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ORIGINAL PAPER

Assessing the nutritional and functional properties of mushrooms from North-Eastern Portugal

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Abstract
Mushrooms are recognized as functional foods with noteworthy nutritional, culinary, and pharmacological properties, leading to their growing consumption. The present study aimed to compare the chemical composition and biological properties of six wild species harvested in north-eastern Portugal and two cultivated species (*Lentinula edodes and Pleurotus citrinopileatus*, purchased in Portuguese retail markets) to evaluate their potential as sources of nutrients and bioactive compounds. The results showed diverse macronutrient proximate profiles, characterized by high carbohydrate, dietary fibre, and protein, along with low-fat content and with moderate antioxidant activity. Notably, glucans were present in high amounts, with β-glucans representing the major fraction. Despite species-specific variations, potassium and phosphorus were the predominant mineral elements. Additionally, lysine and arginine were the most abundant free amino acids in the samples. Overall, this manuscript provides a comprehensive insight into the chemical composition, bioactive properties, and nutritional potential of commercially available and wild mushrooms, supplying the first detailed glucan, mineral and antioxidant profile for five under-studied wild species from north-eastern Portugal.

Keywords Wild mushrooms · Functional foods · Nutraceuticals · Glucans

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Introduction

Mushrooms are recognized for their culinary and medicinal properties, making them a nutraceutical food, with a rich history of consumption in different cultures [1–3]. There are around 2189 edible species of wild mushrooms worldwide, nevertheless the number cultivated for commercial purposes is much lower, which shows that there is room for exploitation of these resources [4].

Beyond their flavour and texture characteristics, mushrooms have a rich nutritional profile. They are sources of vitamins and minerals, including vitamin B (such as riboflavin, niacin, and pantothenic acid), and vitamin D (when exposed to sunlight or UV light) [5, 6]. Moreover, mushrooms are notable for their mineral content, with potassium (K) and phosphorus (P) being two prevalent elements. These are followed by calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), zinc (Zn), and copper (Cu), all of which are considered essential for human health [7]. There are significant differences in the mineral content of the mushrooms, especially related to the environmental conditions, for example, the soil, the growing area, and climate factors. Depending on the area of growth, if polluted with toxic compounds such as heavy metals, for example, arsenic (As), chromium (Cr), lead (Pb), or cobalt (Co), the mushrooms will have higher concentrations of these elements, which is considered a serious problem [7].

In addition, mushrooms are a source of amino acids, comprising both essential and non-essential, including tryptophan (try), phenylalanine (phe), leucine (leu), and valine (val) [8]. The amino acid composition of mushrooms can be compared to or even tops the content of soy [9, 10]. Despite their macronutrient and micronutrient content, mushrooms contain diverse bioactive compounds with potential health-promoting properties. Among these, glucans have received the most research attention. They are abundantly present in the cell walls of mushrooms, which are primarily composed of two types of structural polysaccharides: a rigid fibrillary structure of chitin and a matrix-like structure consisting of β-glucans, α-glucans, and glycoproteins. Regarding the α-glucans, the most common are the glucans in which the glycosyl units are bound through α-1,4 and α-1,6 bonds, others solely through α-1,3 bonds, or α-1,3 and α-1,4 bonds [11]. On the other hand, the β- glucans present more variable structures with linear or branched, amorphous or microfibrillar linked by β-1,3 and β-1,6 bonds, often set in crystalline chitin. These polysaccharides exhibit variations in molecular weight, branching patterns, and conformation, influencing their biological activities and functional properties [11–13].

The health benefits attributed to mushrooms extend beyond their nutritional content. Research has demonstrated their potential in modulating immune responses, reducing inflammation, and providing antioxidant protection, which can contribute to the prevention and management of several diseases, including cancer, cardiovascular diseases, and metabolic disorders [14, 15]. Additionally, incorporating mushrooms into diets has been linked to improved gut health due to their prebiotic properties, promoting a healthy microbiome [16].

The existing literature shows that although mushrooms belong to the same species and present specific characteristics, they vary in their biochemical composition. This variability is attributed to differences in genetic strains, the composition of growth substrates, cultivation practices, and diverse environmental and geographical factors such as temperature, humidity and altitude. These factors influence both macronutrient content and the profile of bioactive compounds in mushrooms. Understanding these factors is important for optimizing cultivation strategies and enables the target selection of mushroom varieties adapted to specific nutritional needs and industrial or therapeutic uses [17]. This study explores the chemical composition and biological properties of two commercialized mushrooms (*Lentinula edodes* and *Pleurotus citrinopileatus*) and six wild mushrooms from the northeast of Portugal (*Amanita caesarea*, *Boletus aereus*, *Boletus edulis*, *Boletus radicans*, *Boletus regius*, and *Neoboletus luridiformis*) to assess these products as sources of nutrients and nutraceuticals. Commercial mushroom species were included in the study to provide a comparative baseline, allowing for a more contextual evaluation of the nutritional and biochemical profiles of the wild mushrooms under analysis. Chemical analysis included the determination of protein, fat, ash, carbohydrates, dietary fibre and individual profiles in glucans and minerals content. This comparison not only highlights the properties of the wild edible species from north-eastern (NE) of Portugal but also supports the potential application of these as alternative or complementary sources of nutrients, highlighting their relevance as valuable components of a healthy diet and their potential applications in functional food products. By providing unprecedented β -/ α -glucan ratios, mineral baselines and antioxidant benchmarks for these taxa, the present study fills a critical gap in European mushroom research.

Materials and methods

Mushrooms samples

Eight mushroom species were analysed in this study. The commercial samples of *L. edodes* and *P. citrinopileatus* were acquired from different supermarkets. The remaining

species, including *(A) caesarea*, *(B) aereus*, *B. edulis*, *B. radicans*, *B. regius*, and *N. luridiformis* were collected in a mixed stand at Vinhais, Bragança, located at the North of Portugal in 2023.

Cultivated specimens (*L. edodes* and *P. citrinopileatus*) were purchased in triplicate from the three leading national retail chains that collectively account for >90% of fresh mushroom sales in Portugal [18], thereby mirroring typical consumer exposure and ensuring uniform post-harvest handling. Wild specimens were harvested by mycologists within Montesinho Natural Park (41°53′ N, 6°49′ W). This protected area was selected for its well-characterised macrofungal biodiversity and minimal anthropogenic contamination [19, 20]. All samples were collected during the peak fructification period (mid-October to late-November 2023; mean daily temperature 8–14 °C; cumulative precipitation ≈ 110 mm) to capture a representative biochemical snapshot while minimising inter-seasonal variability [21]. The supermarket procurement radius (< 10 km) centred on Bragança (41°48′ N, 6°45′ W), a demographically stable city devoid of heavy industry and frequently used as a reference region for dietary-exposure studies. This dual-origin strategy balances commercial relevance with ecological conservation contexts, thereby broadening the applicability of the results. The morphological identification of the wild macro fungi was made till species according to macro- and microscopic characteristics [22]. Six wild taxa, *(A) caesarea*, *(B) aereus*, *B. edulis*, *B. radicans*, *B. regius* and *N. luridiformis*, were examined by two independent mycologists. Diagnostic macro-characters (pileus colour/viscosity, hymenophore configuration, stipe ornamentation, context staining reactions, odour and taste) were cross-checked with micro-characters (basidiospore dimensions/ornamentation, presence of clamp connections, cystidia morphology) obtained from 5% KOH and Congo-red mounts viewed under a Nikon Eclipse E-200 microscope (400–1000 ×). Identification followed Courtecuisse & Duhem [23] and Largent et al. [24]. Only collections for which both experts reached complete concordance were retained ($n = 6$); any ambiguous basidiomata were discarded. Cultivated samples (*Lentinula edodes*, *Pleurotus citrinopileatus*) retained their producer-labelled identities and were not re-examined taxonomically. Representative voucher specimens were deposited at the mycological herbarium of Universidade de Trás-os-Montes e Alto Douro (BRESA codes HVR000128 to HVR000133). After taxonomic confirmation, whole fruiting bodies were frozen at − 20 °C, lyophilised (Dura-Dry µP; −41 °C, 500 mTorr), milled to a homogeneous powder, and stored in the dark in hermetically sealed polyethylene bags until analysis.

