

Research article

Chemical composition and nutritional value of nine wild edible mushrooms from Northwestern Tunisia

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Abstract

The chemical composition evaluation and the nutritional value of nine most widespread wild edible mushroom species (*Agaricus arvensis*, *Cantharellus cibarius*, *C. lutescens*, *Craterellus cornucopioides*, *Hericium erinaceus*, *Hydnum repandum*, *Lactarius deliciosus*, *Pleurotus pulmonarius*, and *Ramaria flavescens*) and much collected in northwestern Tunisia, have been analyzed and determined according to standard methodologies. The chemical composition of edible mushrooms was validated by statistical PCA analyzes. Overall results showed that most of the studied species had interesting values for almost all measured variables. In particular, the species *H. erinaceus* had the highest quantity of carbohydrates (89.70%), oleic (24.05%), and docosahexaenoic acid (3.19%), phenolic compounds (11.25 mg g⁻¹ dw), flavonoids (57.5 mg g⁻¹ d.w), and minerals K, Mg and Ca but also the lowest content of proteins (4.80%) and carbohydrates (3.96%). On the other hand, the species *P. pulmonarius* had the lowest lipid content (7.30%) and the lowest caloric value (371.76 Kcal). These promising data can be exploited by taking advantage of the high-quality nutritional value of these interesting species.

Keywords

Hericium erinaceus, *Pleurotus pulmonarius*, mineral composition, phenolics

Introduction

A wide diversity of wild mushrooms can be found in nature, of which about 2,300 are known as useful species (FAO, 2009). However, 1,154 species are considered as edible. They were particularly preferred for their culinary and organoleptic characteristics (source of aromatic substances with

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unique flavors) (Reis et al., 2017). They were recognized much later for their good quantity of nutritional elements (protein, carbohydrate total sugars, lipid, and composition of fatty acid) and their nutraceutical properties (bioactive compounds, phenolics including flavonoids and tannins) essential for human health (Gargano et al., 2017).

They were becoming more important in the human diet for their crude dietary fiber content, mineral elements (Falandyasz and Borovička, 2013) and proteins that can serve as a good substitute for animal proteins in vegetarian diet (Wang et al., 2014). These functional characteristics are mainly due to their content of different types of biologically active compounds in fruit bodies, cultured mycelia, and cultured broth (Chang and Wasser, 2017), essentially depending on species and substratum. In addition, Vetrivka et al. (2019) had demonstrated, by clinical trials, the high medicinal value of some edible mushrooms which contain physiological agents and metabolites having antitumour, cardioprotective, antiviral, antibacterial, immunoregulatory properties. On the other hand, (i) the first annotated checklist of macromycetes in northern Tunisia (Ouali et al., 2018) with 123 species recorded, (ii) the updated checklist with 145 new species recorded (Ouali et al., 2021) and (iii) the record of new species in Tunisia (Jaouani et al., 2015; Ouali et al., 2020) show that this country harbors a large reservoir of fungal diversity especially in northwestern areas. Therefore, it is stimulating to better exploit this diversity, particularly species of important commercial interest such as edible and medicinal mushrooms by determining their chemical composition and studying their nutritional value to better valorize these resources. Despite the significant economic of wild edible and medicinal mushrooms in the collecting areas (around 50 t/year) according to the National Forest Exploitation Authority, Ministry of Agriculture, 2019 (personal communication), the study of their chemical composition and their medicinal potential remains little studied, under-estimated and limited to few mushrooms (Palazzolo et al., 2012; Kacem Jedidi et al., 2016). Thereby, other species should be analyzed to exploit their nutritional, nutraceutical and medicinal potential. The aim of this paper was to investigate the chemical composition of nine wild edible mushrooms species (eight species are well known, the most collected and sold by villagers, and one rare species (*Hericium erinaceus*) recently recorded (Ouali et al., 2020) in northwestern Tunisia. The study was focused on identifying the mineral and biochemical constituents that contribute most to their nutritional value and highlighting species that had the best correlation between these elements.

Material and Methods

Sample collection, identification and preparation

The sporomata of nine species of wild edible mushrooms were collected from two sites in northern Tunisia (Fig. 1, Table 1): eight species were frequently harvested in the area, showing a great economic interest as a source of income for local population, and a rare species known for its medicinal properties (*H. erinaceus*). The macro-morphological and microscopic identification of collected mushrooms was performed on fresh material according to the methodology adopted by Ouali et al. (2018). The mushroom basidiomata (cap and stipe) were cleaned of forest debris and lyophilized (Unicryo MC4L, UniEquip GmbH, Germany), reduced to fine dried powder, and mixed to obtain homogeneous samples and stored, at room temperature, in opaque glass boxes, protected from light, until further analyses.



Fig. 1 - Map of Tunisia; study area: I = Jendouba: AinDraham - OuedEzzen forest; II = Beja: Nefza - Bellif 1 forest.

Table 1 - Edible wild mushrooms studied with habitat characteristics (Em: Ectomycorrhizal; Pn: Parasite necrotrophic; Sh: lignicolous saprotroph; St: terricolous saprotroph).

Specimens	Family	Ecological categories	Governorates (Region - Site - GPS coordinates)	Host vegetation
<i>Cantharellus lutescens</i> Fr.	Cantharellaceae	Em	Jendouba (Ain Draham – Oued Ezzen forest; 36°46'01,7 N; 8°47'39,3 E; 554 m)	Conifer woods of <i>Pinus halepensis</i>
<i>Lactarius deliciosus</i> (L.) Gray	Russulaceae	Em		Mixed oak woods of <i>Quercus canariensis</i> and <i>Q. suber</i>
<i>Agaricus arvensis</i> Schaeff.	Agaricaceae	St		
<i>Hericium erinaceus</i> (Bull.) Pers.	Hericiaceae	Sh		
<i>Hydnum repandum</i> L.	Hydnaceae	Em		
<i>Ramaria flavescens</i> (Schaeff.)	Gomphaceae	Em		
<i>Cantharellus cibarius</i> Fr.	Cantharellaceae	Em	Beja (Nefza - Bellif 1 forest; 37°02'19,2 N; 9°08'01,2 E; 183 m)	
<i>Craterellus cornucopioides</i> (L.)		Em		
<i>Pleurotus pulmonarius</i> (Fr.) Quél.	Pleurotaceae	Pn		

Analytical methods

Ash content was determined by sample calcination at 525 °C after 4 h. The assay of minerals (K, P, Mg, Ca, Na, Fe, Cu, Zn, Mn, and Cr) was analyzed by atomic emission spectrometer with induction coupled plasma (Perkin Elmer, Optima, 7300DV) according to ISO 11885 standards (2007). The assay of total nitrogen (TN) was determined using modified Kjeldahl method, according to ISO 11261 standards (1995). The crude protein content was estimated by multiplying the nitrogen content by the factor 4.38 (= 4.38 × N) (Barros et al., 2007). Total carbohydrates were extracted using the method described by Barros et al. (2007) and their profiles were obtained by high performance liquid chromatography HPLC (Agilent 1200 series, USA) equipped with a quaternary pump model coupled to a refractometer index (RI) detector and with Aminex HPX-87 H ion-exchange columns (300 × 7.8 mm, Bio-Rad) as described by Heleno et al. (2009). The mobile phase was sulfuric acid 5 mmol l L⁻¹ (in milliQ water) at a flow rate of 0.5 mL 1/min. This HPLC was connected to a computer running WINILAB III software (Perichrom, France).

