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Chromatographic and Chemometric Characterization of the Two Wild Edible Mushrooms *Fistulina hepatica* and *Clitocybe nuda*: Insights into Nutritional, Phenolic, and Antioxidant Profiles

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Abstract

Fistulina hepatica (Schaeff.) With. and *Clitocybe nuda* (Bull.) H.E. Bigelow & A.H. Sm. are wild edible mushrooms with nutritional and functional potential that remain insufficiently characterized. This study provides the first comparative assessment of their nutritional profiles, phenolic composition, and antioxidant activity, using specimens collected from Montesinho Natural Park (Portugal). Proximate composition, organic and phenolic acids, free sugars, and fatty acids were analyzed by chromatographic methods, and antioxidant capacity was assessed through OxHLIA and TBARS assays. *F. hepatica* showed higher carbohydrates (9.3 ± 0.2 g/100 g fw) and estimated energy values (43 ± 1 kcal/100 g fw), increased phenolic acids content (2.7 ± 0.1 mg/g extract), and the exclusive presence of *p*-coumaric and cinnamic acids, along with OxHLIA activity (IC_{50} = 126 ± 5 µg/mL at Δt = 60 min). *C. nuda* displayed higher protein (2.5 ± 0.1 g/100 g dw) and quinic acid contents (4.13 ± 0.02 mg/g extract), a PUFA-rich profile, and greater TBARS inhibition (EC_{50} = 303 ± 17 µg/mL). These findings highlight distinct and complementary bioactive traits, supporting their valorization as natural functional ingredients. Their compositional features offer promising applications in sustainable food systems and nutraceutical development, encouraging further investigations into safety, bioaccessibility, and formulation strategies. Notably, *F. hepatica* is best consumed at a young developmental stage, as its sensory properties tend to decline with maturity.

Keywords: *Fistulina hepatica*; *Clitocybe nuda*; phenolic acids; antioxidant activity; wild edible mushrooms

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1. Introduction

The Montesinho Natural Park (MNP) in northeastern Portugal is renowned for its rich biogeographical diversity, particularly its extensive ecosystems and varied taxonomic groups. Among these, a vibrant community of macrofungi thrives, supported by the park's mountainous terrain and favorable edaphoclimatic conditions. Over 200 species of edible mushrooms have been identified here, many of which are traditionally foraged and enjoyed by local communities. These fungi are valued not only for their taste and culinary attributes, but also for their nutritional benefits, making them an integral part of the local culture and diet [1,2].

Wild edible mushrooms are gaining recognition as functional foods due to their rich composition, which includes high-quality proteins, dietary fiber, unsaturated fatty acids, essential vitamins, and minerals. Additionally, they contain a range of bioactive compounds, such as phenolic acids, polysaccharides, sterols, and terpenoids. These compounds are linked to various health benefits, including antioxidant, anti-inflammatory, antimicrobial, and immunomodulatory properties, making them valuable for use in health-promoting food products [3,4]. However, the chemical composition of mushrooms can vary significantly based on species, substrate, developmental stage, and environmental factors [5]. This highlights the importance of conducting species-specific nutritional and phytochemical profiling, particularly for wild species that are not well characterized.

The comprehensive chemical characterization of wild edible mushrooms is essential for assessing their nutritional quality and informing sustainable use and valorization strategies. Such data can facilitate the integration of these mushrooms into food systems as alternative nutritional resources, enhance controlled cultivation practices, and support the conservation and rational management of fungal biodiversity. Understanding the specific chemical profiles of these mushrooms allows for the optimization of their benefits in human diets while ensuring sustainability and preservation in natural ecosystems [6,7].

The Food and Agriculture Organization (FAO) and United Nations Environmental Programme (UNEP) recognize edible mushrooms as key resources for improving nutrition, enhancing food security, and sustaining rural livelihoods. Their responsible exploitation can help maintain forest ecosystems while providing added value to local economies [8,9].

Among the edible fungal species found in MNP, *Fistulina hepatica* (Schaeff.) With., commonly known as "beefsteak fungus," and *Clitocybe nuda* (Bull.) H.E. Bigelow & A.H. Sm., known as "wood blewit," are of particular interest. *F. hepatica,* a member of the Fistulinaceae family, is characterized by its fleshy texture, acidic flavor, and reddish coloration, and has been reported to contain phenolic compounds and other metabolites with antioxidant and antimicrobial properties [10]. *C. nuda*, a basidiomycete of the Tricholomataceae family, is appreciated for its aromatic profile and protein content, which while modest in absolute terms, is relatively higher compared to many other wild mushrooms. Moreover, it has been studied for its antioxidant activity and potential immunomodulatory effects [11]. It is worth noting that, although still widely referred to as *Clitocybe nuda* in food and applied research, this species has recently been reassigned to the genus *Lepista*, according to updated taxonomic revisions.

Despite their ethnomycological relevance and documented bioactivities, comparative studies focusing on the nutritional, phenolic, and antioxidant profiles of these two species are lacking. Therefore, the present study aimed to carry out a detailed chemical characterization of *F. hepatica* and *C. nuda* collected from MNP. The analyses included proximate composition, organic and phenolic acids, free sugars, and fatty acid profiles, determined through standardized chromatographic methods. Additionally, the antioxidant potential of their extracts was evaluated using OxHLIA and TBARS assays. The generated data intend to support the functional valorization of these underutilized mushrooms and provide a scientific foundation for their potential integration into sustainable food and nutraceutical applications. To date, no study has been found that directly compares the nutritional and antioxidant characteristics of these two wild mushroom species collected under the same ecological conditions.

2. Materials and Methods

2.1. Mushroom Samples, Chemical and Standards

Specimens of *F. hepatica* and *C. nuda* mushrooms were collected in the MNP between October 2021 and May 2022. *F. hepatica* samples were collected throughout the Oleiros

locality (41°53′11.47″ N 6°49′49.00″ W), whereas *C. nuda* specimens were gathered across the Cova da Lua area (41°50′45.95″ N 6°50′45.95″ W). For all analyses, both the caps and stems of the fruiting bodies were used. Fungal species were identified based on their macroscopic morphological characteristics, including color, shape, size, and hyphal structure. The samples were washed with distilled water, lyophilized, and then ground into a fine powder (20-mesh). They were subsequently stored at −80 °C until further analysis.

The chemicals and standards used in this study are listed in Table S1 of the Supplementary Materials.

2.2. Nutritional and Chemical Composition

2.2.1. Nutritional Analysis

The nutritional composition of the mushrooms was evaluated through the quantification of proteins, fats, ashes, and available carbohydrates, following the official methods established by AOAC [12]. Protein content was determined by the macro-Kjeldahl method (AOAC 920.152) based on the quantification of total nitrogen (equipment specifications in Table S2), which was converted to protein using a nitrogen-to-protein conversion factor of 6.25 [13]. Crude fat was determined gravimetrically after Soxhlet extraction with petroleum ether (purity ≥99%), using the standard procedure described in AOAC Official Method 920.85. Ash content was determined by incinerating the samples in a muffle furnace at 550 °C for 5 h and calculated based on the residual mass after combustion (AOAC 940.26). Available carbohydrate content was calculated by difference, subtracting the sum of moisture, ash, protein, and fat contents from the total weight [14]. All results were expressed in g/100 g (fw). The energetic value of the mushrooms was also calculated according to Regulation (EU) No 1169/2011, using the following formula: energy (kcal/100 g) = 4 × (g protein + g available carbohydrates) + 9 × (g fat) [15].