Representative voucher specimens were deposited at the mycological herbarium of Universidade de Trás-os-Montes e Alto Douro. After taxonomic identification, the mushrooms were immediately stored at −20 °C, freeze-dried (Dura Dry TM µP, −41 °C and 500 mTorr), and ground to a fine powder. The samples were kept in the dark in hermetically sealed plastic bags up to analysis.

Macronutrient analysis

All analyses were performed in triplicate. Dry matter content was determined for all hot-air dried samples to ensure comparability. Each sample was mixed with sea sand and heated at 103 ± 2 °C until a constant weight was achieved. The weight after heating was divided by the initial weight to calculate the dry matter content [3]. The moisture content was determined using an infrared moisture analyzer (SMO 01, Scaltec Instruments, Germany). Ash content was determined by incinerating the sample in a muffle furnace at 550 °C, as described by Pérez-Bassart et al. [2]. The protein content was estimated using the Kjeldahl method, applying a nitrogen-to-protein conversion factor of 6.25. Total fat content was determined by Soxhlet extraction, while the total carbohydrate content was determined by the difference between the remaining macronutrients [25]. The results were presented as percentage of dry weight (% dw). Dietary fibre was determined by enzymatic-gravimetric method, with insoluble dietary fibre (IDF) and soluble dietary fibre (SDF) quantified separately [26, 27]. Total dietary fibre (TDF) was calculated as the sum of IDF and SDF, and results were expressed in g/100 g dry weight.

The total energy content was calculated in kcal/100 g using the equation:

$$\text{Energy (kcal/100 g)} = 4 \times (\text{g proteins} + \text{g carbohydrates}) + 9 \times (\text{g fat})$$

The total energy content in kJ/100 g was also determined using the conversions factor of 4.2:

$$\text{Energy (kJ/100 g)} = \text{Energy (kcal/100 g)} \times 4.2$$

Determination of glucans content

The protocol described by Lemieszek et al. [28], was followed to determine the glucan content, using only the crude polysaccharide extract. 700 mL of 80% ethanol was added to 100 g of the sample powder and heated at 80 °C with stirring for 1 h. The resulting suspension was filtered through glass fibre to remove alcohol-insoluble residues, which were then washed twice with 50 mL of 80% ethanol and dried in a forced-air oven at 50 °C for 12 h. To extract water-soluble polysaccharides, 100 g of the dried alcohol-insoluble residue (AIR) was suspended in 700 mL of boiling water and stirred for 1 h. The suspension was then centrifuged (6000

rpm, 20 min, 4 °C) to separate the water-insoluble material from the supernatant. The insoluble fraction was washed with water and centrifuged again under the same conditions. The collected supernatants were combined, dialyzed (MW cut-off: 12–14 kDa) against water with six water renewals, and finally freeze-dried to obtain the water-soluble glucan fraction.

Glucans (total, α and β). 1,3/1,6-β-glucans were quantified in quadruplicate using a yeast–mushroom enzymatic kit (Megazyme, Bray, Ireland), following the manufacturer's protocol. Freeze-dried powders were milled (IKA analytical mill), sieved (0.5 mm) and weighed (100 mg) into culture tubes. For total glucan, samples were treated with 1.5 mL concentrated HCl (37%) at 30 °C for 45 min, diluted with 10 mL water and heated in a boiling water bath for 2 h. After neutralisation with 2 M KOH, volumes were adjusted to 100 mL with sodium acetate buffer (pH 5.0). Aliquots (0.1 mL) were incubated with exo-1,3-β-glucanase (20 U/mL) and β-glucosidase (4 U/mL) at 40 °C for 60 min, followed by addition of GOPOD reagent (3 mL) and incubation at 40 °C for 20 min.

For α-glucan (starch/glycogen), 100 mg of powder were stirred with 2 mL KOH (2 M) in an ice-water bath for 20 min, then mixed with 8 mL sodium acetate buffer (pH 3.8) and amyl glucosidase (0.2 mL; 1630 U/mL) and incubated at 40 °C for 30 min. Aliquots (0.1 mL) were combined with 0.1 mL sodium acetate buffer (pH 5.0) and 3 mL GOPOD and incubated at 40 °C for 20 min.

Absorbance was read at 510 nm (LKB Biochrom photometer) against reagent blanks. A yeast β-glucan standard and an internal mushroom-powder control were run with each batch. Values were corrected for free glucose and glucose released from sucrose/oligosaccharides according to the kit instructions. β-Glucan was calculated by difference:

$$\beta - \text{glucan (g/100 g dw)} = \text{Total glucan (g/100 g dw)} - \alpha\text{ - glucan (g/100 g dw)}$$

Results are expressed as g/100 g on a dry-weight basis. We used this specific enzymatic approach (which resolves α-vs total glucan and yields β-glucan by difference) rather than the non-specific phenol–sulfuric-acid assay [29]. Reported assay precision is typically < 5%, and its performance for β-glucan determination in fungal matrices has been validated previously [30, 31].

Antioxidant activity

Antioxidant activity was determined using the ABTS assay (2.2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid), following the method described by Garcia et al., [32] with some differences. A stock solution was prepared by dissolving 0.192 g of ABTS and 0.033 g of potassium persulfate in 50 mL of phosphate-buffered saline (PBS, pH 7.4), followed by overnight stirring. Before analysis, the ABTS·+ radical cation solution was diluted in PBS to achieve an initial absorbance of 0.70 ± 0.02 at 734 nm. Subsequently, 230 µL of the ABTS·+ solution was mixed with 20 µL of each sample, and the absorbance was measured at 734 nm after 6 min incubation at room temperature in darkness. Measurements were conducted in microplates using a SpectraMax iD3 (Molecular Devices) spectrophotometer. A calibration curve was generated using 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), and antioxidant capacity was expressed as mM Trolox equivalents/g (mM TE/g), using Trolox as the standard reference.

The FRAP assay was conducted following the protocol described by Costa et al. [33]. Briefly, 20 µL of the extract was mixed with 180 µL of FRAP working solution, which consisted of 10 volumes of acetate buffer (300 mM, pH 3.6), 1 volume of ferric chloride (20 mM in water), and 1 volume of TPTZ (10 mM dissolved in hydrochloric acid). The microplate was then protected from light and incubated at 37 °C for 30 min. Absorbance was measured at 593 nm, and results were expressed mM TE/g, using Trolox as the standard reference. All the determinations were performed in triplicate.

Element analysis

The essential trace elements in mushroom samples were analysed by fluorimetry following a modified nitric acid/hydrogen peroxide digestion, with subsequent reaction with 2,3-diaminonaphthalene (DAN) [34]. Precisely weighed samples (0.010–0.10 g) were placed in acid-washed glass culture tubes (16 × 160 mm) and treated with 1mL of concentrated nitric acid and 0.5 mL of hydrogen peroxide. The mixture was vortexed and incubated at room temperature overnight. The digestion process involved sequential heating: 50 °C for 1 h, 100 °C for 1 h, 120 °C for 1 h, and finally 155 °C overnight, or until the solution became clear. Acid-washed glass marbles were used to cover the tubes during digestion and were removed before complete drying at 155 °C. Once cooled to room temperature, 0.5 mL of 5 M HCl was added, and the tubes were sealed with Teflon-faced screw caps. The mixture was heated at 100 °C for 30 min, cooled, and then treated with 10 mL of 10 mM EDTA solution and 2 mL of DAN (0.1% in 0.1 M HCl). After vortex-mixed, the solution was heated at 60 °C for 30 min. Following cooling, 5mL of cyclohexane was added, thoroughly mixed, and allowed to separate. The organic phase (3 mL) was extracted and analysed fluorometrically using an FP-777 spectrofluorometer (Jasco) at excitation and emission wavelengths of 375 nm and 525 nm, respectively.

Results are expressed in mg/kg on dry weight basis, and all analyses were performed in triplicate.

