each compound, a 7-level calibration curve was constructed using the peak-area ratio between the sugars and IS *versus* concentration ratio between the standards and IS. The average of triplicate determinations for each level was used. Fructose was used as IS because it was not detected in the analyzed mushrooms. An internal standard should be similar to the substance to be quantified, have a proximate, but different, retention time to the substance, not react with the substance or other components present in the matrices, and not be present in the sample [31]; fructose presented all these characteristics. The method validation was performed using mannitol and trehalose because these sugars were detected in all the analysed samples, and their presence was also reported for other species [12,15,17,18].

The correlation coefficients were always higher than 0.999 for all the compounds (Table 2). The limits of detection (LOD), calculated as the concentration corresponding to three times the calibration error divided by the slope, was 0.07 mg/ml. The limits of quantification (LOQ) were calculated using the concentration corresponding to 10 times the calibration error divided by the slope, and ranged from 0.22 mg/ml to 0.24 mg/ml.

In order to evaluate the instrumental precision, the sample (*T. imbricatum*) extract was injected six times. The chromatographic method proved to be precise (CV% between 0.82% and 1.47%, Table 3). Repeatability was evaluated by applying the whole extraction procedure 6 times to the same sample. All the obtained values were low (CV% ranging from 1.02% to 2.09%, Table 3). The accuracy of the method was evaluated by the standard addition procedure (% of recovery) with three addition levels (1 mg/ml, 10 mg/ml and 20 mg/ml, each one in duplicate). The standard mixture was added to the sample, and all the extraction procedure was carried out. The results demonstrate good recovery for the compounds under study (91.04% and 92.11%).

Table 5
Fatty acid composition (percent) of the wild mushrooms (mean ± SD; $n = 3$). Different letters mean significant differences ($p < 0.05$).