2.2.2. Chemical Composition

Free sugars were quantified using high-performance liquid chromatography (HPLC) equipped with a refractive index detector (Equipment specifications in Table S3). The extraction procedure followed the protocol outlined by Leichtweis et al. [16], incorporating raffinose as the internal standard into 1 g dried sample powder, and submitting it to 30 min stirring extraction with 40 mL of 80% ethanol at 80 °C. The obtained suspension was centrifuged at 15,000× *g* for 10 min. The resulting supernatant was concentrated at 40 °C under reduced pressure and subsequently defatted three times with successive 10 mL portions of ethyl ether. After a second concentration step at 40 °C, the remaining solid residues were dissolved in water to a final volume of 5 mL and filtered through 0.2 µm HPLC filters. Chromatographic separation was conducted in a normal-phase column maintained at 40 °C. The mobile phase consisted of acetonitrile and deionized water (70:30, *v*/*v*), delivered at a flow rate of 1 mL/min. The identification of individual sugars was based on retention time comparison with those of authentic standards [17], while quantification was performed using the internal standard approach and calibration curves generated for fructose, manitol, trehalose, and raffinose. The results were expressed in mg/100 g (fw).

Organic acids were analyzed according to the method developed by Liava et al. [18], employing an ultra-fast liquid chromatography (UFLC) system coupled with a photodiode array (PDA) detector operating at 215 nm (Equipment specifications in Table S3). Extraction was carried out by submitting 2 g of each sample to a stirring extraction with 25 mL of 4.5% meta-phosphoric acid at 25 °C for 45 min. The mixture was then filtered through Whatman No. 4 filter paper and the extracts were further filtered through 0.2 µm nylon membrane filters. Separation was performed on a reverse phase maintained at 35 °C. The

mobile phase consisted of 3.6 mM sulfuric acid, delivered at a flow rate of 0.8 mL/min. Compounds were identified by matching their retention times and spectral characteristics with those of analytical standards. Quantification was performed using external calibration curves constructed for oxalic, malic, and fumaric acids with high linearity and well-defined limits of detection and quantification, previously validated and followed throughout the analysis (Table S4). The results were expressed in mg/100 g (fw).

Fatty acid profiles were obtained after derivatization of the lipid fraction into fatty acid methyl esters (FAMEs), following Soxhlet extraction and transesterification as described by Petropoulos et al. [19]. The FAMEs were analyzed by gas chromatography with flame ionization detection (GC-FID) (equipment specifications in Table S3). The injection was made in spitless mode with an injector temperature of 250 °C. The oven temperature was programmed as follows: initial temperature at 100 °C (held for 2 min), followed by a ramp of 10 °C/min to 140 °C, 3 °C/min to 190 °C, and 30 °C/min to 260 °C, with a final hold at 260 °C for 2 min. The carrier gas was hydrogen, with a flow rate of 1.1 mL/min. Identification was achieved by comparing retention times with those of an external commercial FAME standard mixture. The results were expressed as the relative percentage of each fatty acid within the total fatty acid profile.

2.3. Mushroom Extracts Preparation

One gram of lyophilized mushroom sample was combined with 30 mL of a methanol–water solution (80:20, *v*/*v*) at −20 °C for 2 h, following the procedure described by Heleno et al. [20]. After extraction, the samples were sonicated in a conventional ultrasonic bath (40 Hz) for 15 min in continuous mode (equipment specifications in Table S2) and subsequently filtered through Whatman No. 4 filter paper (BLANCLABO, Groisy, France). This extraction process was repeated twice using fresh 30 mL aliquots of the same solvent mixture. The combined extracts were concentrated under reduced pressure at 40 °C using a rotary evaporator to remove methanol.

For antioxidant activity assays, the resulting aqueous phase was freeze-dried (equipment specifications in Table S2) and reconstituted in the respective solvents. For chromatographic analysis, the aqueous part was first washed with *n*-hexane and then partitioned through liquid–liquid extraction using diethyl ether (3 × 30 mL), followed by ethyl acetate (3 × 30 mL). The *n*-hexane wash served as a defatting step to remove non-polar and lipophilic impurities, while the diethyl ether and ethyl acetate extractions were used to selectively recover semi-polar and polar phenolic compounds, respectively, from the aqueous phase. This purification strategy was designed to eliminate interfering substances and reduce matrix complexity. By minimizing the matrix effect the analytical signal becomes more accurate and reliable, improving both sensitivity and reproducibility. The collected organic fractions were evaporated to dryness at 40 °C and re-dissolved in methanol–water (20:80, *v*/*v*) for subsequent injection.

2.4. Analysis of Phenolic Profile

The dried extract was reconstituted in 20% methanol to a final concentration of 10 mg/mL. Prior to analysis, the solution was filtered through a 0.22 µm membrane filter. Analysis was carried out by HPLC-DAD-ESI/MS^n (instrument specifications in Table S3), following the methodology described by Heleno et al. [20]. Separation was achieved with a reverse-phase column at 25 °C. The mobile phases consisted of solvent A (0.1% formic acid in water) and solvent B (acetonitrile). The elution gradient was programmed as follows: 10–15% B over 5 min, 15–25% B over the next 5 min, 25–35% B over 10 min, followed by an isocratic step at 50% B for 10 min, and final re-equilibration of the column. The flow rate was maintained at 0.5 mL/min. Compound identification was based on the comparison of

retention times, UV–Vis spectra, and mass spectral data with those of commercial reference standards. When reference standards were unavailable, identification was supported by previously reported chromatographic data from the literature. Quantification was performed using calibration curves generated from commercial standards (Table S4), and results were expressed as mg of standard equivalents per g of extract.

2.5. Antioxidant Activity

The antioxidant capacity of the mushroom hydromethanolic extracts was assessed through two complementary in vitro assays. First, the inhibition of lipid peroxidation was determined using the thiobarbituric acid reactive substances (TBARS) assay, in which porcine brain homogenates served as the lipid-rich biological matrix. The method followed the procedure outlined by Pereira et al. [21], and the results were expressed as EC_{50} values, corresponding to the extract concentration required to inhibit 50% of TBARS formation. Extract concentrations ranged from 39 to 5000 μg/mL. Second, the protective effect against oxidative hemolysis was evaluated using the Oxidative Hemolysis Inhibition Assay (OxHLIA), as described by Lockowandt et al. [22]. This method measures the ability of the extracts to delay hemolysis in sheep erythrocytes under oxidative stress conditions, with IC_{50} values indicating the concentration necessary to postpone the hemolysis of the 50% of the erythrocytes by 60 min in both assays. For this assay, extract concentrations ranged from 69 to 540 μg/mL. Trolox was used as a positive control in both assays for comparative purposes.