Amino acids determination by high-performance liquid chromatography with fluorescence detection (HPLC-FLD)

Amino acids, including histidine (his), arginine (arg), serine (ser), asparagine + aspartic acid (asx), glutamic acid + glutamine (glx), threonine (thr), glycine (gly), alanine (ala), proline (pro), valine (val), phenylalanine (phe), isoleucine (ile), leucine (leu), lysine (lys) and tyrosine (tyr), were analysed by reversed-phase high-performance liquid chromatography with fluorescence detection (HPLC-FLD), as described by Baltazar et al. [35]. Samples were subjected to acidic hydrolysis (6 M HCl, 110 °C, 24 h) before derivatization. Chromatographic analysis was performed using an integrated HPLC system equipped with Thermo Scientific Dionex Ulti Mate 3000 Series system (Thermo Fisher Scientific, Inc., Waltham, MA, USA), composed of a RS quaternary pump, a WPS-3000RS autosampler (maintained at 4 °C), a TCC-3000RS column compartment (maintained at 35 °C), and a FLD-3400RS fluorescence detector (excitation and emission wavelength were set to 250 and 395 nm, respectively). Separation was achieved using an ACE 5 C 18 column (5 µm, 150 mm x 4.6 mm i.d., Advanced Chromatography Technologies Ltd., Aberdeen, Scotland). Chromatographic data were analysed using Chromeleon software version 7.2 (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Amino acids were identified by comparing retention times with authentic standards, and quantification was performed using the internal standard method. Results are expressed in mg/100 g on dry weight basis, and all analyses were performed in triplicate.

Statistical analysis

All data are presented as mean ± standard deviation (Mean ± SD). One-way ANOVA was used to evaluate the significance of differences among treatments, followed by Tukey's post-hoc test for multiple comparisons. A p-value of < 0.05 was considered statistically significant. All statistical analyses were performed using Excel and JASP.

Results and discussion

Proximate composition and glucans content

The following Table 1 present the results of the proximate composition of the commercial (*L. edodes and P. citrinopileatus*) and wild (*A. caesarea*, *B. aereus*, *B. edulis*, *N. luridiformis*, *B. regius*, *B. radicans*) mushroom species, confirming the variation in their macronutrient profiles, ash, and energy content.

Table 1 Proximate composition (%), moisture (%), and dry matter (%) of commercial (*L. edodes* and *P. citrinopileatus*) and wild (*A. caesarea*, *B. aereus*, *B. edulis*, *N. luridiformis*, *B. regius*, *B. radicans*) mushrooms species (whole fruiting bodies) on dry weight basis, including energy values expressed in kcal/100 g and kJ/100 g. Data are presented as mean ± SD

Mushroom species	Moisture	Dry matter	Protein	Fat	Ash	Carbohydrates	Energy (kcal/100 g)	Energy (kJ/100 g)
L. edodes	$9.38 \pm 0.45^{a, b}$	93.98 ± 2.98^{a}	17.90 ± 0.21^{c}	$5.57 \pm 0.72^{c, d}$	5.73 ± 0.21^{f}	70.81 ± 1.23^{a}	$404.93 \pm 2.79^{c, d}$	$1700.69 \pm 11.70^{c, d}$
P. citrinopileatus	$9.72 \pm 0.28^{a, b}$	$89.55 \pm 3.96^{a, b,c}$	33.99 ± 1.93^{a}	$9.08 \pm 1.01^{b, e}$	7.88 ± 0.13^{b}	49.05 ± 1.84^{e}	$413.90 \pm 5.60^{a, d,e}$	$1738.38 \pm 23.51^{a, d,e}$
A. caesarea	$9.57 \pm 0.09^{a, b}$	$91.42 \pm 3.96^{a, b}$	$21.71 \pm 0.02^{b, c}$	13.33 ± 0.79^{a}	11.39 ± 0.11^{a}	53.58 ± 0.92^{e}	$421.06 \pm 3.49^{a, b}$	$1768.44 \pm 14.67^{a, b}$
B. aereus	$9.05 \pm 0.89^{a, b}$	$82.56 \pm 2.42^{c, d}$	26.06 ± 0.79^{b}	4.43 ± 0.39^{c}	7.13 ± 0.16^{c}	62.38 ± 0.88^{b}	393.63 ± 2.58^{c}	1653.24 ± 10.85^{c}
B. edulis	10.26 ± 0.29^{a}	80.32 ± 3.93^{d}	32.49 ± 1.50^{a}	$6.94 \pm 0.89^{c, e,f}$	$6.88 \pm 0.04^{c, d}$	53.69 ± 1.19^{e}	$407.19 \pm 4.29^{b, c,d}$	$1710.19 \pm 18.02^{b, c,d}$
N. luridiformis	8.43 ± 0.30^{b}	$85.59 \pm 5.96^{b, c,d}$	18.12 ± 0.04^{c}	$8.02 \pm 0.13^{b, d,f}$	$6.45 \pm 0.03^{d, e}$	$67.41 \pm 0.11^{a, c,d}$	$414.30 \pm 0.52^{a, d,e}$	$1740.05 \pm 2.20^{a, d,e}$
B. regius	$8.79 \pm 0.16^{a, b}$	$90.06 \pm 3.65^{a, b,c}$	19.88 ± 0.97^{c}	10.09 ± 0.72^{b}	$6.21 \pm 0.12^{e, f}$	$63.82 \pm 1.97^{b, c}$	425.64 ± 4.10^{a}	1787.68 ± 17.24^{a}
B. radicans	8.15 ± 0.08^{b}	78.89 ± 1.56^{d}	19.34 ± 0.33^{c}	$7.05 \pm 0.68^{c, e}$	7.70 ± 0.22^{b}	$65.91 \pm 0.43^{b, d}$	$404.44 \pm 2.54^{c, e}$	$1698.66 \pm 10.66^{c, e}$

All values refer to the portion obtained after removal of basal mycelium and substrate particles; no peel or rhizome material was present

Wild = collected in NE Portugal; Cultivated = purchased in Portuguese retail markets

Carbohydrates by difference includes dietary fibre

ANOVA one way with Tukey post hoc test, significant differences for ($p < 0.05$)

Different letters (a–f) in the same column indicate significant differences ($p < 0.05$) between samples

The moisture content of the freeze-dried mushrooms samples ranged from 8.15% in *B. radicans* to 10.26% in *B. edulis*. Dry matter content ranged from 78.89% in *B. radicans* to 93.98% in *L. edodes*. Protein content varied between 17.90% in *L. edodes* to 33.99% in *P. citrinopileatus*. Fat content ranged from 4.43% in *B. aereus* to 13.33% in *A. caesarea*. Total carbohydrates, calculated by difference including dietary fibre ranged from 49.05% in *P. citrinopileatus* to 70.81% in *L. edodes*, representing the major macronutrient fraction, contributing to the overall energy content, with variations likely influenced by species-specific metabolic traits and environmental growing conditions [25]. Ash content varied between 5.73% in *L. edodes* to 11.39% in *(A) caesarea*. The energy values ranged from 393.63 kcal/100 g in *(B) aereus* to 425.64 kcal/100 g in *B. regius.* Comparing our results with the existing literature, *L. edodes* exhibited a higher carbohydrate content (70.81% dw) than previously reported (58–60%), while its protein content (17.90% dw) was slightly lower (20–23%). Fat content (5.57% dw) exceeded prior findings (1.3%), whereas ash content (5.73% dw) remained unchanged. Its high carbohydrate composition contributes to a total energy value of 404.93 kcal/ 100 g dw [25]. The analysis of *P. citrinopileatus* showed compositional differences compared to previous reports. In this study the fat content was higher (9.08% vs. 2.2–3.5%), moderate protein (33.99% vs. 16.2–37.6%), and lower carbohydrate levels (49.05% vs. 52.7–64.9%) [25]. Compared to previous findings, *(A) caesarea* in this study showed lower protein content (21.71% vs. 24–34.77%), higher fat (13.33% vs. 3.5% – 5.6%), and carbohydrate levels (53.58%) like those reported for western Macedonia and Epirus (55.63%) but higher than those from Thessaly (31.9%) [25]. *Boletus* spp. revealed a variety of macronutrient compositions. For instance, *(B) edulis* in the present study contained higher protein (32.49% vs. 21.9%) and fat (6.94% vs. 2.6%), but lower carbohydrates (53.69% vs. 59.2%) than previously reported by Dimopoulou et al. [25]. These differences resulted in a higher energy value (407.19 vs. 347 kcal/100 g). Regarding the species *B. aereus* the results show lower carbohydrates (62.38% vs. 82.58%) but remarkably higher protein content (26.06% vs. 7.86%) compared to Stojković et al. [36]. *N. luridiformis* (previously identified as B. erythropus) showed a carbohydrate-rich profile (67.41%) but compared to Petrovska et al. [37], it exhibited lower protein (18.02% vs. 22.01%), higher fat (8.02% vs. 2.38%), and consequently higher energy value (1740.05 vs. 1586.29 kJ/ 100 g). *B. regius* proximate composition profile compared to Leal et al. [38], showed higher protein content (19.88% vs. 5.22%), higher fat (10.09% vs. 1.59%), lower carbohydrates (63.82% vs. 88.79%), and higher energy values (425.64 vs. 390.36 kcal/ 100 g). For *B. radicans* (*Caloboletus radicans* or *Rooting bolete*), literature data are scarce, however, compared to *B. edulis* analysed in this study, showed lower protein (19.34% vs. 32.49%), higher fat content (7.05% vs. 6.94%), higher carbohydrates (65.91% vs. 53.69%), and slightly lower energy value (404.44 vs. 407.19 kcal/ 100 g). Considering the results obtained, the comparison of proximate composition between commercial and wild mushrooms highlights their similarity regarding the macronutrient profiles, particularly in their low fat, and relatively high protein and carbohydrate content. Despite the differences of the origin, for example the cultivation methods applied to the commercial mushrooms, sawdust/ log substrates for *L. edodes*, and pasteurized straw/sawdust in bag culture for *P. citrinopileatus*, our results shows that wild mushrooms can offer comparable macronutrient contributions to the human diet [39, 40].