	C. glaucopus	*F. hepatica*	*H. aurantiaca*	*H. capnoides*	*L. laccata*	*L. salmonicolor*	*L. inversa*	*R. delica*	*S. mediterraneensis*	*T. imbricatum*
C6:0	0.44 ± 0.04	0.05 ± 0.00	0.06 ± 0.00	0.30 ± 0.02	0.10 ± 0.01	0.26 ± 0.02	0.09 ± 0.01	0.12 ± 0.02	0.04 ± 0.00	0.28 ± 0.00
C8:0	0.04 ± 0.01	0.08 ± 0.00	0.06 ± 0.00	6.38 ± 0.39	0.03 ± 0.00	0.06 ± 0.00	0.08 ± 0.00	0.05 ± 0.00	0.01 ± 0.00	0.17 ± 0.00
C10:0	0.03 ± 0.00	0.72 ± 0.10	0.07 ± 0.00	0.17 ± 0.03	0.01 ± 0.00	3.74 ± 0.20	0.04 ± 0.01	0.19 ± 0.01	0.04 ± 0.00	0.24 ± 0.02
C12:0	0.11 ± 0.01	0.06 ± 0.01	0.22 ± 0.01	0.44 ± 0.03	0.03 ± 0.00	0.38 ± 0.01	0.05 ± 0.01	0.16 ± 0.01	0.05 ± 0.00	0.08 ± 0.00
C13:0	nd	0.01± 0.00	0.02 ± 0.00	0.11 ± 0.03	nd	nd	nd	0.03 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
C14:0	0.41 ± 0.02	0.16 ± 0.01	0.27 ± 0.01	1.10 ± 0.02	0.12 ± 0.00	0.23 ± 0.04	0.28 ± 0.02	1.44 ± 0.05	0.20 ± 0.01	0.20 ± 0.02
C15:0	0.98 ± 0.02	0.27 ± 0.03	0.60 ± 0.01	0.87 ± 0.00	0.05 ± 0.00	0.43 ± 0.04	0.68 ± 0.03	0.31 ± 0.00	0.63 ± 0.01	1.09 ± 0.06
C16:0	12.05 ± 0.02	10.42 ± 0.64	9.97 ± 0.05	16.43 ± 0.16	11.64 ± 0.07	7.35 ± 0.51	16.36 ± 0.23	12.02 ± 0.01	11.93 ± 0.08	7.44 ± 0.15
C16:1	0.42 ± 0.01	0.61 ± 0.11	2.18 ± 0.01	1.24 ± 0.04	0.24 ± 0.00	0.27 ± 0.01	0.30 ± 0.01	3.59 ± 0.06	0.66 ± 0.02	0.19 ± 0.01
C17:0	0.19 ± 0.00	0.13 ± 0.02	0.10 ± 0.00	0.44 ± 0.02	0.06 ± 0.00	0.22 ± 0.03	0.11 ± 0.00	0.17 ± 0.00	0.18 ± 0.00	0.23 ± 0.01
C18:0	2.98 ± 0.00	2.54 ± 0.12	0.92 ± 0.00	4.10 ± 0.07	2.02 ± 0.00	40.13 ± 0.47	1.71 ± 0.03	10.34 ± 0.10	3.56 ± 0.01	4.10 ± 0.01
C18:1n9c	24.01 ± 0.85	31.51 ± 0.07	17.82 ± 0.00	16.98 ± 0.53	60.68 ± 0.01	18.45 ± 0.04	28.78 ± 0.08	41.20 ± 0.06	36.42 ± 0.03	51.53 ± 0.42
C18:2n6c	54.99 ± 1.00	52.37 ± 1.23	55.45 ± 0.26	35.67 ± 0.17	20.45 ± 0.14	26.44 ± 0.20	44.58 ± 0.08	27.15 ± 0.05	43.72 ± 0.17	33.03 ± 0.14
C18:3n3	nd	0.16 ± 0.01	8.16 ± 0.03	0.55 ± 0.03	0.39 ± 0.03	0.47 ± 0.04	4.64 ± 0.22	0.39 ± 0.01	0.02 ± 0.00	0.19 ± 0.00
C20:0	0.30 ± 0.04	0.09 ± 0.01	0.31 ± 0.04	0.62 ± 0.26	0.25 ± 0.00	0.16 ± 0.01	0.33 ± 0.07	0.58 ± 0.01	0.38 ± 0.00	0.13 ± 0.02
C20:1c	nd	nd	nd	nd	0.25 ± 0.04	0.12 ± 0.00	nd	nd	0.15 ± 0.00	nd
C20:2c	0.10 ± 0.01	0.05 ± 0.01	0.15 ± 0.00	nd	0.05 ± 0.01	0.04 ± 0.00	0.06 ± 0.01	0.09 ± 0.00	0.14 ± 0.00	0.04 ± 0.00
C20:3n3+C21:0	nd	0.02 ± 0.00	0.05 ± 0.00	0.31 ± 0.03	0.01 ± 0.00	nd	0.06 ± 0.01	0.03 ± 0.00	0.07 ± 0.00	0.03 ± 0.00
C20:5n3	0.19 ± 0.02	0.09 ± 0.01	0.36 ± 0.07	nd	0.12 ± 0.01	0.11 ± 0.00	0.21 ± 0.05	0.25 ± 0.00	0.09 ± 0.00	nd
C22:0	0.73 ± 0.04	0.16 ± 0.03	0.93 ± 0.02	2.64 ± 0.17	0.61 ± 0.01	0.24 ± 0.02	0.54 ± 0.09	0.65 ± 0.5	0.40 ± 0.02	0.25 ± 0.00
C22:1n9	nd	nd	nd	nd	0.18 ± 0.00	nd	nd	nd	0.33 ± 0.00	nd
C23:0	0.26 ± 0.01	0.09 ± 0.00	0.25 ± 0.01	2.53 ± 0.64	0.12 ± 0.00	0.25 ± 0.02	nd	0.32 ± 0.00	0.20 ± 0.00	0.23 ± 0.01
C22:6n3	nd	nd	0.32 ± 0.01	nd	nd	nd	nd	nd	nd	nd
C24:0	1.06 ± 0.01	0.33 ± 0.01	1.00 ± 0.09	8.23 ± 0.77	0.41 ± 0.00	0.54 ± 0.06	1.03 ± 0.04	0.82 ± 0.01	0.39 ± 0.01	0.46 ± 0.01
C24:1	0.69 ± 0.06	0.07 ± 0.02	0.64 ± 0.01	0.89 ± 0.13	2.19 ± 0.02	0.09 ± 0.02	0.06 ± 0.00	0.10 ± 0.00	0.36 ± 0.02	0.09 ± 0.00
Total SFA	19.60 ± 0.21^{e}	15.11 ± 0.96^{g}	14.75 ± 0.25^{g}	44.37 ± 0.58^{b}	15.44 ± 0.10^{g}	54.00 ± 0.29^{a}	21.30 ± 0.01^{d}	27.20 ± 0.06^{c}	18.04 ± 0.11^{f}	14.91 ± 0.27^{g}
Total MUFA	25.12 ± 0.80^{g}	32.19 ± 0.22^{e}	20.76 ± 0.03^{h}	19.11 ± 0.70^{i}	63.64 ± 0.06^{a}	18.93 ± 0.04^{i}	29.14 ± 0.10^{f}	44.89 ± 0.00^{c}	37.92 ± 0.06^{d}	51.80 ± 0.41^{b}
Total PUFA	55.28 ± 1.01^{b}	52.70 ± 1.18^{c}	64.49 ± 0.22^{a}	36.53 ± 0.11^{f}	21.03 ± 0.18^{i}	27.07 ± 0.25^{h}	49.56 ± 0.10^{d}	27.91 ± 0.06^{h}	44.03 ± 0.17^{e}	33.29 ± 0.14^{g}