2.6. Statistical Analysis

The experiments were conducted in triplicate, with each replicate consisting of an identical portion of the same bulk mushroom sample. These bulk samples were composed of pooled fruiting bodies collected from multiple locations within each locality of the MNP and during different periods throughout the year, ensuring both geographical and seasonal representativeness. The triplicates served as technical replicates, used to assess instrumental variability. Data are presented as mean values ± standard deviation (SD). The decimal place of the uncertain digit of the mean value was determined by rounding the SD value to one significant figure. Statistical tests were performed at a 5% significance level using IBM SPSS Statistics software (22.0. IBM Corp.: Armonk, NY, USA). Statistical differences between samples were assessed using a two-tailed paired Student's *t*-test.

Multivariate statistical analysis was performed using OriginPro 2019b version 9.6.5.169 (OriginLab Corporation, Northampton, MA, USA). Principal component analysis (PCA) was employed to explore patterns and groupings within the dataset. Prior to PCA, all variables were auto-scaled, mean-centered and normalized by their standard deviation to ensure the equal contribution of each variable regardless of scale. The PCA was conducted using a correlation matrix-based extraction method. The resulting biplot displays both scores (samples) and loadings (variables) projected onto the first two principal components (PC1 and PC2), which captured the entire variance structure in the data. Variables are represented as vectors to reflect their contribution to the principal components and their influence on sample separation. Interpretation was based on the spatial arrangement and directional alignment of variables and samples within the biplot, providing insights into underlying compositional differences.

3. Results and Discussion

3.1. Nutritional Value

F. hepatica and *C. nuda* are edible mushrooms traditionally consumed fresh, prized for their unique flavor and appealing texture. These species are widely used in culinary

applications, including sautéed dishes, soups, and sauces, and have gained increasing attention in gourmet cuisine and functional food development. Due to their nutritional profile and bioactive compound content, characterizing their composition in fresh form is critical to support their utilization in the food and nutraceutical industries.

The nutritional profiles of *F. hepatica* and *C. nuda* (Table 1) reveal notable differences in their macronutrient composition, which may guide their selective use in food formulations with distinct functional purposes. *C. nuda* exhibits significantly higher moisture and proteins contents, two attributes commonly associated with improved nutritional quality in fresh mushrooms and frequently valued in low-calorie, nutrient-dense foods. The elevated protein level in *C. nuda* suggests that it may be eligible for the "high protein" nutrition claim, as defined in Regulation (EC) No 1924/2006 [23], provided that the final formulation meets the required energy-to-protein ratio. This property makes *C. nuda* a promising candidate for inclusion in high-protein plant-based products, functional foods, or sports nutrition formulations. In contrast, *F. hepatica* showed higher concentrations of carbohydrates, crude fats, and ash, resulting in a significantly greater caloric value, which points to its potential as an energy-rich food component. These patterns generally align with trends reported for wild edible mushrooms, such as those described by Ouzouni et al. [24], although differences in proportions should be interpreted cautiously due to the influence of analytical and ecological variables. Their data indicate a composition of approximately 86.24% moisture, 0.44 g/100 g fat, 3.11 g/100 g protein, 9.08 g/100 g carbohydrates, and 1.12 g/100 g ash for *F. hepatica*, while *C. nuda* (reported as *Lepista nuda*) exhibited higher moisture (91.34%) but lower fat (0.28 g/100 g, fw), protein (2.98 g/100 g, fw), carbohydrate (4.88 g/100 g, fw), and ash (0.52 g/100 g, fw) contents. These estimations, derived from dry weight data and moisture levels, support the robustness of the compositional differences highlighted in the current study.

Table 1. Proximate composition, energy, free sugars, and organic acids composition of *F. hepatica* and *C. nuda*.

	F. hepatica	***C. nuda***
Moisture (g/100 g fw)	88 ± 1^{b}	94 ± 1^{a}
Proteins (g/100 g fw)	1.22 ± 0.05^{b}	2.5 ± 0.1^{a}
Ash (g/100 g fw)	1.01 ± 0.01^{a}	0.60 ± 0.01^{b}
Crude fat (g/100 g fw)	0.143 ± 0.002^{a}	0.094 ± 0.001^{b}
Available carbohydrates (g/100 g fw)	9.3 ± 0.2^{a}	2.1 ± 0.1^{b}
Energy (kcal/100 g)	43 ± 1^{a}	19.3 ± 0.7^{b}
Fructose (mg/100 g fw)	1.79 ± 0.03	-
Mannitol (mg/100 g fw)	0.032 ± 0.003^{a}	0.018 ± 0.002^{b}
Trehalose (mg/100 g fw)	0.16 ± 0.01^{b}	0.237 ± 0.002^{a}
Σ Free sugars (mg/100 g fw)	1.98 ± 0.05^{a}	0.26 ± 0.1^{b}
Oxalic acid (mg/100 g fw)	19.4 ± 0.7^{a}	18.0 ± 0.2^{b}
Malic acid (mg/100 g fw)	608 ± 2	-
Fumaric acid (µg/100 g fw)	152.6 ± 0.2	102.6 ± 0.2
Σ Organic acids (mg/100 g fw)	627 ± 3^{a}	18.0 ± 0.2^{b}

The results are presented as mean ± standard deviation. In each line, different letters (a–b) denote statistically significant differences between samples ($p < 0.05$), as determined by two-tailed paired Student's *t*-test.

Significant differences were also observed in the caloric contents of the two mushrooms. *F. hepatica* displayed a notably higher energy value of 43 kcal/100 g, attributed to its lower moisture and higher carbohydrate and fat contents, while *C. nuda* had an energy value of only 19.3 kcal/100 g. These findings align with previous research by Ouzouni et al. [24], who reported 52.72 kcal/100 g for *F. hepatica* and 33.96 kcal/100 g for *C. nuda*. This suggests

that *F. hepatica* may be more suitable for energy-dense food preparations, particularly for individuals with increased caloric needs, such as those recovering from illness, experiencing undernutrition, or following vegetarian diets that require energy compensation without animal fats. Additionally, the higher energy density could facilitate the development of shelf-stable mushroom-based concentrates or spreads.

Beyond caloric value, the significantly higher ash content in *F. hepatica* (1.01 g/100 g fw) indicates a greater contribution of mineral elements. This aligns with previous studies that have positioned *F. hepatica* among the richest wild mushrooms in essential minerals such as magnesium, potassium, and zinc [25]. These elements play crucial physiological roles, and their intake is often suboptimal in modern diets [26]. Therefore, *F. hepatica* may be favorably positioned for inclusion in mineral-rich or micronutrient-supportive formulations targeting specific nutritional gaps. On the other hand, the pronounced protein content in *C. nuda* reinforces its value as a lean, plant-based protein source, suitable for dietary approaches emphasizing satiety, muscle maintenance, or metabolic regulation.