Total lipids were extracted by the mixture chloroform/methanol (2:1, v:v) as the solvent containing 0.01% butylated hydroxyl toluene (BHT) as antioxidant. The organic solvent was evaporated under a stream of nitrogen and the lipid content was determined gravimetrically. Then, extracted lipids were suspended in chloroform/methanol (2:1 v/v) and stored at -30 °C prior to chromatographic analysis. For fatty acid analysis, lipids extracts were esterified according to ISO 5509 (2000) standards trans-esterification method. Fatty acids were determined by gas-liquid chromatography with flame ionization detection (GLC-FID)/capillary column. The fatty acid profile was analyzed with a chromatograph (HP 6890) equipped with a split-split less injector, a flame ionization detector (FID). The temperatures of the injector and detector were maintained at 250 °C and 275 °C, respectively. Separation was achieved on 30 m × 250 µm i.d fused Innowax capillary column (HP) coated with a 0.25 µm film. Nitrogen was used as carrier gas at an internal pressure of 120 kPa. The column temperature was programmed to rise from 50 to 180 °C at a rate of 4 °C/min, then programmed to increase from 180 °C to 220 °C at 1.33 °C/min and then held for 7 min. Fatty acids were identified through comparison of their retention times with those of a mixture of methyl esters (SUPELCO PUFA-3). Methyl nonadecanoate C19:0 (Sigma) was used as internal standard, and then fatty acid peaks were integrated and analyzed using HP chemstation software.

Total carbohydrates were calculated by difference:

Total carbohydrates = 100 – (g moisture + g protein + g fat + g ash).

Total energy was calculated according to the following equations:

Energy (kcal) = [4 × (g protein + g carbohydrate)] + [9 × (g lipid)]

Extraction of phenolic compounds, flavonoids and tannins, from freeze-dried carpophores was carried out according to the slightly modified protocol described by Palacios et al. (2011). The total phenolic and flavonoids content in the mushroom methanolic extracts was estimated using the colorimetric assays of the Folin-Ciocalteu and Aluminum Chloride (AlCl₃), respectively. Gallic acid was used to calculate the standard curve for the phenolic total content. The results were expressed as milligrams of gallic acid equivalents per gram of dried mushroom (mg GAE g⁻¹ dw). The total content of the tannins in the mushroom methanolic extracts was evaluated by the colorimetric test with vanillin 4%. Catechin was employed to calculate the standard curve of the described method. For the total contents of flavonoids and tannins, the results are expressed in milligrams of catechin equivalents per gram of dried mushroom (mg CE g⁻¹ dw). The absorbance was measured with spectrophotometer (Perkin Elmer, Lambda 25, UV-VIS) at 760 nm, 510 nm and 500 nm for the phenolic compounds, flavonoids, and tannins contents, respectively.

Statistical analysis

For the ash, as well as mineral elements, phenolic compounds, flavonoids and tannins, the effect of the fixed factor species was tested with an analysis of variance (ANOVA). The residual normality as well as homogeneity of variances assumptions was assessed using Shapiro test and Levene's test, respectively. LS-means were calculated and compared with Tukey test. These statistical analyses were carried out using SAS 9.4 (SAS Institute Inc, Cary, North Carolina, USA). A principal component analysis (PCA) was separately performed on the two groups of variables measured on mushroom species: (1) mineral composition, and (2) biochemical composition (protein, carbohydrate, total sugars, lipid, and composition of fatty acid) that were centered and reduced beforehand. Then, the relationship between these two variable groups was examined using a co-inertia analysis (CoIA) (Dolédéc and Chessel, 1994). The PCA and CoIA were executed using respectively Vegan (Oksanen et al., 2019), and ade4 (Dray et al., 2019) R-packages R-3.6.1.

Results and discussion

Mineral composition

All the measured mineral variables varied significantly according to the species characteristics (Table 2). Ash content ranged from 4.8 g kg⁻¹ to 17.6 g kg⁻¹ dw, with an average (11.7 ± 3.9 g kg⁻¹ dw). The lowest mean values of ash were recorded in *H. erinaceus*, followed by *L. delisiosus*, and the highest one was monitored in *C. cibarius* (Table 2). These data were higher than those found on similar species in Tunisia (Kacem Jedidi et al., 2016) and in other countries such as Turkey (Onbaşılı et al., 2015) and Bangladesh (Colak et al., 2009). They indicate that mushrooms absorb major and trace elements more than plants. Indeed, mushrooms have effective mechanisms of mineral absorbency notably using low molecular weight organic acids (Mleczek et al., 2016).

The K content varied considerably from 15.75 to 71.15 g kg⁻¹ dw (mean 34.57 ± 15.00 g kg⁻¹ dw), whereas P content varied slightly from 1.0 to 12.9 g kg⁻¹ dw (mean: 4.19 ± 3.30 g kg⁻¹ dw). Both K and P contents were more abundant than Mg (0.5–2.0 g kg⁻¹; mean: 1.13 ± 0.47 g kg⁻¹), Ca (0.1–1.7 g kg⁻¹; mean: 0.72 ± 0.42 g kg⁻¹ dw) and Na (0.02–2.15 g kg⁻¹ dw; 0.52 ± 0.58 g kg⁻¹ dw) (Table 2). These results confirm those of Andres and Baumann (2012), reporting that the main constituents in mushroom ashes were K and P (60% totally).

Hericium erinaceus had the highest average contents of K, Mg and Ca elements, whereas *A. arvensis* had the highest average of P content and the lowest mean contents of Ca and Na. *Cantharellus lutescens* had the highest Na content while K, Mg and Ca contents in *C. cibarius* were higher, and Na content was considerably lower, than those reported by Kacem Jedidi et al. (2016). However, *H. repandum* had the same K content but a very low Na content compared to that reported by Kacem Jedidi et al. (2017). The accumulation of these major elements in all species may be due to their abundance in the soil or to the composition of substratum (Vaishaly et al., 2015).