In Table 2 is represented the values of dietary fibre content (g/100 g dw), demonstrate the richness of the mushrooms compared to other conventional sources of dietary fibre (cereals, legumes, fruits and vegetables) [41].

The insoluble dietary fibre values ranged from 5.1 g/100 g in *(A) caesarea* to 35.7 g/100 g in *(B) radicans*, these results are consistent with previous research, being IDF the major component of TDF [42]. Additionally, the values of the soluble dietary fibre varied between 2.0 g/100 g in both *P. citrinopileatus* and *(A) caesarea* to 9.2 g/100 g in *(B) regius*. Finally, the sum of insoluble and soluble dietary fibre yielded total dietary fibre with values ranging from 7.1 g/100 g in *(A) caesarea* to 43.6 g/100 g in *(B) radicans*. The results obtained in the present study shows that the commercial and wild mushrooms, surpassed the TDF content of some fruit pulps (6.71–25.9 g/100 g) and wholegrains (2.65–13.97 g/100 g), being even comparable to fruit peels (39.25–67.43 g/100 g) [42, 43]. Zhang et al. [42],

Table 2 Dietary fibre content (insoluble, soluble, and total in g/100 g, dry weight basis) in commercial (*L. edodes* and *P. citrinopileatus*) and wild (*A. caesarea*, *B. aereus*, *B. edulis*, *N. luridiformis*, *B. regius*, *B. radicans*) mushrooms. Data are presented as mean ± SD

Mushroom Species	Insoluble dietary fibre	Soluble dietary fibre	Total dietary fibre
L. edodes	31.5 ± 2.3^{d}	4.7 ± 0.5^{b}	$36.2 \pm 2.8^{c, d}$
P. citrinopileatus	22.1 ± 2.2^{c}	2.0 ± 0.4^{a}	24.1 ± 2.6^{b}
A. caesarea	5.1 ± 0.7^{a}	2.0 ± 0.2^{a}	7.1 ± 0.9^{a}
B. aereus	21.5 ± 1.5^{c}	$5.0 \pm 0.3^{b, c}$	$26.5 \pm 1.8^{b, c}$
B. edulis	25.0 ± 1.2^{c}	3.0 ± 0.3^{a}	$28.0 \pm 1.5^{b, c}$
N. luridiformis	15.8 ± 0.9^{b}	6.9 ± 0.4^{c}	22.7 ± 1.3^{b}
B. regius	$32.7 \pm 3.1^{d, e}$	9.2 ± 1.1^{d}	41.9 ± 4.2^{d}
B. radicans	35.7 ± 2.2^{e}	7.9 ± 0.9^{d}	43.6 ± 3.1^{d}

Wild = collected in NE Portugal; Cultivated = purchased in Portuguese retail markets.

ANOVA one way with Tukey post hoc test, significant differences for ($p < 0.05$).

Different letters (a–f) in the same column indicate significant differences ($p < 0.05$) between samples.

demonstrated that *L. edodes*, originated from China, had higher TDF (54.23 g/100 g) than the results we achieved (36.2 g/100 g). The results obtained by Fernandes et al., [44], regarding *B. edulis* correspond to those obtained in the present study (28.0 g/100 g). The same author affirms the variability of dietary fibre content among different species, which is consistent with the present results [44].

The following Table 3 presents the results of the percentage of glucan composition (g/100 g, dry weight basis) of the eight mushroom species analysed. To our knowledge, this is the first quantitative side-by-side determination of β- and α-glucans for *(A) caesarea*, *(B) regius*, *B. radicans*, *B. aereus* and *N. luridiformis*, thereby extending the current glucan database for wild *Boletaceae*.

Total of glucans varied between 16.71 g/100 g dw in *P. citrinopileatus* and 67.96 g/100 g dw in *B. edulis*. The α-glucans content ranged from 3.94 g/100 g dw in *P. citrinopileatus* to 14.10 in *B. edulis*. β-glucans content was calculated by difference and varied between 12.78 g/100 g dw in *P. citrinopileatus* and 53.86 g/100 g dw in *B. edulis*.

B. edulis and *B. aureus*, display the highest total and β-glucans values, suggesting that wild mushrooms may be superior sources of β-glucans compared to the commercial *L. edodes* and *P. citrinopileatus* mushrooms, which showed lower values. Regarding the α-glucans content was consistently lower than the β-glucans across all species, reflecting the expected structural composition of fungal cell walls. These results emphasize the variability in glucan content among mushrooms, with β-glucans being the principal polysaccharide, known for their immune-modulating properties. Tan et al. [45] further supports these findings with their report of *B. edulis* containing 25.26 g/kg of α-glucans and 64.5 g/kg of β-glucans. This high concentration of β-glucans, particularly in B. edulis, suggests that this species, along with *L. edodes*, is a rich source of bioactive polysaccharides. Although *B. radicans* is not recommended for culinary use, its whole-body β-glucan content (18.11 g/100 g dw) places it among the richest *Boletaceae* quantified in the present study. This highlights non-culinary species as promising feedstocks for β-glucan extraction, provided that standard purification and safety evaluations are met. Such valorisation complements the food-focused relevance of the edible taxa. The findings by Sari et al. [3] and Tan et al. [45] highlight the diversity in glucan content, emphasizing the need for a standardized method of glucan extraction and analysis, as the bioactivity of β-glucans is influenced by their molecular conformation, such as the triple-helix structure detected by the Congo red method. Furthermore, findings of high β-glucan content across studies support the immunomodulatory potential of these compounds, particularly in mushrooms like *B. edulis* and *Pleurotus* spp., as demonstrated by McCleary and Draga [46]. Taken together, the glucan contents observed across *(A) caesarea*, *(B) aereus*, *B. edulis*, *B. regius*, *N. luridiformis* and the non-culinary *B. radicans* indicate that both edible and non-edible wild taxa can serve as viable sources of β-glucans for ingredient production, complementing their (where applicable) gastronomic relevance. These variations highlight the importance of continued research to fully understand the therapeutic potential of β-glucans, as well as the necessity for precise extraction and analysis techniques to assess their efficacy across different species [13]. To the best of our knowledge, there is a significant lack of literature concerning the detailed nutritional composition and biochemical properties of *B. regius*, *B. aureus*, *(A) caesarea*, *N. luridiformis*, and *(B) radicans*. While the genus *Boletus* has been the focus of research, particularly with well-known species such as *B. edulis*, comprehensive studies on the nutritional profiles, mineral content, and bioactive compounds of these lesser-studied species remain scarce. This gap in literature limits the understanding of their potential health benefits, ecological roles, and contributions to human nutrition. Hence, with our results it is possible to conclude that these eight mushroom species are rich and can be a great source of glucans with therapeutic potential.