Both mushrooms exhibited very low levels of crude fat (0.143 g/100 g, fw in *F. hepatica* and 0.094 g/100 g, fw in *C. nuda*), which qualifies them both to be labeled as "fat-free" in accordance with the criteria established by Regulation (EC) No 1924/2006 [23]. This attribute further enhances their appeal in the formulation of health-oriented foods, particularly for consumers seeking low-fat or cardiovascular-friendly options. Their fat-free nature, combined with their plant/mushroom origin and high nutritional density, also aligns with current consumer trends favoring clean-label, low-lipid products with natural functional value.

Taken together, these distinctions highlight the complementary nutritional roles of *F. hepatica* and *C. nuda*. *F. hepatica* stands out as a fat-free, energy-dense source of protein and minerals, with potential application in formulations requiring caloric support or micronutrient enrichment. Meanwhile, *C. nuda* emerges as a fat-free, high-protein mushroom with low energy value, suitable for dietetic foods, high-protein snacks, or functional meals aimed at muscle maintenance, weight control, or metabolic health. These findings, which mirror the patterns observed by Ouzouni et al. [24], reinforce the reproducibility of nutritional profiles across different ecological contexts and support the strategic valorization of each species according to its compositional strengths. Moreover, their potential eligibility for regulated nutrition claims, including "fat-free", "source of protein" (*F. hepatica*), and "high protein" (*C. nuda*), adds marketing and regulatory value for their incorporation into novel food products within the European Union framework.

3.2. Chemical Composition

Sugars and organic acids play a pivotal role in shaping both the nutritional value and sensory attributes of edible mushrooms. These compounds are key contributors to the flavor profile, imparting sweetness, acidity, and subtle umami notes that influence consumer preference and culinary versatility. Free sugars, particularly mannitol and trehalose, are among the most abundant carbohydrates in many fungal species. They function not only as energy reserves but also as osmoprotectants, stabilizing cellular structures and maintaining turgor pressure under environmental stress. This osmoprotective role becomes especially relevant during post-harvest handling and storage, where these compounds can mitigate desiccation and oxidative damage, ultimately extending shelf life and preserving textural quality [27]. Meanwhile, organic acids such as malic, fumaric, and oxalic acids, are integral intermediates in cellular metabolic pathways. Beyond their metabolic function, many of these acids exhibit bioactive properties, including antioxidant, anti-inflammatory, and potential neuroprotective effects, which may contribute to the health-promoting potential of mushrooms when consumed as part of a balanced diet [28,29]. Their presence also

influences pH and microbial stability, playing a functional role in food preservation and safety [30].

The qualitative and quantitative profiles of both free sugars and organic acids can vary significantly between mushroom species, and even among different ecological conditions or developmental stages. This biochemical variability provides a valuable tool for species differentiation and chemotaxonomic studies. It also enables the targeted selection of mushroom species for the development of functional foods, nutraceutical formulations, and high-end gourmet products, where specific sensory or health-related properties are desired. Thus, a comprehensive characterization of these compounds is essential not only for nutritional assessment, but also for enhancing the economic and gastronomic valorization of wild and cultivated mushrooms.

The sugar and organic acid compositions of *F. hepatica* and *C. nuda* exhibit notable differences, as Table 1 shows (chromatograms in Figures S1 and S2 of Supplementary Materials), reflecting their distinct metabolic pathways and potential nutritional applications.

The species *F. hepatica* exhibits a notably higher total sugar content (1.98 ± 0.05 g/100 g), largely attributed to its elevated fructose concentration (1.79 ± 0.03 g/100 g), which accounts for approximately 90% of the total sugar pool. This monosaccharide, commonly associated with sweetness and energy availability, may play a critical role in the metabolic and sensory properties of *F. hepatica* [31]. The presence of fructose, alongside measurable levels of mannitol (0.032 ± 0.003 g/100 g) and trehalose (0.16 ± 0.01 g/100 g), suggests a diverse carbohydrate profile that could reflect specific physiological adaptations, such as osmoregulation strategies aligned with the ecological characteristics of the habitat this species inhabits [32]. In contrast, *C. nuda* shows a significantly lower total sugar content (0.26 ± 0.1 g/100 g), with trehalose (0.237 ± 0.002 g/100 g) as the predominant sugar and a minor contribution from mannitol (0.018 ± 0.002 g/100 g). The absence of detectable fructose in *C. nuda* is consistent with previous literature, where trehalose and mannitol are typically reported as the major free sugars in this species, with no mention of fructose presence [33]. Trehalose is a well-known disaccharide with roles in stress tolerance, cellular protection against desiccation, and thermal stability. Its dominance in *C. nuda* may reflect an ecological strategy geared toward coping with environmental fluctuations, including low temperatures or limited water availability, which are common in the forest habitats where this species typically thrives [34].

Regarding organic acids, *F. hepatica* exhibits a notably diverse and abundant profile, with a total organic acid content of 667 ± 3 mg/100 g. Malic acid is by far the most predominant compound (608 ± 2 mg/100 g), followed by lower amounts of oxalic acid (19.4 ± 0.7 mg/100 g) and fumaric acid (152.6 ± 0.2 µg/100 g). This composition reflects the involvement of these acids in fundamental metabolic processes and suggests a well-developed capacity for organic acid biosynthesis in this species [35]. The predominance of malic acid not only contributes to the characteristic acidic flavor of *F. hepatica*, but also points to its potential functional role, as malic acid has been associated with antioxidant, antimicrobial, and metabolic-regulating properties in various food matrices [36].

Fumaric acid, although present in lower concentration, is also of interest due to its role as an intermediate metabolite and its documented antioxidant and neuroprotective activities [37]. Oxalic acid, detected in both species at similar concentrations (*F. hepatica*—19.4 ± 0.7 mg/100 g; *C. nuda*—18.0 ± 0.2 mg/100 g), is a secondary metabolite commonly secreted by fungi into their surrounding environment. Beyond its presence in fruiting bodies, it plays a key ecological role in fungi by facilitating lignocellulose degradation. As a low-molecular-weight compound, it enhances the breakdown of cellulose and lignin, particularly in wood-decaying and saprotrophic species, supporting nutrient acquisition and contributing to carbon cycling in forest ecosystems [37].

In contrast, *C. nuda* showed a far more limited organic acid profile, with only oxalic and fumaric acids detected. The absence of malic acid may reflect species-specific metabolic characteristics, or a lower activity of metabolic pathways involved in their biosynthesis. This simplified profile suggests that *C. nuda* may contribute less to the acidic or functional properties typically associated with organic acids, though its oxalic and fumaric acids content still holds biochemical relevance.

These differences underscore the interspecific variability in organic acid metabolism among wild mushroom species, and highlight the potential of *F. hepatica* as a source of bioactive organic acids for functional food applications. Furthermore, the richness and diversity of organic acids in *F. hepatica* may enhance its nutritional and sensory appeal, and its higher acid content could also play a role in natural preservation, contributing to microbial stability in mushroom-derived products. The findings are consistent with previous reports on *F. hepatica* and reinforce the importance of organic acid profiling in the characterization and valorization of edible fungi [38].