The trace elements, represented by Fe (mean: 280 ± 260 mg kg⁻¹ dw) and Zn (39.2 ± 27.8 mg kg⁻¹ dw), were more abundant than the other elements, Cu (18.5 ± 16.2 mg kg⁻¹ dw), Mn (8.4 ± 11.8 mg kg⁻¹ dw) and Cr (1.7 ± 3 mg kg⁻¹ dw) which were lower in traces. This observation is in agreement with Vaishaly et al., (2015), reporting that mushrooms are known as Zn accumulators. All specimens possess lower Zn content and higher Fe content than reported by Colak et al. (2009), except for lignicolous *H. erinaceus* which had the lowest Fe content and null contents of Cu, Zn, Mn and Cr. This exception is probably due to the saprotrophic ecology of *H. erinaceus* and to the specific bioaccumulation metabolism of metals by fungi. On the other hand, terricolous *A. arvensis* had the highest content for both Cu and Zn.

In this study, Fe, Mn and Cr contents of *C. cibarius* were higher than those reported by Colak et al. (2009) for the same species. These metal levels can be interesting for human health and assured their daily recommended intake. In contrast, Fe and Zn contents of this species were higher, and its Cu content was considerably lower than those reported by Kacem Jedidi et al. (2016), on the same species, collected in the same areas in northwestern Tunisia in 2012-2013. This may be due to yearly or microclimate variations, to the environmental conditions (site with ferralitic soil) of the mushroom growth that have influence on the concentration of metal absorption by mushrooms. These concentrations in Cu are higher than those in vegetables and should be considered as a nutritional source of this element. Indeed, the *Recommended Dietary Allowances* (RDA) for adults is 0.9 mg Cu/day (Andres and Baumann, 2012). Cu contents of the sampled mushrooms are not considered as a health risk. This element acts as an antioxidant and contributes to the good functioning of the nervous and immune system (EFSA NDA Panel 2009). In the present study, mushrooms were shown to be safe in terms of heavy metal contents. Indeed, the studied fungi showed negligible and acceptable levels of heavy metals (Fe, Zn, Cu, Mn and Cr) by reference to other data reported by Galgowska and Pietrzak-Fiećko (2020) and Širić et al. (2016). The mushrooms are characterized by high contents of assimilated minerals that depend not only on the species, but also on the collecting site, the nature of substratum (acidic and organic matter content of the soil) and even the distance from sources of pollution (Andres and Baumann, 2012). However, the relationship between soil contamination with trace elements and their content in sporomas is not tight enough to enable usage of mushroom species as a reliable bio-indicator of local pollution (Andres and Baumann, 2012). This study shows that *H. erinaceus* and *P. pulmonarius* are the species with the best mineral composition for human consumption with average contents of major elements (K, P, Ca, and Mg) and low contents of heavy metals (Cr, Mn, Zn).

Biochemical composition

Wild mushrooms were rich sources of protein and carbohydrates, and had low amounts of fat (Barros et al., 2007). In this study, mushroom protein content was ranging from 4.8 g/100 g dw (for *H. erinaceus*) to 33.94 g/100 g dw (for *A. arvensis*). Indeed, some mushrooms had protein content below meats, but well above most other foods, including milk (25.2%), cereals (7.3% in rice, 12.7% in wheat and 9.4% in corn) and most of wild plants. The protein content in *A. arvensis* (33.94 g/100 g of dw) was higher not only than that reported by Kumar et al. (2013), for the same species, but also higher than that of milk and other wild and cultivated mushrooms. Several studies have proven mycoproteins' nutritional, health and environmental benefits and affirm their role in a healthful diet. Indeed, mycoproteins are rich in essential amino acids and can help maintain healthy blood cholesterol levels, promote muscle synthesis, control glucose and insulin levels, and increase satiety (Finnigan et al., 2019). Study on human volunteers has revealed that the biological value of proteins in mycoproteins is similar to that of milk proteins (Hashempour-Baltork et al., 2020). Hence, mycoproteins constitute a sustainable and satisfying alternative protein source that can replace environmentally-damaging animal-based foods (Xu et al., 2021). Several authors (Barros et al., 2008; Colak et al., 2009; Kumar et al., 2013; Kacem Jedidi et al., 2016) reported higher protein contents in *C. cibarius*, *C. cornucopioides*, *H. repandum* and *P. pulmonarius*. This variation in mushroom protein content could be depending on species, development stage, level of available nitrogen in the soil or substrate and the location.

Table 2 - Chemical composition/energetic value of Tunisian wild edible mushrooms: mean \pm standard deviation (SD); n = 3.