Table 3 Percentage of glucan content (total glucans, *α*-glucans, and β-glucans in g/100 g, dry weight basis) in commercial (*L. edodes* and *P. citrinopileatus*) and wild (*A. caesarea*, *B. aereus*, *B. edulis*, *N. luridiformis*, *B. regius*, *B. radicans*) mushrooms. Data are presented as mean ± SD

Mushroom Species	Total of Glucans	α-Glucans	β-Glucans
L. edodes	38.87 ± 3.54^{c}	$4.39 \pm 1.07^{d, e}$	34.48 ± 2.47^{c}
P. citrinopileatus	16.71 ± 2.09^{f}	3.94 ± 0.34^{e}	12.78 ± 1.72^{f}
A. caesarea	$18.87 \pm 3.54^{e, f}$	$4.39 \pm 1.07^{d, e}$	$14.48 \pm 2.47^{e, f}$
B. aereus	55.20 ± 4.53^{b}	10.77 ± 1.11^{b}	44.44 ± 3.42^{b}
B. edulis	67.96 ± 3.90^{a}	14.10 ± 2.19^{a}	53.86 ± 1.71^{a}
N. luridiformis	31.73 ± 2.35^{d}	7.65 ± 0.79^{c}	24.09 ± 1.56^{d}
B. regius	$21.21 \pm 2.26^{e, f}$	$6.03 \pm 0.82^{c, d, e}$	$15.18 \pm 1.44^{e, f}$
B. radicans	24.66 ± 2.34^{e}	$6.56 \pm 1.32^{c, d}$	18.11 ± 1.02^{e}

Wild = collected in NE Portugal; Cultivated = purchased in Portuguese retail markets

ANOVA one way with Tukey post hoc test, significant differences for ($p < 0.05$)

Different letters (a–f) in the same column indicate significant differences ($p < 0.05$) between samples

Antioxidant activity

Figure 1 Antioxidant capacity of commercial (*L. edodes* and *P. citrinopileatus*) and wild (*A. caesarea*, *B. aereus*, *B. edulis*, *N. luridiformis*, *B. regius*, *B. radicans*) mushrooms species, determined by ABTS and FRAP assays expressed in

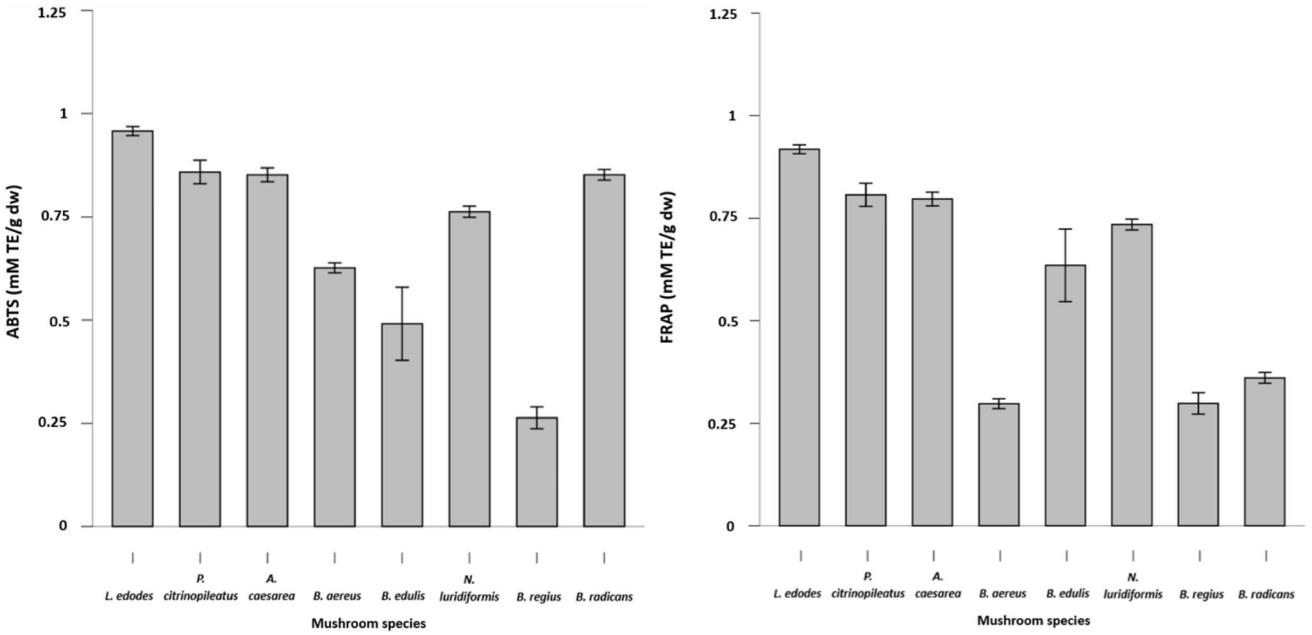

Fig. 1 Shows the results of the antioxidant activity of the eight mushrooms species being studied, measured using the ABTS and FRAP assays

mM TE/g. Error bars represent SD from triplicate measurements ($n=3$). Significant differences for ($p>0.05$).

Concerning the results obtained from the ABTS assay, these revealed significant variability among the samples, with results between 0.96 mM Trolox/g dw in *L. edodes* to 0.32 mM Trolox/g dw in *B. regius*. Overall, these results highlight the diverse antioxidant properties within the samples species, demonstrating the differences in their bioactive compound profiles. The FRAP assay results further emphasized the differences in the antioxidant activities among the studied mushrooms. Similarly with the results obtained by the ABTS assay, *L. edodes* exhibited the highest FRAP capacity with a value of 0.93 mM Trolox/g dw and *B. regius* the lowest with 0.31 mM Trolox/g dw. In agreement with existing literature, Bach et al. [47] investigated five commercial mushroom species, reporting ABTS assay results that ranged from 34.57 to 128.60 µmol TE/g dw. Particularly, *L. edodes* exhibited the lowest antioxidant capacity in their study, a finding not illustrated in our research. Regarding the FRAP assay, the results indicated values from 14.66 to 48.26 µmol TE/g dw, with *L. edodes* presenting a value of 26.77 µmol TE/g dw. There is a difference between the ABTS and FRAP assay values of *L. edodes*, that also can be seen in the present study. Additionally, Sharma and Gautam [48], evaluated the antioxidant potential of twenty wild culinary mushroom species, revealing ABTS EC50 values ranging from 1.45 to 4.26 mg/ml, and FRAP assay values between 0.78 and 2.16 µmol Fe^{2+} equivalents/g dw, the wild species *A. caesarea* demonstrated ABTS and FRAP values of 1.45 mg/ml and 1.86 µmol Fe^{2+} equivalents/g dw, respectively. In the present work there is a higher antioxidant activity in the commercialized mushrooms species, which agrees with the existent literature. Nevertheless, in our research the wild mushroom species also have remarkable antioxidant potential.

Heavy metal and essential minerals composition

The following Table 4 presents the heavy metals content results of the eight mushrooms species in analysis.

Aluminium (Al) was present in moderate amounts across all species, with *(A) caesarea* having the highest concentration (9.77 mg/kg) and *(B) radicans* the lowest (4.89 mg/kg). As levels were low overall, with *(A) caesarea* showing the highest amount (0.127 mg/kg), and *(B) aureus* the lowest (0.021 mg/kg). Cd was found to be low in concentrations in all species, with *(A) caesarea* showing the highest value (0.033 mg/kg) and *N. luridiformis* the lowest (0.008 mg/kg). Co concentrations were generally low, with *(B) edulis* having the highest amount (0.049 mg/kg) and *P. citrinopileatus* and *B. radicans* exhibiting the lowest (0.005 mg/kg and 0.004 mg/kg, respectively). Cr was detected only in *(A) caesarea* (0.0809 mg/kg), *(B) edulis* (0.0419 mg/kg), and *N. luridiformis* (0.0007 mg/kg), while it was undetectable in the remaining species. Pb was present in very low concentrations across all species, with *A. caesarea* showing the highest value (0.0032 mg/kg) and *P. citrinopileatus* the lowest (0.0002 mg/kg).