Fatty acids represent a key component of the lipid fraction in edible mushrooms, and contribute to both their nutritional value and potential health benefits. Although present in relatively low concentrations compared to other food sources, the fatty acid profile of mushrooms is characterized by a favorable balance of saturated and unsaturated fatty acids, particularly linoleic and oleic acids. These compounds are essential for cellular membrane integrity and play roles in inflammatory regulation and cardiovascular health [39]. Moreover, certain fatty acids influence the sensory qualities of mushrooms, contributing to aroma, mouthfeel, and oxidative stability. The composition of fatty acids can vary substantially between species, developmental stages, and environmental conditions, offering valuable insight into the physiological status of the fungi and revealing their potential use in functional foods, or as nutritionally valuable meat alternatives [40,41].

The fatty acid profiles of *F. hepatica* and *C. nuda* reveal marked interspecific differences that reflect distinct metabolic traits and potential nutritional implications (Table 2 and Figure S3 of Supplementary Materials). In the present study, *C. nuda* exhibited a more unsaturated lipid profile, particularly characterized by significantly higher proportions of oleic acid (C18:1*n*-9, 44.5%) and linoleic acid (C18:2*n*-6, 33.0%) compared to *F. hepatica* (36.4% and 29.7%, respectively). These two C18 unsaturated fatty acids are commonly reported as dominant in mushroom species and are known to contribute not only to human health, specially through cardiovascular benefits and anti-inflammatory effects [42,43], but also to the development of desirable sensory attributes, such as the formation of aroma-active compounds like 1-octen-3-ol [44].

The high concentration of polyunsaturated fatty acids (PUFAs) in *C. nuda* (78.2%) emphasizes its potential as a functional food ingredient. Linoleic acid, an essential fatty acid obtained solely through diet, is linked to enhanced lipid metabolism and a decreased risk of chronic diseases. Additionally, the increased oleic acid content contributes to the lipid quality index of *C. nuda*, as this monounsaturated fatty acid (MUFA) is recognized for its stability and health benefits, akin to those present in olive oil [43].

Conversely, *F. hepatica* displayed a higher content of saturated fatty acids (SFA), amounting to 29.8%, which was significantly greater than that of *C. nuda* (19.8%). This was mainly due to higher concentrations of palmitic acid (C16:0, 19.7%), stearic acid (C18:0, 5.8%), and myristic acid (C14:0, 2.5%). Although saturated fats are generally considered less desirable from a nutritional standpoint, their presence can confer technological advantages such as increased oxidative stability and improved texture, particularly in processed or gourmet food applications [45]. These traits may favor *F. hepatica* in culinary formulations where flavor preservation and structural consistency are important.

Table 2. Fatty acid (%, relative percentage) profiles of *F. hepatica* and *C. nuda*.

Fatty Acid (%, Relative Percentage)	*F. hepatica*	*C. nuda*
C6:0	nd	0.382 ± 0.006
C8:0	nd	0.10 ± 0.02
C10:0	nd	0.134 ± 0.009
C12:0	0.87 ± 0.04 [a]	0.178 ± 0.003 [b]
C14:0	2.5 ± 0.2 [a]	0.60 ± 0.03 [b]
C14:1	0.083 ± 0.004 [a]	0.057 ± 0.001 [b]
C15:0	0.29 ± 0.04 [a]	0.303 ± 0.004 [a]
C15:1	nd	0.012 ± 0.003
C16:0	19.7 ± 0.2 [a]	15.1 ± 0.1 [b]
C16:1	1.6 ± 0.2 [a]	0.865 ± 0.004 [b]
C17:0	0.180 ± 0.003 [a]	0.071 ± 0.001 [b]
C18:0	5.8 ± 0.5 [a]	1.84 ± 0.04 [b]
C18:1*n*-9	36.4 ± 0.7 [b]	44.5 ± 0.4 [a]
C18:2*n*-6	29.7 ± 0.9 [b]	33.038 ± 0.001 [a]
C18:3*n*-3	2.3 ± 0.3 [a]	0.482 ± 0.002 [b]
C20:0	0.45 ± 0.04 [a]	0.25 ± 0.01 [b]
C20:1	nd	0.145 ± 0.002
C20:2	nd	0.029 ± 0.006
C21:0	nd	0.17 ± 0.03
C20:3*n*-6	nd	0.118 ± 0.002
C22:0	nd	0.29 ± 0.03
C24:0	nd	0.36 ± 0.03
C24:1	nd	0.655 ± 0.005
Fatty Acid Class		
SFA	29.8 ± 0.8 [a]	19.81 ± 0.07 [b]
MUFA	1.7 ± 0.2 [a]	1.732 ± 0.006 [a]
PUFA	69 ± 1 [b]	78.2 ± 0.4 [a]

The results are presented as mean ± standard deviation. C6:0—caproic acid, C8:0—caprylic acid, C10:0—capric acid, C12:0—lauric acid, C14:0—myristic acid, C14:1—myristoleic acid, C15:0—pentadecanoic acid, C15:1—pentadecanoic acid, C16:0—palmitic acid, C16:1—palmitoleic acid, C17:0—heptadecanoic acid, C17:1—heptadecenoic acid, C18:0—stearic acid, C18:1*n*9—oleic acid, C18:2*n*6—linoleic acid, C18:3*n*-3—α-linolenic acid, C20:0—arachidic acid, C20:1—eicosenoic acid, C20:2—eicosadienoic acid, C21:0—heneicosanoic acid, C20:3*n*-6—8,11,14-eicosatrienoic acid, C22:0—behenic acid, C24:0—lignoceric acid, C24:1—nervonic acid, SFS—saturated fatty acids, MUFA—monounsaturated fatty acids, PUFA—polyunsaturated fatty acids. nd—not detected. In each line, different letters (a–b) indicate statistically significant differences ($p < 0.05$) between samples according to the two-tailed paired Student's *t*-test.

Interestingly, while *F. hepatica* had a broader spectrum of fatty acids overall, including higher amounts of some long-chain SFA and trace levels of C18:3*n*-3 (α-linolenic acid), *C. nuda* exhibited a more diverse range of very-long-chain fatty acids (e.g., C20:1, C20:2, C24:0, C24:1), albeit at lower concentrations. These minor components may still play roles in modulating biological membranes and contributing to physiological functions, and their presence reflects species-specific lipid metabolism [45].

When compared with the previous literature, our results for *F. hepatica* align with those reported by Ribeiro et al., who also identified linoleic and oleic acids as predominant, although in different relative proportions [46]. These variations are likely attributable to differences in sample origin (e.g., environmental conditions, substrate), developmental stage, and extraction methodologies. Similarly, our findings for *C. nuda* are consistent with those reported by Noël-Suberville et al., who analyzed individual anatomical parts (gills) and reported linoleic and oleic acids as major constituents [47]. Although absolute values were lower in that study due to the specific tissue analyzed and potentially lower sensitivity, the proportional trends remain comparable.