	<i>A. arvensis</i>	<i>C. cibarius</i>	<i>C. lutescens</i>	<i>C. cornucopioides</i>	<i>H. erinaceus</i>	<i>H. repandum</i>	<i>L. deliciosus</i>	<i>P. pulmonarius</i>	<i>R. flavescens</i>
Ash (%)	12.90 \pm 0.35 d	17.50 \pm 0.05 a	11.30 \pm 0.45 ef	16.40 \pm 0.01 b	5.50 \pm 0.01 h	14.40 \pm 0.01 c	6.40 \pm 0.2 g	10.60 \pm 0.53 f	12 \pm 0.02 ed
K (g kg ⁻¹ dw)	43.04 b	37.69 c	26.64 f	35.78 d	70.91 a	28.51 e	16.99 g	26.82 f	26.87 f
P (g kg ⁻¹ dw)	12.50 a	2.18 d	1.10 e	2.48 d	4.91 c	3.81 d	3.04 d	6.04 b	2.55 d
Mg (g kg ⁻¹ dw)	1.66 ba	0.88 d	0.64 d	1 d	1.98 a	0.75 d	0.96 d	1.60 b	1.22 b
Ca (g kg ⁻¹ dw)	0.10 e	1.02 b	0.98 b	0.79 b	1.57 a	0.66 cb	0.33 e	0.53 c	0.70 c
Na (g kg ⁻¹ dw)	0.03 f	0.41 cbd	2.15 a	0.43 cd	0.86 b	0.25 ced	0.15 ed	0.52 cb	0.14 e
Fe (g kg ⁻¹ dw)	0.05 c	0.77 a	0.28 b	0.60 ba	0.02 c	0.57 a	0.08 c	0.05 c	0.26 b
Cu (g kg ⁻¹ dw)	0.05 a	0.05 a	0.02 a	0.02 a	0 a	0.01 a	0.01 a	Trace a	0.02 a
Zn (g kg ⁻¹ dw)	0.12 a	0.03 ba	0.05 ba	0.08 ba	0 b	0.03 ba	0.08 ba	0.04 ba	0.05 ba
Mn (g kg ⁻¹ dw)	trace cd	0.04 a	trace cd	trace cd	0 d	0.01 cb	trace d	0.02 b	0.02 b
Cr (g kg ⁻¹ dw)	trace b	0.01 a	trace b	trace b	0 b	trace b	trace b	trace b	trace b
Total nitrogen (%)	7.75	2.31	1.95	2.69	1.10	2.58	2.82	3.18	2.70
Proteins (%)	33.94 \pm 2.25	10.10 \pm 1.50	8.55 \pm 0.20	11.79 \pm 1.08	4.80 \pm 0.08	11.28 \pm 1.12	12.35 \pm 0.17	13.91 \pm 0.08	11.80 \pm 0.02
Carbohydrates (%)	21.46 \pm 0.42	35.94 \pm 0.86	59.16 \pm 2.25	6.20 \pm 1.42	89.70 \pm 1.25	51.64 \pm 1.42	53.13 \pm 0.65	62.63 \pm 0.50	10.67 \pm 0.72
Mannitol (%)	6.06 \pm 0.05	12.15 \pm 0.04	15.46 \pm 0.15	15.02 \pm 0.20	2.58 \pm 0.58	5.81 \pm 0.88	13.43 \pm 0.50	1.35 \pm 0.50	10.69 \pm 1.23
Trehalose (%)	0.53 \pm 0.00	9.63 \pm 0.02	0.57 \pm 0.05	0.20 \pm 0.01	1.37 \pm 0.36	9.11 \pm 0.31	5.75 \pm 0.05	7.63 \pm 1.40	1.04 \pm 0.75
Total sugars (%)	6.60 \pm 0.05	21.79 \pm 0.55	16.03 \pm 0.22	15.23 \pm 0.18	3.96 \pm 0.50	14.93 \pm 0.50	19.18 \pm 0.01	8.98 \pm 1.66	11.73 \pm 1.50
Total Lipids (%)	26.80 \pm 1.51	32.50 \pm 0.75	35.20 \pm 0.62	61.65 \pm 0.05	26.78 \pm 0.75	18.28 \pm 1.55	23.62 \pm 0.75	7.30 \pm 2.01	60.97 \pm 0.05

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Table 2 - Continued

Fatty acids									
C14:0 (%)	0.30 ± 0.04	0.65 ± 0.02	0.33 ± 0.04	0.50 ± 0.05	0.24 ± 0.08	0.02 ± 0.00	0.28 ± 0.01	0.15 ± 0.01	0.04 ± 0.04
C15:0 (%)	0.29 ± 0.04	0.60 ± 0.02	0.62 ± 0.04	0.68 ± 0.04	0.14 ± 0.00	0.89 ± 0.07	1.13 ± 0.02	0.13 ± 0.01	0.16 ± 0.00
C16:0 (%)	12.60 ± 0.25	12.50 ± 0.10	13.96 ± 1.87	13.86 ± 1.15	24.29 ± 0.25	14.30 ± 0.50	14.74 ± 0.08	15.13 ± 0.56	17.06 ± 1.20
C16:1 (%)	0.65 ± 0.05	0.32 ± 0.02	0.93 ± 0.75	0.50 ± 0.00	0.18 ± 0.00	0.43 ± 0.03	1.22 ± 0.02	3.44 ± 0.34	0.78 ± 0.79
C17:0 (%)	17.30 ± 0.55	18.72 ± 1.15	4.18 ± 1.46	8.88 ± 2.00	14.83 ± 1.91	8.79 ± 0.75	12.23 ± 0.15	16.98 ± 0.82	6.43 ± 0.81
C18:0 (%)	3.83 ± 0.03	3.77 ± 0.02	4.96 ± 0.83	5.67 ± 0.35	3.60 ± 0.78	2.09 ± 0.01	17.17 ± 0.05	3.73 ± 0.42	2.57 ± 0.22
C18:1 (%)	2.05 ± 0.12	1.33 ± 0.01	14.76 ± 1.02	20.26 ± 0.22	24.05 ± 1.64	6.51 ± 0.10	9.18 ± 0.42	3.56 ± 0.15	20.43 ± 0.09
C18:2 (%)	35.4 ± 1.12	1.88 ± 0.03	19.41 ± 1.75	21.02 ± 1.55	11.76 ± 0.91	26.11 ± 0.55	11.89 ± 0.15	15.45 ± 0.45	29.82 ± 0.90
C18:3 (%)	0.07 ± 0.00	0.08 ± 0.00	0.07 ± 0.00	0.05 ± 0.00	0.00 ± 0.00	0.15 ± 0.00	0.17 ± 0.02	0.09 ± 0.05	0.07 ± 0.00
C20:0 (%)	0.60 ± 0.01	1.08 ± 0.01	0.50 ± 0.02	0.24 ± 0.01	0.21 ± 0.01	0.38 ± 0.01	0.46 ± 0.03	0.80 ± 0.51	0.26 ± 0.02
C20:1 (%)	0.53 ± 0.02	0.13 ± 0.00	0.03 ± 0.12	0.61 ± 0.20	0.46 ± 0.04	0.36 ± 0.01	0.77 ± 0.01	0.70 ± 0.22	0.50 ± 0.20
C20:2n-6 (%)	0.14 ± 0.00	0.50 ± 0.01	0.13 ± 0.03	0.18 ± 0.02	0.04 ± 0.01	0.13 ± 0.00	0.25 ± 0.00	0.05 ± 0.01	0.08 ± 0.00
C20:3n-6 (%)	0.40 ± 0.01	0.25 ± 0.00	23.14 ± 1.40	10.94 ± 1.50	0.02 ± 0.02	20.25 ± 1.50	1.59 ± 0.05	0.17 ± 0.02	2.28 ± 0.05
C20:4n-6 (%)	0.37 ± 0.01	0.06 ± 0.00	0.21 ± 0.04	0.11 ± 0.00	0.01 ± 0.02	0.12 ± 0.00	0.15 ± 0.00	0.04 ± 0.00	0.01 ± 0.01
C21:0 (%)	0.20 ± 0.05	0.18 ± 0.01	0.17 ± 0.02	0.16 ± 0.00	0.09 ± 0.00	0.17 ± 0.00	0.24 ± 0.01	0.10 ± 0.00	0.14 ± 0.02
C22:0 (%)	0.26 ± 0.03	0.48 ± 0.02	0.12 ± 0.02	0.27 ± 0.04	0.10 ± 0.01	0.14 ± 0.00	0.30 ± 0.01	0.33 ± 0.00	0.36 ± 0.05
C24:0 (%)	0.91 ± 0.05	0.10 ± 0.00	0.35 ± 0.01	1.13 ± 0.57	0.75 ± 0.03	0.25 ± 0.01	1.02 ± 0.07	2.32 ± 0.12	0.72 ± 0.04
C22:6n-3 (%)	0.33 ± 0.02	0.42 ± 0.05	0.39 ± 0.05	0.69 ± 0.32	3.19 ± 0.57	1.23 ± 0.75	0.21 ± 0.01	0.22 ± 0.04	0.69 ± 0.70
C24:1 (%)	0.03 ± 0.00	1.80 ± 1.12	0.33 ± 0.85	0.50 ± 0.02	1.07 ± 0.36	0.61 ± 0.10	0.32 ± 0.00	0.31 ± 0.05	0.16 ± 0.01
Total SFA (%)	60 ± 1.51	78.93 ± 2.25	40.59 ± 1.50	45.15 ± 0.58	59.22 ± 1.20	44.09 ± 0.50	74.26 ± 0.05	75.87 ± 0.74	45.18 ± 1.35
Total MUFA (%)	3.26 ± 0.85	3.60 ± 0.95	16.06 ± 0.36	21.86 ± 0.24	25.76 ± 1.57	7.91 ± 0.40	11.49 ± 0.12	8.02 ± 0.65	21.87 ± 1.11
Total PUFA (%)	36.70 ± 1.05	3.21 ± 0.90	43.35 ± 0.50	32.99 ± 0.33	15.02 ± 0.95	47.99 ± 0.50	14.26 ± 0.15	16.03 ± 0.21	32.95 ± 1.01
Energy (Kcal) (%)	462.80 ± 0.03	476.66 ± 0.05	514.96 ± 0.70	626.83 ± 0.02	378 ± 0.01	416.20 ± 0.05	474.50 ± 0.05	371.86 ± 0.02	638.59 ± 0.02