In Table 5 is presented the results of the minerals composition of commercial (L. edodes and P. citrinopileatus) and wild (*A. caesarea*, *B. aereus*, *B. edulis*, *N. luridiformis*, *B. regius*, *B. radicans*) mushrooms species.

Table 4 Heavy metals composition expressed in mg/kg on dry weight basis of commercial (*L. edodes* and *P. citrinopileatus*) and wild (*A. caesarea*, *B. aereus*, *B. edulis*, *N. luridiformis*, *B. regius*, *B. radicans*) mushrooms species. Data are presented as mean ± SD

Heavy Metals	Mushrooms species							
	L. edodes	*P.* citrinopileatus	A. caesarea	B. aereus	B. edulis	*N.* luridiformis	B. regius	B. radicans
Al	7.47 ± 0.81^{a}	8.00 ± 1.60^{a}	9.77 ± 0.00^{a}	7.76 ± 2.85^{a}	6.61 ± 0.41^{a}	8.05 ± 0.81^{a}	6.61 ± 0.41^{a}	4.89 ± 0.41^{a}
As	0.114 ± 0.143^{a}	0.062 ± 0.017^{a}	0.127 ± 0.132^{a}	0.021 ± 0.009^{a}	0.029 ± 0.009^{a}	0.054 ± 0.015^{a}	0.050 ± 0.011^{a}	0.036 ± 0.009^{a}
Cd	0.022 ± 0.010^{c}	$0.018 \pm 0.007^{c, d}$	0.033 ± 0.016^{f}	$0.022 \pm 0.007^{d, e}$	$0.029 \pm 0.012^{e, f}$	0.008 ± 0.000^{a}	$0.010 \pm 0.000^{a, b}$	0.014 ± 0.001^{b}
Co	$0.022 \pm 0.013^{a, b}$	0.005 ± 0.000^{a}	0.008 ± 0.003^{a}	0.007 ± 0.002^{a}	0.049 ± 0.015^{b}	0.005 ± 0.000^{a}	0.0111 ± 0.003^{a}	0.004 ± 0.000^{a}
Cr	0.0000 ± 0.0000^{a}	0.0000 ± 0.0000^{a}	0.0809 ± 0.0080^{c}	0.0000 ± 0.0000^{a}	0.042 ± 0.009^{b}	0.0007 ± 0.0010^{a}	0.0000 ± 0.0000^{a}	0.0000 ± 0.0000^{a}
Pb	0.003 ± 0.002^{a}	0.0002 ± 0.0000^{a}	0.0032 ± 0.0010^{a}	0.0002 ± 0.0000^{a}	0.0001 ± 0.0000^{a}	0.0014 ± 0.0003^{a}	0.0003 ± 0.0000^{a}	0.0002 ± 0.0000^{a}

Wild = collected in NE Portugal; Cultivated = purchased in Portuguese retail markets

ANOVA one way with Tukey post hoc test, significant differences for ($p < 0.05$)

Different letters (a–f) in the same line indicate significant differences ($p < 0.05$) between samples

Calcium (Ca) content varied considerably, with *L. edodes* exhibiting the highest level (21.04 mg/kg), while *B. aureus* (1.81 mg/kg) and *B. regius* (1.84 mg/kg) contained the lowest amounts. For Cu, *B. edulis* had the highest concentration (2.19 mg/kg), followed by *(A) caesarea* (1.29 mg/kg), while *P. citrinopileatus* contained the least (0.33 mg/kg). Iron (Fe) levels were highest in *P. citrinopileatus* (1.98 mg/kg) and *N. luridiformis* (1.08 mg/kg), with *L. edodes* having the lowest value (0.53 mg/kg). Potassium (K) was abundant in all species, especially *(B) radicans* (2521.4 mg/kg) and *(A) caesarea* (2307.9 mg/kg), with *(B) regius* (985.8 mg/kg) showing the lowest level. Magnesium (Mg) levels were highest in *P. citrinopileatus* (54.1 mg/kg) and *B. radicans* (49.2 mg/kg), whereas *B. edulis* contained the lowest amount (2.2 mg/kg). Manganese (Mn) was most concentrated in *(A) caesarea* (1.03 mg/kg), followed by *(B) edulis* (0.57 mg/kg), and was lowest in *B. radicans* (0.10 mg/kg). Sodium (Na) levels were relatively consistent across species, with *B. radicans* showing the highest concentration (22.64 mg/kg) and *N. luridiformis* the lowest (14.71 mg/kg). Phosphorus (P) content varied significantly, with *B. regius* containing the highest level (1.79 mg/kg) and *A. caesarea* the lowest (0.19 mg/kg). Zinc (Zn) levels were relatively uniform, with *A. caesarea* having the highest concentration (2.40 mg/kg) and *L. edodes* the lowest (1.43 mg/kg).

These results constitute the first mineral and heavy-metal baseline for the six target species from NE Portugal, confirming that all toxic elements remain below EU limits while revealing exceptionally high K in *B. radicans* [49, 50]. These findings highlight the varied mineral compositions of these mushroom species, each offering unique nutritional benefits and potential health risks. Both *(A) caesarea* and *(B) edulis* stand out for their high elemental concentrations, particularly in Fe, Cu, and P, making them valuable additions to a balanced diet. However, the presence of trace heavy metals like As and Cd underscores the need for further research into the factors influencing heavy metal uptake in mushrooms. The mineral composition of mushrooms differs significantly depending on species, region, and environmental factors such as soil and climate. Studies have highlighted the high levels of essential minerals like K, P, Ca, Mg, Na, and Fe in wild-growing mushrooms, which play a crucial role in human nutrition [7, 10, 51]. For instance, *B. edulis* collected in Shanxi, China, showed elevated levels of Na (14.9 g/kg dw), Ca (6.60 g/kg dw), and Fe (5.77 g/kg dw), compared to samples from other regions, accordingly to Tan et al. [45]. Mushrooms are also known for their capability to accumulate trace minerals like Zn and Cu [45]. Such variability in mineral content has been reported in studies from China and Europe, highlighting the importance of understanding regional and species-specific mineral profiles to precisely assess the nutritional benefits of mushroom

Table 5 Minerals composition expressed in mg/kg on dry weight basis of commercial (*L. edodes* and *P. citrinopileatus*) and wild (*A. caesarea*, *B. aereus*, *B. edulis*, *N. luridiformis*, *B. regius*, *B. radicans*) mushrooms species. Data are presented as mean ± SD

Minerals	Mushrooms species							
	L. edodes	*P.* citrinopileatus	A. caesarea	B. aereus	B. edulis	*N.* luridiformis	B. regius	B. radicans
Ca	21.04 ± 0.13^{c}	5.65 ± 1.26^{b}	5.37 ± 0.09^{b}	1.81 ± 0.17^{a}	2.52 ± 0.04^{a}	3.38 ± 0.22^{a}	1.84 ± 0.30^{a}	2.70 ± 0.30^{a}
Cu	0.44 ± 0.02^{a}	0.33 ± 0.01^{a}	1.29 ± 0.08^{d}	0.96 ± 0.01^{c}	2.19 ± 0.02^{f}	0.71 ± 0.01^{b}	0.77 ± 0.02^{b}	1.47 ± 0.06^{e}
Fe	0.53 ± 0.05^{a}	1.98 ± 0.00^{c}	$0.69 \pm 0.00^{a, b}$	$0.76 \pm 0.09^{a, b}$	$0.73 \pm 0.05^{a, b}$	1.08 ± 0.18^{b}	0.60 ± 0.14^{a}	0.56 ± 0.18^{a}
K	1117.1 ± 10.9^{a}	1276.2 ± 68.2^{a}	2307.9 ± 140.8^{b}	1378.5 ± 52.7^{a}	1346.9 ± 122.1^{a}	1212.7 ± 270.8^{a}	985.8 ± 15.4^{a}	2521.4 ± 69.7^{b}
Mg	29.0 ± 0.20^{b}	54.1 ± 1.9^{d}	46.6 ± 1.0^{c}	$27.6 \pm 0.4^{a, b}$	22.2 ± 2.02^{a}	$25.5 \pm 0.1^{a, b}$	$26.1 \pm 1.2^{a, b}$	$49.2 \pm 1.2^{c, d}$
Mn	$0.35 \pm 0.15^{b, c,d}$	$0.39 \pm 0.06^{c, d}$	1.03 ± 0.02^{e}	$0.17 \pm 0.03^{a, b,c}$	0.57 ± 0.00^{d}	$0.13 \pm 0.01^{a, b}$	$0.13 \pm 0.02^{a, b}$	0.10 ± 0.00^{a}
Na	20.59 ± 2.30^{a}	18.70 ± 4.12^{a}	19.37 ± 1.16^{a}	18.43 ± 4.84^{a}	18.90 ± 0.19^{a}	14.71 ± 1.67^{a}	16.60 ± 2.07^{a}	22.64 ± 7.03^{a}
P	$0.46 \pm 0.32^{a, b}$	$0.44 \pm 0.13^{a, b}$	0.19 ± 0.12^{a}	0.91 ± 0.23^{c}	0.99 ± 0.08^{c}	0.99 ± 0.38^{c}	1.79 ± 0.54^{d}	$0.68 \pm 0.22^{b, c}$
Zn	1.43 ± 0.06^{a}	2.26 ± 0.11^{b}	2.40 ± 0.00^{c}	$2.33 \pm 0.00^{b, c}$	$2.35 \pm 0.00^{b, c}$	$2.23 \pm 0.06^{b, c}$	2.16 ± 0.05^{b}	2.14 ± 0.06^{b}