Taken together, these findings confirm that both species are valuable sources of unsaturated fatty acids, yet with different lipid profiles that may support distinct applications. *C. nuda*, with its high PUFA and oleic acid content, is particularly well-suited for inclusion in functional foods aimed at cardiovascular health promotion. In contrast, *F. hepatica*, with its more balanced lipid composition and higher SFA content, may be advantageous for food products requiring greater oxidative stability, such as savory snacks, pâtés, or mushroom-based seasonings. These insights reinforce the importance of species-specific biochemical characterization for the strategic valorization of wild edible mushrooms in the development of health-oriented or gourmet food products.

3.3. Phenolic Profile

Phenolic compounds are a diverse group of secondary metabolites widely recognized for their health-promoting properties. They exhibit strong antioxidant activity and have been associated with various biological effects, including anti-inflammatory, cardioprotective, neuroprotective, and anticancer potential. In the context of human health, these compounds can neutralize reactive oxygen species, modulate enzyme activity, and influence cellular signaling pathways. As such, the identification and quantification of phenolic compounds in natural sources is essential for evaluating their functional value and potential applications in nutraceutical and pharmaceutical formulations [48].

As shown in Table 3, four phenolic acids and quinic acid were detected and tentatively identified in the hydroethanolic extracts of *F. hepatica* and *C. nuda* using HPLC-DAD-ESI/MS^n (chromatogram in Table S4 of Supplementary Materials). Compound **1**, tentatively identified as quinic acid, showed a deprotonated molecule $[M - H]^-$ at m/z 191, with characteristic MS^2 fragment ions at m/z 111 (100%) and 173 (18%), and a maximum absorption maximum at 193 nm. Compound **2** exhibited a pseudomolecular ion $[M - H]^-$ at m/z 169 and produced a main MS^2 fragment at m/z 125 (100%), with maximum absorbance at 280 nm. This compound was identified as gallic acid. Compound **3** showed a deprotonated ion $[M - H]^-$ at m/z 153 and a single fragment at m/z 109 (100%), along with a maximum absorbance at 259 nm, allowing its identification as protocatechuic acid. Compound **4** showed a $[M - H]^-$ ion at m/z 163, with fragment ions at m/z 145 (100%) and 119 (17%), and maximum absorbance at 310 nm. These features correspond to *p*-coumaric acid. Compound **5** had a $[M - H]^-$ ion at m/z 147, which fragmented to m/z 119 (100%), and presented a maximum absorbance at 284 nm, leading to its identification as cinnamic acid.

Quantitative analysis revealed clear interspecific differences in both the presence and concentration of the identified compounds. *C. nuda* showed a significantly higher content of quinic acid (4.13 $\pm$ 0.02 mg/g extract) compared to *F. hepatica* (0.818 $\pm$ 0.001 mg/g), suggesting a species-specific accumulation of this precursor molecule. This higher concentration may be related to metabolic pathways oriented toward phenolic biosynthesis or to stress-related metabolite storage. Quinic acid was the only non-phenolic compound quantified under the applied conditions, and in *C. nuda* it represented the major constituent of the hydroethanolic extract. Quinic acid itself has been associated with antioxidant and hepatoprotective activities [49,50], and its presence in *C. nuda* may enhance the extract's overall bioactivity.

Table 3. HPLC-DAD-ESI/MS^n identification and quantification of phenolic compounds in *F. hepatica* and *C. nuda* extracts.

Peak	Rt (min)	λ_{max} (nm)	$[M-H]^-$ (*m*/*z*)	MS^2 (*m*/*z*)	Compound	Identification	Content (mg/g Extract)	
							F. hepatica	***C. nuda***
1	4.53	193	191	111(100); 173(18)	Quinic acid	MS/DAD	0.818 ± 0.001^{b}	4.13 ± 0.02^{a}
2	5.51	280	169	125(100)	Gallic acid	STD	1.103 ± 0.006^{a}	0.858 ± 0.06^{b}
3	6.26	259	153	109(100)	Protocatechuic acid	STD	0.97 ± 0.09^{a}	0.829 ± 0.005^{b}
4	16.41	310	163	145(100); 119(17)	*p*-Coumaric acid	STD	0.493 ± 0.006	nd
5	24.72	284	147	119(100)	Cinnamic acid	STD	0.112 ± 0.002	nd
					Σ Organic acids		0.818 ± 0.001^{b}	4.13 ± 0.02^{a}
					Σ Phenolic acids		2.7 ± 0.1^{a}	1.69 ± 0.04^{b}

Quantitative results are expressed as mean ± standard deviation, and different letters (a–b) in each row denote statistically significant differences between samples ($p < 0.05$), as determined by Student's *t*-test. Quinic acid was quantified using chlorogenic acid standard. The remaining compounds were quantified using their respective authentic standards.

In contrast, *F. hepatica* exhibited a significantly higher total phenolic acid content (2.70 ± 0.10 mg/g extract) than *C. nuda* (1.69 ± 0.04 mg/g extract), indicating a broader and more concentrated phenolic profile. Among the identified phenolic acids, gallic acid and protocatechuic acid were dominant in both species, but in higher concentrations in *F. hepatica*. Gallic acid is a potent antioxidant, known for its ability to scavenge free radicals and chelate metal ions, and has also been reported to exert antimicrobial and cytoprotective effects [51]. Protocatechuic acid similarly possesses antioxidant and anti-inflammatory properties, and has been investigated for its role in modulating lipid metabolism and protecting against oxidative damage in cellular systems [52]. The co-occurrence of both acids suggests a synergistic antioxidant effect in the extracts, especially in *F. hepatica*.

A particularly relevant finding is the exclusive detection of *p*-coumaric acid and cinnamic acid in *F. hepatica*, at concentrations of 0.493 ± 0.006 and 0.112 ± 0.002 mg/g extract, respectively. These hydroxycinnamic acids are well known for their capacity to inhibit oxidative enzymes and their potential to act as radical scavengers. Moreover, *p*-coumaric acid has been linked to anti-inflammatory [53], antimicrobial [54], and anticancer properties [55], while cinnamic acid has shown neuroprotective and vasorelaxant activities in vitro and in vivo [56,57]. Their absence in *C. nuda* and presence in *F. hepatica* may contribute to the distinct biological profiles and offer a biochemical basis for species-specific health claims or functional applications.

These results suggest differential metabolic allocations between the two species—*C. nuda* concentrates more in organic acid synthesis, particularly quinic acid, while *F. hepatica* invests in a chemically diverse set of phenolic acids. This could be a reflection of species-specific stress responses, habitat-driven metabolic plasticity, or developmental stage at the time of harvest. From a functional standpoint, the broader phenolic spectrum of *F. hepatica* supports its potential use in antioxidant-rich supplements or as a bioactive ingredient in functional foods aiming to prevent oxidative stress-related conditions. In contrast, the high quinic acid content in *C. nuda* may be advantageous in formulations targeting liver support or general detoxification.