The carbohydrates contents of the sampled mushrooms showed high variation from 6.2 to 89.70 g/100 g dw, in *C. cornucopioides* and *H. erinaceus*, respectively (Table 2), indicating that mushroom carbohydrates are also an abundant macronutrient. Carbohydrates are the most important energy source for human nutrition. They support the nervous and immune system, help control blood glucose and insulin metabolism, participate in cholesterol and triglyceride metabolism, and help with fermentation (Holesh et al., 2021). Nevertheless, all samples had lower carbohydrates contents than those found before (Colak et al., 2009; Kumar et al., 2013; Kacem Jedidi et al., 2016), except for *P. pulmonarius*, (62.63%). For total sugars contents, the highest (21.79 g/100 g dw) and the lowest (3.96 g/100 g dw) were revealed in *C. cibarius* and *H. erinaceus*, respectively. To our knowledge this is the first report of sugar profiles for *A. arvensis*, *C. lutescens*, *C. cornucopioides*, *H. erinaceus*, *P. pulmonarius*, and *R. flavescens*. Mannitol and trehalose were detected in all analyzed samples as the most abundant sugars in fungi (Andres and Baumann, 2012). In this study, mannitol was the main dominant sugar in all the studied species, varying from 1.35 to 15.46 g/100 g dw in *P. pulmonarius* and *C. lutescens*, respectively. Trehalose content was ranging from 0.2 to 9.63 g/100 g dw in *C. cornucopioides* and *C. cibarius* respectively. Mannitol and trehalose contents obtained in *C. cibarius* and *C. cornucopioides* were higher than those reported by Barros et al. (2008). *H. repandum* had twice as much trehalose content and low mannitol content compared to that previously reported by Fernandes et al. (2013).

The lipid content of the analyzed mushrooms was remarkable; it varied between 7.3 and 61.65 g/100 g of dw in *P. pulmonarius* and *C. cornucopioides* respectively. Based on the immediate analysis, it can be calculated that an edible portion of 100 g of these dry mushrooms provides on average 484 kcal (2,022 kJ). The highest value was shown by *R. flavescens* (638.59 Kcal) while *P. pulmonarius* had the lowest energy contribution (371.86 Kcal) due to its low fat content (7.3 g/100 g of dw). This result confirmed those reported by Fernandes et al. (2013). Thereby, *P. pulmonarius* could rebalance or supplement the menus too rich in lipids or integrate in hypo caloric diets (Gargano et al., 2017). The chemical composition and energetic value of *H. repandum* agreed with the previous works of Fernandes et al. (2013).

The results of fatty acid composition, total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) of the studied mushrooms were also shown in Table 2. Fatty acid composition analysis allowed the quantification of 19 fatty acids which varies from one species to another. The main fatty acids detected at high contents were palmitic (C16: 0), margaric (C17: 0), oleic (C18: 1) and linoleic acid (C18: 2). This finding agreed with the results reported for mushrooms from Portugal (*A. arvensis*, *C. cibarius*, *C. cornucopioides* and *L. deliciosus*) in which linoleic, oleic and palmitic acids were considered the most specific fatty acids (Barros et al., 2007, 2008). SFA were the most abundant group of fatty acids in all the studied mushrooms, ranging from 40.59% to 78.93%. The main SFA found in studies species is palmitic acid followed by margaric acid. *C. cibarius* revealed the highest SFA content due to the contribution of palmitic, margaric acid, and the lowest contents of MUFA and PUFA, respectively. As reported by Helano et al. (2015), SFA in *H. erinaceus* predominate over PUFA and MUFA due to the high contents of palmitic and margaric acid. Compared to Barros et al. (2007, 2008) studies, palmitic acid content was higher (the double) in *C. cornucopioides*, while it was lower in *A. arvensis* and *C. cibarius*. For *A. arvensis*, *C. cibarius*, *C. cornucopioides* and *L. deliciosus*, both linoleic and margaric acid contents were higher than that found by Barros et al. (2007, 2008). The highest percentage of oleic acid and linoleic acid were respectively observed in *H. erinaceus* and *A. arvensis*. The most predominant UFA was linoleic acid followed by oleic acid, confirming what was found by Helano et al. (2015). Linoleic acid (Omega 6) is the most abundant fatty acid in all studied species. It is the main aromatic compound of most mushrooms, known as mushroom alcohol. Linoleic acid may contribute to the flavor of mushrooms and is necessary for the human body (Ribeiro et al., 2009). *Agaricus arvensis* had the highest content