nd—not detected.

In what concerns sugar composition (Table 4), mannitol and trehalose were detected in all the samples. For *R. delica* (18.41 g/100 g), *L. salmonicolor* (13.48 g/100 g) and *T. imbricatus* (10.53 g/100 g) mannitol was the most abundant sugar (Fig. 1), while trehalose predominates in *C. glaucopus*, *H. aurantiaca*, *L. laccata*, *L. inversa* and *H. capnoides*, ranging from 1.58 g/100 g to 18.66 g/100 g. The accumulation of the disaccharide trahalose and sugar alcohol mannitol in the fruit-bodies of other species was already reported [12,15,17,18]. Arabinose was the main sugar for *F. hepatica* (7.76 g/100 g) and *S. mediterraneensis* (4.03 g/100 g), and it was detected for the first time in mushrooms samples. *C. glaucopus* revealed the highest sugar contents (19.72 g/100 g), while *H. capnoides* revealed the lowest levels (1.96 g/100 g).

3.3. Fatty acids profile by GC

The results for fatty acid composition, total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) of the studied mushrooms are shown in Table 5. In general, the major fatty acid found in the studied samples was linoleic acid (C18:2), followed by oleic acid (C18:1) and palmitic acid (C16:0). It is known that linoleic acid is the precursor of 1-octen-3-ol, known as the alcohol of fungi, which is the principal aromatic compound in most fungi and might contribute to mushrooms flavour [32]. For *L. salmonicolor* the most abundant fatty acid was stearic acid (C18:0). In previous reports, we found that this fatty acid is characteristic of *Lactarius* sp., being abundant in *L. deliciosus* and *L. piperatus* [33].

Besides the four main fatty acids already described, 21 more were identified and quantified. PUFA were the main group of fatty acids in *C. glaucopus*, *F. hepatica*, *H. aurantiaca*, *L. inversa* and *S. mediterraneensis*, where MUFA were the main group in *L. lacata*, *R. delica* and *T. imbricatum*. For *H. capnoides* and *L. salmonicolor* SFA were the main group (Table 5). UFA predominate over SFA in all the studied species, with exception of *L. salmonicolor* (due to the presence of high amounts of stearic acid), and ranged from 56% to 85%. This is consistent with the observations that, in mushrooms, unsaturated fatty acids predominate over the saturated, in the total fatty acid content [7,10,18]. Considering total PUFA content, *H. aurantiaca* had the highest value due to the high contribution of linoleic acid. The abundance of this essential fatty acid in other edible mushrooms has been described [34]. Considering total MUFA content, *L. lacata* had the highest value due to the high contribution of oleic acid. Both linoleic and oleic acids have been related to decreased risk of cardiovascular disease, contributing to the recommendation of mushrooms in the diets of people with high blood cholesterol [34]. *Trans* isomers of unsaturated fatty acids were not detected in the studied mushrooms.

Overall, the rich nutritional composition (high contents in protein and carbohydrates, low contents in fat with the precious contribution of unsaturated fatty acids, and absence of *trans* fatty acids) makes wild mushrooms very special. The method optimized for the analysis of free sugars proved to be sensitive, reproducible and accurate, being all the compounds separated in a short period of 10 min. This study contributes to the documentation of the nutritional composition of wild mushrooms, which are highly consumed and appreciated, but most of the times without a scientific base of support.

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