Wild = collected in NE Portugal; Cultivated = purchased in Portuguese retail markets

ANOVA one way with Tukey post hoc test, significant differences for ($p < 0.05$)

Different letters (a–f) in the same line indicate significant differences ($p < 0.05$) between samples

consumption [25]. The mineral content results of the present study reflected these findings, revealing considerable differences in mineral concentrations. Potassium (K) was the most abundant element, with *B. radicans* exhibiting the highest value (2521.4 mg/kg), which aligns with Wang et al. [10] research highlighting mushrooms as K-rich foods. Calcium (Ca) concentrations were highest in *L. edodes* (21.04 mg/kg), consistent with previous studies that emphasized its Ca-rich profile [25]. In contrast, the Na levels observed in the current study (ranging from 14.71 mg/kg in *N. luridiformis* to 22.64 mg/kg in *B. radicans*) were much lower than those reported in *B. edulis* from Tibet (3.52 g/kg dm) [45]. Trace elements like Zn and Cu were present in moderate amounts, with *(A) caesarea* and *(B) edulis* showing higher Cu accumulation (1.29 mg/kg and 2.19 mg/kg, respectively), reflecting the selective mineral uptake capacities of different species [33]. Furthermore, the detection of toxic elements such as Cd and As in low concentrations suggests that while these mushrooms offer nutritional benefits, careful monitoring of toxic element accumulation is necessary to ensure safe consumption, particularly for species with higher accumulation tendencies [25]. Regarding metallic elements accumulation, macro fungi have exhibited a remarkable capacity to accumulate high concentrations, even when growing in soils comparatively depleted in metal content. The bioaccumulation factor, which represents the ratio of an element's concentration in the fungal fruit body or mobility in soil, varies significantly among different elements [52]. Elements commonly accumulated in macro fungi include As, Cd, chlorine (Cl), Cu, mercury (Hg), and Zn. Conversely, elements with typically low accumulation in macro fungi include Co, Cr, and rare earth elements. Lead (Pb) is frequently detected in mushrooms due to its relative abundance in soil and background contamination. However, fungi have a limited capacity to bioaccumulate Pb, ensuring that its content in edible mushrooms remains below the threshold for tolerable dietary intake [52]. According to Kalač [53], both wild and cultivated edible mushrooms are rich sources of K, Ca, and Na, with K being the predominant element across species, while Ca and Na are present in lower concentrations. The results obtained in the present study align with this observation. Wild edible mushrooms represent a valuable food source, being low in fat and calories while providing significant protein, carbohydrate, and dietary fibre content, nevertheless, there is still a risk of exposure to toxic elements, particularly when collected from polluted areas. In general, species from unpolluted environments and cultivated mushrooms grown on clean substrates are considered safe, as their toxic element concentration remain below the regulation's limits [50, 54]. Considering the heavy metals and minerals profiles obtained in this study, we can understand that there is variability between species, but that this is not so evident when comparing between the commercial and wild mushrooms. These findings emphasize the importance of controlled cultivation practices, but also a continued monitoring of these products.

Free amino acids profiles

In Table 6 shows the amino acid profiles of the commercial *L. edodes* and *P. citrinopileatus*, and the wild *(A) caesarea*, *(B) aereus*, *B. edulis*, *N. luridiformis*, *B. regius*, *B. radicans* mushrooms species, to both essential and non-essential amino acid, including leu, ileu, val, lys, phe, thr, his, ala, arg, asx, glx, gly, pro, ser, and finally tyr. These results show significant differences, highlighting the unique nutritional and functional properties of mushrooms. The variability observed across the species is possibly attributed to intrinsic metabolic or genetic differences and environmental factors [55].

Regarding the essential amino acids, it is possible to observe the wide variations among the mushroom species.

Table 6 Free amino acid profiles expressed in mmol/g, dry weight basis of commercial (*L. edodes* and *P. citrinopileatus*) and wild (*A. caesarea*, *B. aereus*, *B. edulis*, *N. luridiformis*, *B. regius*, *B. radicans*) mushrooms species. Data are presented as mean ± SD

amino acids	Mushroom species							
	L. edodes	*P.* citrinopileatus	A. caesarea	B. aereus	B. edulis	*N.* luridiformis	B. regius	B. radicans
His	0.224 ± 0.180[a]	0.060 ± 0.023[a]	0.086 ± 0.007[a]	0.156 ± 0.002[a]	0.113 ± 0.006[a]	0.080 ± 0.020[a]	0.132 ± 0.026[a]	0.139 ± 0.072[a]
Ileu	0.092 ± 0.052[a]	0.031 ± 0.016[a]	0.046 ± 0.006[a]	0.073 ± 0.005[a]	0.047 ± 0.001[a]	0.034 ± 0.002[a]	0.053 ± 0.009[a]	0.049 ± 0.010[a]
Leu	0.162 ± 0.061[a]	0.062 ± 0.032[a]	0.093 ± 0.013[a]	0.156 ± 0.009[a]	0.095 ± 0.000[a]	0.074 ± 0.002[a]	0.111 ± 0.023[a]	0.100 ± 0.015[a]
Lys	0.377 ± 0.035[a]	0.294 ± 0.057[a]	0.410 ± 0.077[a]	0.434 ± 0.023[a]	0.270 ± 0.026[a]	0.362 ± 0.085[a]	0.345 ± 0.082[a]	0.355 ± 0.036[a]
Phe	0.048 ± 0.008[a, b]	0.025 ± 0.008[b]	0.031 ± 0.002[b]	0.054 ± 0.001[a]	0.036 ± 0.004[a, b]	0.032 ± 0.004[a, b]	0.042 ± 0.007[a, b]	0.034 ± 0.005[a, b]
Thr	0.315 ± 0.236[a]	0.079 ± 0.044[a]	0.119 ± 0.026[a]	0.177 ± 0.011[a]	0.127 ± 0.010[a]	0.075 ± 0.016[a]	0.180 ± 0.027[a]	0.146 ± 0.045[a]
Val	0.162 ± 0.083[a]	0.058 ± 0.018[a]	0.068 ± 0.011[a]	0.113 ± 0.009[a]	0.078 ± 0.007[a]	0.055 ± 0.002[a]	0.086 ± 0.010[a]	0.089 ± 0.032[a]
Ala	0.025 ± 0.021[a]	0.013 ± 0.010[a]	0.005 ± 0.004[a]	0.052 ± 0.054[a]	0.007 ± 0.000[a]	0.005 ± 0.004[a]	0.011 ± 0.003[a]	0.008 ± 0.004[a]
Arg	0.478 ± 0.053[a]	0.325 ± 0.042[a]	0.440 ± 0.117[a]	0.387 ± 0.072[a]	0.370 ± 0.015[a]	0.325 ± 0.065[a]	0.387 ± 0.029[a]	0.349 ± 0.005[a]
Asx	0.367 ± 0.294[a]	0.129 ± 0.084[a]	0.156 ± 0.032[a]	0.209 ± 0.029[a]	0.163 ± 0.008[a]	0.098 ± 0.016[a]	0.157 ± 0.023[a]	0.214 ± 0.055[a]
Glx	0.290 ± 0.200[a]	0.090 ± 0.046[a]	0.139 ± 0.013[a]	0.274 ± 0.005[a]	0.145 ± 0.014[a]	0.120 ± 0.012[a]	0.171 ± 0.025[a]	0.136 ± 0.016[a]
Gly	0.027 ± 0.021[a]	0.009 ± 0.006[a]	0.010 ± 0.002[a]	0.015 ± 0.001[a]	0.011 ± 0.000[a]	0.008 ± 0.000[a]	0.013 ± 0.003[a]	0.015 ± 0.008[a]
Pro	0.115 ± 0.070[a]	0.043 ± 0.014[a]	0.053 ± 0.008[a]	0.099 ± 0.019[a]	0.059 ± 0.004[a]	0.044 ± 0.001[a]	0.066 ± 0.013[a]	0.062 ± 0.016[a]
Ser	0.445 ± 0.529[a]	0.039 ± 0.020[a]	0.097 ± 0.073[a]	0.090 ± 0.001[a]	0.081 ± 0.025[a]	0.042 ± 0.001[a]	0.071 ± 0.014[a]	0.112 ± 0.086[a]
Tyr	0.036 ± 0.016[a]	0.006 ± 0.005[a]	0.010 ± 0.003[a]	0.031 ± 0.011[a]	0.012 ± 0.005[a]	0.006 ± 0.002[a]	0.018 ± 0.006[a]	0.016 ± 0.008[a]