of linoleic acid, the lowest content of MUFA and high contents of SFA and PUFA. This result agreed with previous ones published by Barros et al. (2007). For *A. arvensis*, *C. cibarius*, *C. cornucopioides* and *L. deliciosus*, linoleic acid contents were lower than that found by Barros et al. (2007, 2008). However, *H. repandum* had higher contents of SFA and PUFA and lower content of MUFA than those reported by Fernandes et al. (2013). Other fatty acids were also detected but in low amounts. *H. erinaceus* had the highest content of docosahexaenoic acid (DHA or Omega-3). The highest content of stearic acid (C18:0) was observed in *L. deliciosus*; it was the main fatty acid present in the genus *Lactarius* as it has been showed by both Fernandes et al. (2013) and Vieira et al. (2014). Compared to Barros et al. (2007) study, *L. deliciosus* had the highest content of palmitic acid and the lowest stearic acid. A remarkable content of homo- γ -linolenic acid (C20:3- ω 6; precursor of the Omega 3 family) was found in *C. lutescens*, *C. cornucopioides* and *H. repandum*. The sample of *H. repandum* had the highest content of PUFA due to the high contribution of linoleic acid and homo- γ -linolenic acid. These two PUFA acids exerting a powerful regulatory role in the treatment of diabetic neuropathy and in cardiovascular disease prevention (Coste et al., 2004). Further studies should be conducted to evaluate other components such as vitamins (i.e., vitamin D2), and sterols (i.e., ergosterol) which have been linked to antioxidant, anticancer, hepatoprotective, antiallergic, antimicrobial, and antiviral activities (Ramos et al., 2019; Hu et al., 2021).

Phenolic compounds, among the most well-known are flavonoids, were the most important groups of secondary metabolites due to their powerful and essential antioxidant properties for human nutrition. The results of phenolic (TP), flavonoids (TF) and tannins (TT) contents in methanolic extract of the analyzed mushrooms were presented in Fig. 2. These contents varied significantly according to *Species*. The TP content varied considerably from 2.49 to 11.25 mg GAE g⁻¹ dw (overall mean: 5.41 \pm 3.36 mg GAE g⁻¹ dw), the TF content varied from 3.05 to 19.34 mg GAE g⁻¹ dw (overall mean: 6.46 \pm 4.51 mg GAE g⁻¹ dw), whereas the TT content varied slightly from 15.23 to 26.84 mg GAE g⁻¹ dw (overall mean: 21.29 \pm 3.66 mg GAE g⁻¹ dw). The analysis of (TP), (TF) and (TT) compound profiles showed that *H. erinaceus* and *R. flavescens* were the richest species in these compounds.

Hericium erinaceus had the highest average content of (TP) and the lowest average content of (TT), while, the highest and the lowest average of (TF) content were recorded in *R. flavescens* and *P. pulmonarius*, respectively. The (TP) average content of *H. repandum* was lower than that reported by Keleş et al. (2011) (2.63 mg GAE g⁻¹ dw versus 4020 g of GAE kg⁻¹ dw). According to Charumathy et al. (2017), the contents of (TP) and (TF) in hot water extract in *H. erinaceus* were higher than that showed in methanolic extract of this study. Compared to Onbaşılı et al. (2015), *L. deliciosus* had higher (TT) content, lower (TP) content and almost double content of (TF). Mushroom samples of *C. cibarius*, *C. cornucopioides* and *L. deliciosus* had higher (TP) compounds and (TF) contents in comparison with data reported by Palacios et al. (2011). The present study confirms the results reported by Charumathy et al. (2017) and Schillaci et al. (2013) showing that *H. erinaceus* and the genus *Pleurotus* had excellent organoleptic qualities, high nutritional value and potential applications in the pharmaceutical and medicinal sectors.

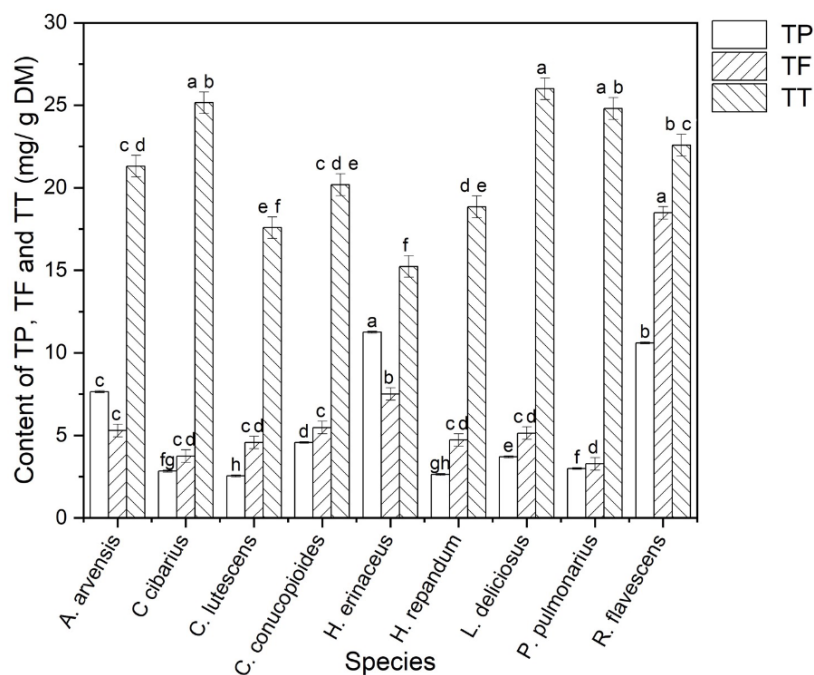


Fig. 2 - Content of total phenolic (TP), flavonoids (TF) and tannin (TT) compounds in Tunisian wild edible mushrooms (mg g⁻¹ dw).

Principal Component Analyses (PCA)

The Principal Component Analyses (PCAs) were performed separately on both mineral and biochemical variables. The PCA was performed on mineral variables and allowed to separate the mushrooms species into four groups. The first group is composed of *A. arvensis*, *H. erinaceus*, *R. flavescens*, *P. pulmonarius*, *C. cornucopioides* and *C. cibarius*. The three other groups are composed each single species (*L. deliciosus*, *C. lutescens* or *H. repandum*) (Fig. 3). According to PCA, the four groups are separate in relation to two origins (Jendouba and Beja). Just mushrooms from Beja were represented only in the first group.