Overall, the phenolic profile of *F. hepatica* appears more chemically diverse and functionally complex, while *C. nuda* is characterized by a dominant single metabolite with high abundance. These compositional patterns have direct implications for their respective bioactivities and potential applications in the nutraceutical and food industries. Future research should include in vitro and in vivo bioactivity assessments, stability studies under processing conditions, and detailed characterizations of their phenolic metabolism to fully unlock the functional potential of these underexplored mushroom species.

3.4. Antioxidant Activity

The antioxidant activity of methanolic extracts from *F. hepatica* and *C. nuda* was assessed using two complementary assays, TBARS and OxHLIA, which simulate biologically relevant oxidative processes. Trolox was employed as the positive control in both cases, allowing for comparative evaluations of the mushroom extracts' efficacy (Table 4).

The results reveal a distinct pattern of activity between the two species, consistent with differences in their phytochemical composition. In the OxHLIA assay, which measures the ability of antioxidants to protect erythrocyte membranes from peroxyl radical-induced hemolysis, *F. hepatica* exhibited significantly stronger activity (IC_{50} = 126 ± 5 µg/mL) than *C. nuda* (182 ± 6 µg/mL). This suggests that *F. hepatica* contains membrane-active compounds capable of stabilizing or scavenging free radicals at the cellular interface, potentially due to its more diverse phenolic profile, including the exclusive detection of *p*-coumaric and cinnamic acids.

Conversely, in the TBARS assay used to evaluate the inhibition of lipid peroxidation in a porcine brain homogenate matrix, *C. nuda* was significantly more effective (EC_{50} = 303 ± 17 μg/mL) than *F. hepatica* (555 ± 29 μg/mL). This may reflect the presence of lipophilic antioxidant compounds, such as polyunsaturated fatty acids or other synergistic metabolites, more efficient in interrupting lipid oxidation chains in complex biological matrices.

Table 4. Antioxidant activity of *F. hepatica* and *C. nuda* extracts and positive controls.

	F. hepatica	***C. nuda***	**Positive Control**
Antioxidant activity			Trolox
OxHLIA (IC_{50}, μg/mL), Δ*t* 60 min	126 ± 5 [b]	182 ± 6 [c]	21.5 ± 0.2 [a]
TBARS formation inhibition (EC_{50}, μg/mL)	555 ± 29 [c]	303 ± 17 [b]	139 ± 5 [a]

The results are presented as mean ± standard deviation. In each line, different letters (a–c) denote statistically significant differences between samples ($p < 0.05$), as determined by two-tailed paired Student's *t*-test.

These contrasting results underscore the need to consider the antioxidant assay model when evaluating biological extracts, as different mechanisms of action may predominate depending on the structural nature of the oxidative target and the chemical environment. The superior performance of *F. hepatica* in the OxHLIA test, together with its more diverse phenolic content, suggests greater potential in applications aimed at protecting cell membranes or redox-sensitive tissues. Meanwhile, the higher TBARS inhibition by *C. nuda* reinforces its suitability for formulations designed to enhance lipid stability.

Comparison with the existing literature further supports the relevance of these findings. Rugolo et al. [58] reported OxHLIA and TBARS IC_{50} values for *C. nuda* of 93 ± 6 μg/mL and 711 ± 185 μg/mL, respectively. While their *C. nuda* extract showed better performance in OxHLIA, our sample displayed superior activity in TBARS. Such discrepancies may result from geographic, seasonal, or methodological factors, including differences in extraction solvent, mushroom maturity, or environmental stressors, which can strongly influence antioxidant compound profiles. Although *F. hepatica* was not directly analyzed in that study, its placement within the *Fistulina* genus reported to contain bioactive metabolites highlights the novelty of our findings. To our knowledge, this is the first report characterizing and comparing the antioxidant properties of *F. hepatica* and *C. nuda* using both TBARS and OxHLIA. These results suggest a differentiated functional potential for each species, offering distinct antioxidant profiles relevant to food, nutraceutical, or biotechnological applications.

3.5. Principal Component Analysis

The extended principal component analysis (PCA) biplot (Figure 1) provides a robust multivariate overview of the biochemical and nutritional differences between *F. hepatica* and *C. nuda*, offering strong statistical support for species-level discrimination based on the selected variables. PC1 explains 100% of the total variance, which is highly uncommon in biological datasets and highlights the presence of a very clear linear separation between both mushroom species. Although PC2 explains no additional variance, the spatial distribution of variables and sample scores along PC1 still yields meaningful biological insights.

On the positive side of PC1, *F. hepatica* is clearly associated with a cluster of variables indicative of higher energy density and antioxidant potential. These include total free sugars (specifically fructose and mannitol), organic acids such as fumaric acid, and key phenolic compounds like gallic acid and protocatechuic acid. In addition, its proximity to fat, ash, and saturated fatty acids (SFA) reinforces its characterization as a nutrient-dense species with potential for energy-rich formulations. The association with OxHLIA further

supports its ability to delay oxidative damage at the cellular level, possibly due to the combined effects of phenolic acids and simpler antioxidants such as sugars and fumaric acid. This pattern suggests that *F. hepatica* may be particularly suited for use in functional foods aimed at oxidative stress modulation, immune support, or energy supplementation.

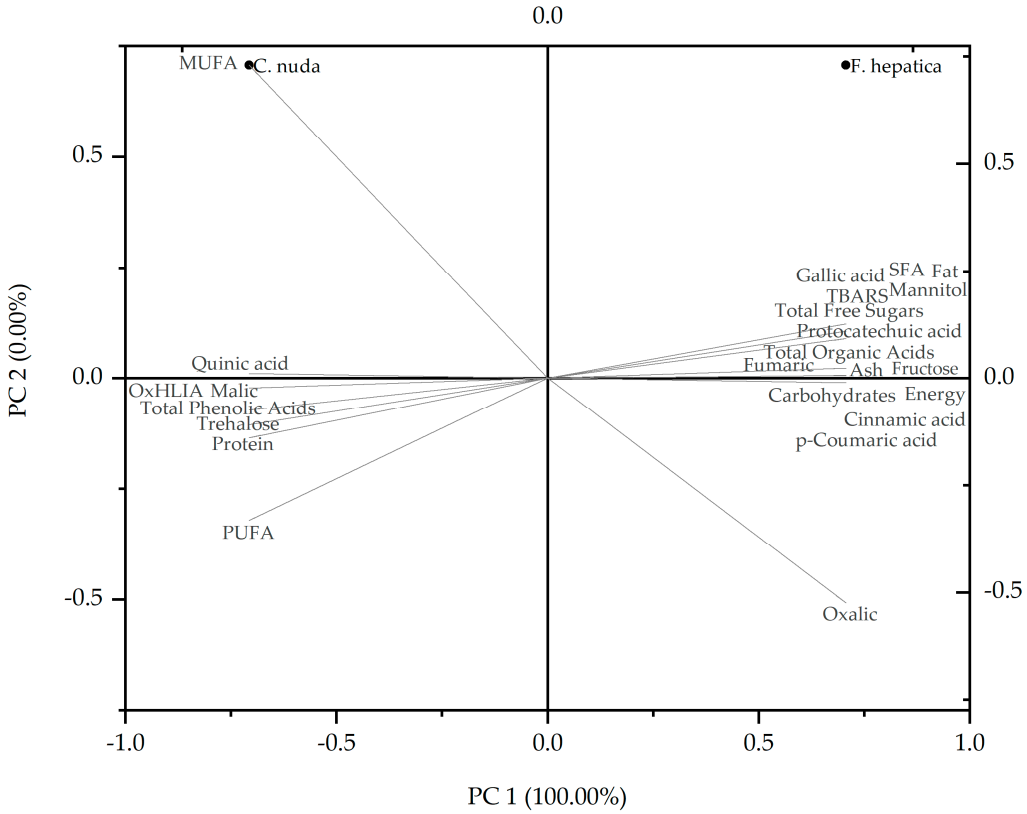

Figure 1. PCA biplot of nutritional, phytochemical, and antioxidant variables of *F. hepatica* and *C. nuda*.