Wild = collected in NE Portugal; Cultivated = purchased in Portuguese retail markets

ANOVA one way with Tukey post hoc test, significant differences for ($p < 0.05$)

Different letters (a–b) in the same line indicate significant differences ($p < 0.05$) between samples

Lysine (lys) emerges as the most abundant essential amino acid, with particularly high value observed in *B. aereus* (0.434 mmol/ g dw) and *(A) caesarea* (0.410 mmol/ g dw). Lysine (lys) is a crucial amino acid for protein synthesis, immune function, and calcium absorption, making these species especially valuable for nutritional purposes [56]. Leucine (leu) and isoleucine (ile), fundamental for muscle metabolism and repair, were also present in different concentrations [57]. *L. edodes* exhibited the highest leu content (0.162 mmol/ g dw), while *P. citrinopileatus* showed the lowest levels of both leu (0.062 mmol/ g dw) and isoleucine (0.031 mmol/ g dw). Threonine (thr) is also an essential amino acid, important for collagen formation and immune response, and was particularly abundant in *L. edodes* (0.315 mmol/ g dw) [57]. In contrast, *P. citrinopileatus* consistently exhibited lower concentrations of most essential amino acids, suggesting that in this case, it has limited utility as a standalone protein source. Phenylalanine (phe) showed low concentrations in general, with *(B) aereus* (0.054 mmol/ g dw) showing the highest content; this amino acid is essential for neurotransmitter production [57]. Valine (val), also crucial for muscle metabolism, was most abundant in *L. edodes* (0.162 mmol/ g dw) [58]. Histidine (his) is an amino acid fundamental for haemoglobin production and tissue repair [57] and showed the highest level in *L. edodes* (0.224 mmol/ g dw) and *B. aereus* (0.156 mmol/ g dw). Concerning the non-essential amino acid profiles, arg acts as a precursor for nitric oxide and polyamines, a potent vasodilator and neurotransmitter, and participates in protein synthesis, immune function, and wound healing [59], being the most prominent in *L. edodes* (0.478 mmol/ g dw) and *(A) caesarea* (0.440 mmol/ g dw). Aspartic acid or asparagine (asx), important for energy metabolism and cellular signalling, showed the highest concentrations in *L. edodes* (0.367 mmol/g dw), followed by *(B) radicans* (0.214 mmol/ g dw) [57]. Glutamic acid or Glutamine (glx) are both vital for neurotransmitter synthesis and immune support, like the previous amino acids presented higher values for *L. edodes* (0.290 mmol/ g dw), followed by *B. aereus* (0.274 mmol/ g dw) [39]. Serine (ser) is another non-essential amino acid important for cell membrane structure and metabolism. *L. edodes* (0.445 mmol/ g dw) showed the highest content with a considerable difference from the other species [57]. Alanine (ala), involved in glucose and tryptophan metabolism was present in small concentrations across all species [58], nevertheless, *B. aereus* (0.052 mmol/ g dw) showed the higher value. Similarly, with lower values across all species were gly and pro fundamental for collagen synthesis, joint health, and immune function, as well as tyr crucial for hormone production and brain function [57]. These three amino acids showed higher concentration in *L. edodes* (0.027 mmol/ g dw; 0.115 mmol/ g dw; 0.036 mmol/ g dw respectively).

Amino acids are constituent building blocks of proteins, the latter having diverse structural and functional roles in the body. Deficiency of amino acids can, therefore, compromise normal health. The results obtained reinforce the potential of mushrooms as high-quality dietary protein sources, particularly for populations consuming predominantly plant-based diets, where certain essential amino acids like lys are often deficient [56]. It is also shown that species such

as *L. edodes*, *(A) caesarea*, and *(B) aereus* have superior nutritional profiles. The combination of essential and non-essential amino acids in these mushrooms reinforces their potential as functional foods [58]. On the contrary, *P. citrinopileatus* displayed the lowest concentrations of nearly all amino acids. While this suggests lower overall protein quality, its adaptability to diverse cultivation environments and low production cost make it an important species for addressing nutritional gaps in regions with limited access to high-quality protein sources. Furthermore, when consumed as part of a balanced diet, *P. citrinopileatus* can complement the amino acid profiles of other foods [60].

Wild species like *A. caesarea* and *Boletus spp.* provide rich amino acid profiles, but their seasonal availability and ecological sensitivity needs careful management to ensure long-term sustainability. In contrast, cultivated species such as *L. edodes* and *P. citrinopileatus* offer scalable and environmentally friendly options, and combine superior amino acid content with the ability to grow on agricultural by-products, making it a highly sustainable protein source [61].

Conclusion

This study provides a comparison between two commercialized (*L. edodes* and *P. citrinopileatus*) and six wild (*A. caesarea*, *B. aereus*, *B. edulis*, *B. radicans*, *B. regius*, and *N. luridiformis*) mushrooms from the NE of Portugal, highlighting their distinct nutritional and bioactive profile. The analysis underscores the nutritional value of these mushrooms, particularly their high fibre content, which is associated with numerous health benefits, including reduced risks of cardiovascular disease, hypertension, diabetes, obesity, and certain gastrointestinal disorders. Moreover, the presence of β-glucans, with their diverse structures and therapeutic potential (immunomodulatory, antineoplastic, antioxidant, and antimicrobial properties), further elevates the functional value of mushrooms. The dataset also identifies a non-culinary species (*B. radicans*) as a plausible β-glucan feedstock, underscoring opportunities for polysaccharide valorisation beyond direct consumption. The standardization of β-glucan extraction and structural characterization is critical for harnessing their therapeutic applications. Mineral analysis revealed that wild mushrooms are sources of K and significant contributors of trace elements such as Mg, Ca, Fe, and Cu, with implications for cardiovascular health and dietary mineral supplementation. However, the low bioavailability of Fe and potential Cu toxicity in certain species needs careful consideration. Amino acid profiling demonstrated that all studied species contain essential and non-essential amino acids, affirming their role as valuable protein sources, particularly for vegetarian diets. The variability in quantitative amino acid profiles across species suggests that combining multiple mushroom types in the diet could provide a more balanced amino acid intake and enhance culinary diversity. The antioxidant activity assessed by ABTS radical scavenging activity and ferric reducing power (FRAP) assays, suggest the capacity to neutralize free radicals and reduce oxidized compounds. Overall, this research emphasizes the nutritional and functional potential of mushrooms, both commercialized and wild, supporting their wider inclusion in human diets and further exploration of their bioactive properties for health-promoting applications.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

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