Mushrooms of the first group showed heterogenous ecological categories (Em, St, Sh, Pn) whereas the mushrooms of groups 2, 3 and 4 were all Em. According to mushroom species mineral composition, the two Principal Components (Axes) explained 66.68% (Fig. 3).

The scaling 1 (species scaling) biplot showed a gradient from left to right, starting with the first single-species group (*L. deliciosus*) which displays the highest Fe, Cr, ash and Mn values, and the lowest K value. The second group of species including *H. erinaceus*, *P. pulmonarius*, *R. flavescens*, *A. arvensis*, *C. cornucopioides* and *C. cibarius* which had intermediate values for almost all mineral measured variables. This group could be subdivided into three subgroups: the first subgroup formed by *H. erinaceus*, *P. pulmonarius* and *R. flavescens*; they were not spread out by the variables contributing to axes 1. Within this subgroup, the only two lignicolous saprotroph species (living on tree trunk), *H.*

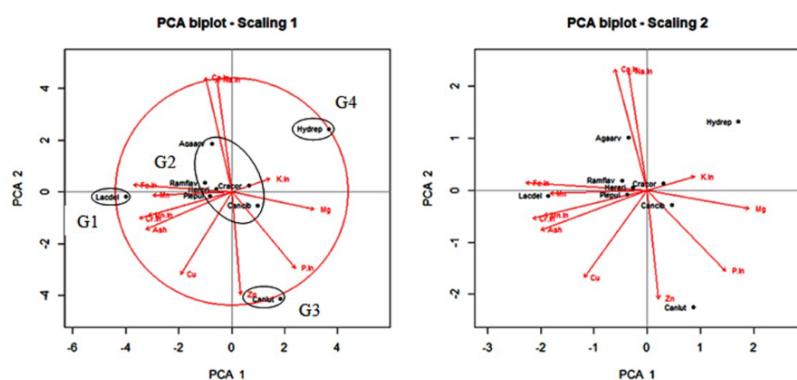


Fig. 3 - Graphical projection of the nine wild edible mushrooms species based on PCA of 11 mineral variables (Ash, K, P, Mg, Ca, Na, Fe, Cu, Zn, Mn, Cr).

erinaceus and *P. pulmonarius*, had null or even trace values of Fe, Cu, Zn, Mn and Cr, and the highest Mg values and an average values Na. Moreover, *H. erinaceus* had the highest K and Ca values and an average value in P, while *P. pulmonarius* had average values of K, P, and Ca. The second subgroup formed by *C. cibarius* and *C. cornucopioides* had almost similar values of all the measured chemical minerals probably because they belong to the same taxonomic family. The last subgroup formed by *A. arvensis* had the highest K value and the lowest Ca and Na values, whereas the third group formed by *C. lutesens* had the highest Na and Ca values and the lowest P and Mg values. The fourth group formed by *H. repandum* had high K value, and low Cr and Mn values.

The scaling 2 biplot (variable scaling) showed that the variables were organized into groups. The lower left part showed that the four ash variables, Cu, Mn and Cr were very highly, positively correlated, and they were very highly, negatively correlated with K. The two variables Ca and Na had a higher contribution than average in comparison to the other mineral variables; they were highly, negatively correlated with both Zn and P. These latter variables as well as Ca and Na were positively related to K and Mg. Fe and Zn content had nearly orthogonal arrows, indicating a correlation close to 0.

The PCA applied on biochemical composition profiles allowed to distinguish four mushroom species groups: 1) *L. deliciosus* and *C. cibarius*; 2) *R. flavescens*, *A. arvensis*, *H. erinaceus* and *P. pulmonarius*; 3) *C. lutesens* and *C. cornucopioides* and 4) *H. repandum* (Fig. 4). Concerning PCA applied on mushroom species biochemical composition, the two first PCAs explained 53.44% (Fig. 4).

The scaling 1 biplot showed also a gradient from left to right, starting with the first group formed by *L. deliciosus* and *C. cibarius* having almost similar (C20:0), (C22:0) and (C16:1) fatty acids contents. The second group of species formed by *R. flavescens*, *A. arvensis*, *H. erinaceus* and *P. pulmonarius* having intermediate values of mannitol, (C20: 3n-6), lipids, PUFA, energy and the lowest values of carbohydrates and (C20:1) fatty acid. *Hericium erinaceus* had average values in total lipids, while *P. pulmonarius* had the highest SFA in particular docosanoic acid (C22:0) and lignoceric (C24:0). Both samples *H. erinaceus* and *P. pulmonarius* have the lowest values in sugars, fatty acids C20:2n, C20:3n, C20:4n, C21:0 and average values in energy, stearic and linoleic acid. A third group formed by *C. cornucopioides* and *C. lutesens* having the highest trehalose, (C17:0) and SFA contents, and

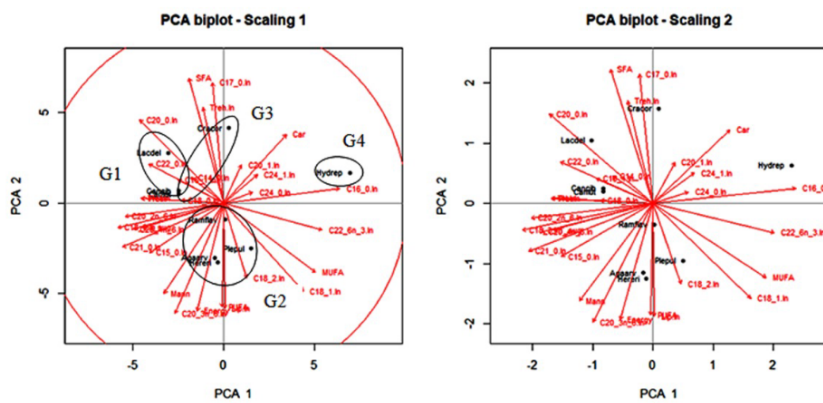


Fig.4 - Graphical projection of the nine wild edible mushrooms species based on PCA of 30 biochemical variables (Total nitrogen, Proteins, Carbohydrates, Mannitol, Trehalose, Total sugar, Lipids and fatty acids).

the lowest (C18: 2), PUFA and lipids contents probably because they belong to the same taxonomic family and same ecological category (ectomycorrhizal species). The fourth group formed by *H. repandum* had high (C16:0) fatty acid content, and low (C20: 2n-6) content.