In contrast, *C. nuda* is positioned on the negative side of PC1, showing strong correlations with unsaturated fatty acids (MUFA and PUFA), higher protein content, and organic acids such as quinic and malic acids. The association with enhanced performance in the TBARS assay suggests the superior inhibition of lipid peroxidation, which, combined with its unsaturated lipid profile, positions *C. nuda* as a valuable ingredient for cardioprotective and anti-inflammatory functional foods. The presence of higher quinic acid and total phenolic acid levels in *C. nuda* also supports its relevance in applications targeting oxidative balance and metabolic regulation. These characteristics may be particularly appealing in plant-based dietary supplements or formulations designed for active lifestyles and cardiovascular health.

The clear spatial separation between the two species also implies divergent metabolic strategies. *F. hepatica* appears more oriented toward carbohydrate metabolism and the accumulation of energy-related metabolites and hydroxybenzoic acids, which are structurally simpler but more potent antioxidants. Conversely, *C. nuda* reflects a strategy focused on membrane protection through unsaturated lipids and phenolic precursors (e.g., quinic acid), suggesting a more lipid-centered antioxidant response.

Importantly, the PCA biplot not only confirms previously discussed univariate differences, but also integrates them into a coherent multivariate pattern, emphasizing the complementarity of the two species. From a product development perspective, these results suggest the possibility of formulating synergistic blends combining the antioxidant potency and energy density of *F. hepatica* with the lipid-stabilizing and protein-rich profile of *C.*

nuda. Such combinations could enhance both the nutritional and functional quality of mushroom-based formulations.

For future research, integrating these biochemical profiles with genomic, transcriptomic, or proteomic analyses could provide deeper insight into the regulatory pathways responsible for the observed metabolite distributions. Such an approach would not only clarify the ecological and physiological drivers of these differences, but also support the biotechnological exploitation of both species in the context of precision nutrition and functional ingredient design.

4. Conclusions

This study provides the first comparative nutritional, chemical, and antioxidant characterizations *of F. hepatica* and *C. nuda* collected from MNP, revealing marked compositional and functional differences between both species. The main findings derived from the results and discussion are summarized below:

- *F. hepatica* showed higher contents of carbohydrates, fat, ash, and energy, as well as a more diverse phenolic acid profile, including the exclusive presence of *p*-coumaric and cinnamic acids. It also exhibited superior antioxidant capacity in the OxHLIA assay, suggesting potential protection at the cellular membrane level;
- *C. nuda* stood out for its greater protein and moisture contents, significantly higher levels of quinic acid, and a lipid profile rich in polyunsaturated fatty acids. It demonstrated a stronger inhibition of lipid peroxidation in the TBARS assay;
- Principal component analysis (PCA) confirmed a clear chemical and bioactive distinction between the two species, reinforcing the importance of integrating nutritional, phytochemical, and functional data for species differentiation;
- These findings support the targeted valorization of *F. hepatica* in energy- and antioxidant-enriched formulations, and of *C. nuda* in high-protein and PUFA-rich dietary products.

Taken together, these findings support the potential use of *F. hepatica* and *C. nuda* in the development of functional food and nutraceutical formulations. The study focused on specimens collected from Montesinho Natural Park, reflecting a deliberate effort to characterize species within a specific ecological and biogeographical context of high conservation and mycological value. While this localized approach enhances the ecological relevance of the data, broader studies encompassing seasonal dynamics, habitat variation, and environmental gradients would be valuable to assessing the consistency and applicability of the observed profiles.

Future investigations should also consider complementary bioactivities such as antimicrobial, anti-inflammatory, or cytoprotective effects, as well as the impacts of postharvest handling and processing on the stability and functionality of the identified compounds. Additionally, exploring the feasibility of ex situ cultivation could contribute to the sustainable use and conservation of these species.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/separations12080204/s1. Table S1: Chemicals and standards used in the analyses; Table S2: Equipment used in nutritional, chemical, and bioactivity analysis. Table S3: Equipment used in chromatographic analysis; Table S4: Calibration curves used to quantify organic acids and phenolic compounds. Figure S1: Chromatograms of free sugars: (**A**) sugar standards, (**B**) *F. hepatica*, and (**C**) *C. nuda*. Peaks correspond to: **1**—fructose, **2**—mannitol, **3**—trehalose, and **4**—raffinose. Figure S2: Chromatograms of organic acids: (**A**) organic acid standards, (**B**) *F. hepatica*, and (**C**) *C. nuda*. Peaks correspond to: **1**—oxalic acid, **2**—malic acid, and **3**—fumaric acid. Figure S3: Chromatographic profiles of fatty acid methyl esters (FAMEs) obtained by GC-FID. (**A**) Commercial

FAME standard; (**B**) *F. hepatica*; (**C**) *C. nuda*. Identified compounds: (**1**) caproic acid (C6:0), (**2**) caprylic acid (C8:0), (**3**) capric acid (C10:0), (**4**) lauric acid (C12:0), (**5**) myristic acid (C14:0), (**6**) myristoleic acid (C14:1), (**7**) pentadecanoic acid (C15:0), (**8**) pentadecenoic acid (C15:1), (**9**) palmitic acid (C16:0), (**10**) palmitoleic acid (C16:1), (**11**) heptadecanoic acid (C17:0), (**12**) heptadecenoic acid (C17:1), (**13**) stearic acid (C18:0), (**14**) oleic acid (C18:1n9), (**15**) linoleic acid (C18:2n-6), (**16**) α-linolenic acid (C18:3n-3), (**17**) arachidic acid (C20:0), (**18**) eicosenoic acid (C20:1), (**19**) eicosadienoic acid (C20:2), (**20**) heneicosanoic acid (C21:0), (**21**) 8,11,14-eicosatrienoic acid (C20:3n-6), (**22**) behenic acid (C22:0), and (**23**) lignoceric acid (C24:0). Figure S4: Chromatograms of phenolic acids: (**A**) phenolic acid standards, (**B**) *F. hepatica*, and (**C**) *C. nuda*. Peaks correspond to: **1**—quinic acid, **2**—gallic acid, **3**—protocatechuic acid, **4**—*p*-coumaric acid, and **5**—cinnamic acid.

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