The species *A. arvensis*, *H. erinaceus*, *R. flavescens*, *C. lutesens*, *C. cibarius*, and *C. cornucopioides* are not spread out by the variables contributing to axes 1 and 2. The lower left part, of the scaling 2 biplot, shows that total sugars and mannitol were very highly, positively correlated, and that these two variables were very highly, negatively correlated with carbohydrates. Furthermore, the group of variables formed by energy, lipids and PUFA were very highly, positively correlated, and that these variables were very highly, negatively correlated with SFA, trehalose and (C17:0). The group of variables SFA and (C17:0) contributed the most to the explained variance by the two axes. These two variables were highly correlated with lipids and PUFA contents group. The carbohydrates and MUFA contents are positively correlated with these two groups.

Total nitrogen, proteins and stearic acid (C18:0) displayed nearly orthogonal arrows to PCA1. As far as that goes, lipids and PUFA displayed nearly orthogonal arrows to PCA2. These variables indicating a correlation almost nil close to 0. While, other variables such as (C14:0), (C20:1), (C24:1) and (C24:0), displayed a shorter arrow, and were less important for the ordination of mushroom species.

Co-inertia analysis (CoIA)

Co-inertia analysis (CoIA) confirms the results of mineral and biochemical variables obtained by PCAs. The mineral and biochemical profile study performed by the PCA and CoIA analysis by confirming the results presented in Table 2. Figure 5 is a global view of the effect of all mineral and biochemical variables based on the results of the principal component analysis (Fig. 3 and 4). The upper right-hand plot (normed species scores) shows the positions of species on the co-inertia axes using mineral composition (origins of the arrows) and biochemical composition (arrowheads) co-inertia weights. The profile of the Co-inertia analysis shows the rapprochement of the chemical composition of the nine species of mushrooms which can be grouped as follows: (i) *H. erinaceus*, *P. pulmonarius*, *R.*

flavescens, *L. deliciosus*, and *A. arvensis*, (ii) *C. cibarius* and *C. lutescens*, (iii) *C. cornucopioides* and (iv) *H. repandum*. The first group has the shortest arrows, showed the best concordance between the two projections of the point of the two variable groups (mineral and biochemical). The lower right-hand pair of plots showed the contributions of these groups of variables. Zn content correlates positively with proteins, content Na and Ca negatively with proteins. P, Mg and K contents are all negatively correlated with total sugars, mannitol, trehalose, lipids and energy since these variables have higher values downstream, and positively correlated with fatty acids (C18: 0), (C18: 2), (C24:

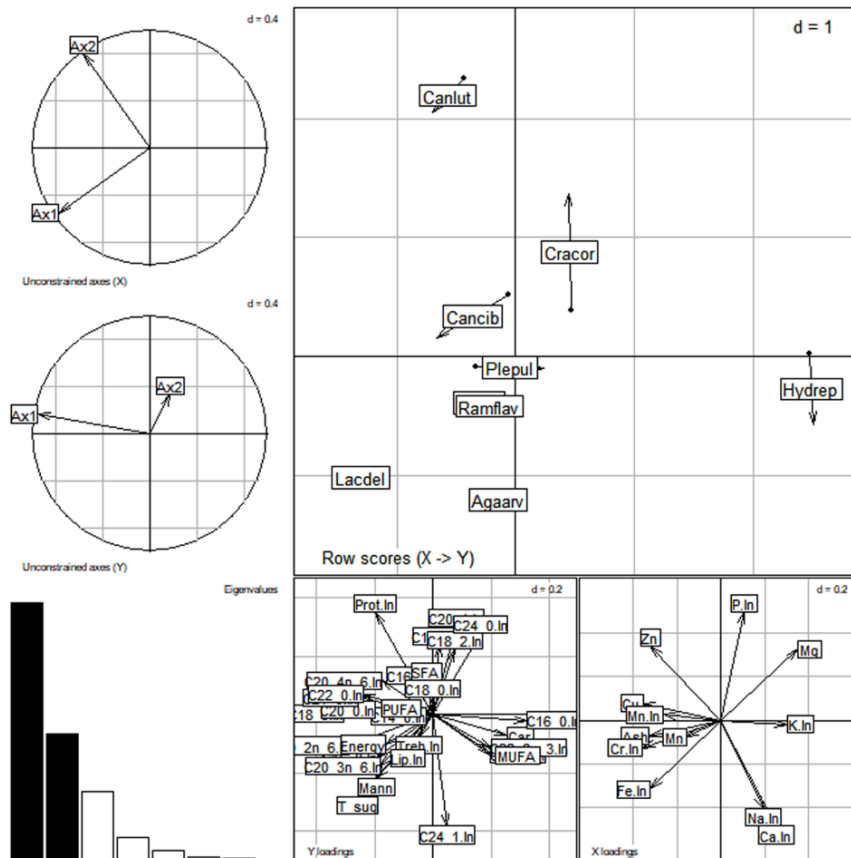


Fig. 5 - Graphic projection of the nine wild edible mushrooms species based on co-inertia analysis of 41 mineral and biochemical variables.

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0) and SFA, which increase downstream. According to the results of co-inertia analysis, P, Mg and Ca contents on the one hand, and proteins, total sugars on the other hand, having the longest vectors contributed more to the nutritional value of species.

Conclusion

This study is the first report on the chemical composition of nine wild edible mushrooms originated from Tunisia. The analyzed mushrooms showed low amounts in fat and calories contents and high levels of protein, carbohydrates, minerals and, phenolics flavonoids and omega-3 fatty acids. Interestingly, *H. erinaceus* appeared to be the most nutritious mushroom; it had the highest amount of carbohydrates, proteins, oleic and docosahexaenoic (DHA) acids, phenolic compounds and flavonoids as well as minerals mainly K, Mg and Ca. Therefore, it could be recommended for the obese, diabetics, and people with cardiovascular diseases. Despite the nutritional and therapeutic importance of *H. erinaceus*, this species remains poorly known in Tunisia and is not protected. Therefore, special awareness program of the importance of this species is needed for mycologists, foresters and general public. Furthermore, *P. pulmonarius* had high carbohydrate content and the lowest lipid content with the lowest caloric value which make them suitable for obesity diet plan. In conclusion our findings support the development and the utilization of edible mushrooms as a complete food ingredient or sustainable alternative material, because of their composition, their safety, and health benefits (Chang and Wasser, 2017). Because of their availability and their low carbon footprint, they could contribute to food security and rural sustainable development. Hence, there is urgent need to a holistic approach for a sustainable management of such a valuable bio-resource. This would include: (i) the conservation of these natural resources and their habitats, (ii) commercial exploitation by improving culture techniques, and (iii) the determination of their biotechnological potential.

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