COMPARATIVE NUTRITIONAL AND FATTY ACID PROFILING OF COMMERCIALLY CULTIVATED AND WILD-HARVESTED *PLEUROTUS OSTREATUS* AND *ARMILLARIA MELLEA* MUSHROOMS

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In this study, the composition of commercially purchased mushrooms was compared with two species of wild mushrooms collected from Romania's flora. The protein content, lipid content, total sugar content, and the chromatographic profile of fatty acids were evaluated. Although the two types of forest-collected mushrooms exhibited significantly higher protein and lipid content, it was observed that commercially cultivated mushrooms had a much higher total carbohydrate content. These aspects are highly relevant from a gastronomic perspective, as mushrooms are traditionally consumed in Romania.

Keywords: *Pleurotus ostreatus, Armillaria mellea*, GC-MS, polyunsaturated fatty acids

1. Introduction

Although certain mushroom species are toxic and unsuitable for consumption, leading to widespread caution, particularly among European populations, in Asia, mushrooms have been cherished for millennia as both food and for their therapeutic properties. Archaeological findings indicate that as early as 6,800 years ago in Neolithic China, the Reishi mushroom, known as "Ling Zhi" or "ghost plant" in Chinese, was utilized. Their cultivation can be traced back to the Tang Dynasty, between 600 and 900 CE. Today, approximately 200 mushroom species have been successfully cultivated in laboratory settings, with nearly 100 edible species domesticated, of which about 60% are grown for commercial purposes [1-3].

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This trend is gaining momentum, becoming increasingly popular in recent years within European culture as well. The market now features a growing variety of edible mushrooms for the culinary industry, as well as dietary supplements, personal care products, and cosmetics containing medicinal mushrooms or therapeutic compounds derived from them.

The increasing consumption of cultivated mushrooms in Romania aligns with global trends in industrial mushroom production, as evidenced by the world ranking of leading producers as presented in Fig. 1, of Devochkina et al. (2019), which underscores the economic and nutritional significance of mushrooms in addressing food security. The analysis also indicates that in 2019, Romania ranked 25th in the global mushroom production hierarchy, with an output of 14,500 t [4].

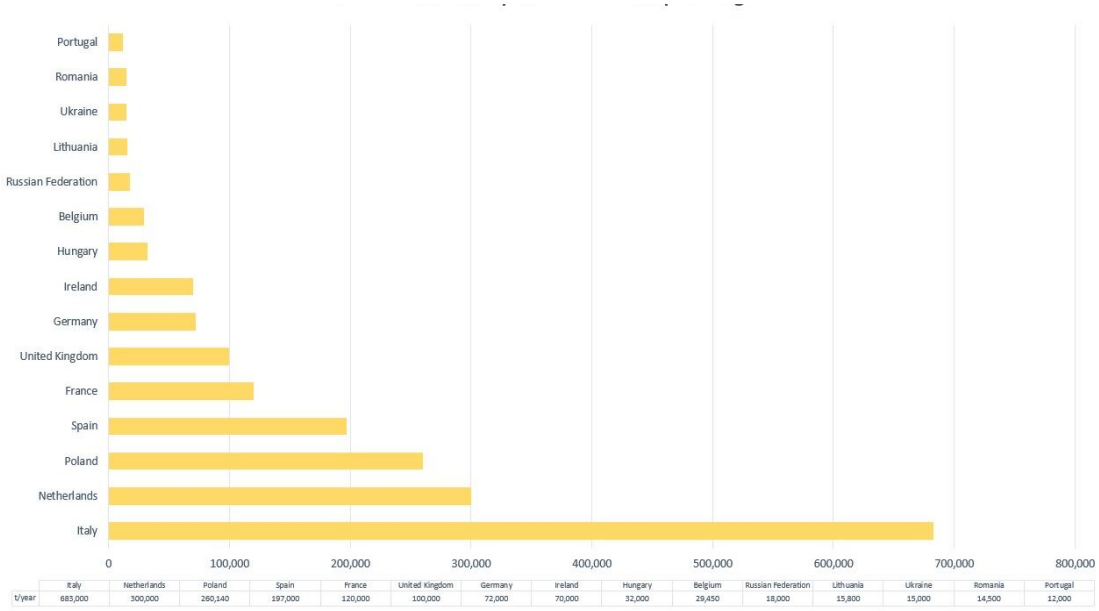

Fig. 1. Mushroom production in the European region [4]

The nutritional value of wild edible mushrooms in Romania, encompassing their lipid content, total protein, carbohydrate composition, and fatty acid profiles, is highlighted by the detailed analysis of Transylvanian species such as *Pleurotus ostreatus*, as documented by Fogarasi et al. (2018), underscoring their importance in dietary and gastronomic applications. They have reported that *Pleurotus ostreatus* has a protein content of approximately 17.92% dry weight, lipid content of 1.26% dry weight, and significant carbohydrate levels, with fatty acid profiles dominated by unsaturated fatty acids like linoleic acid. These findings support the nutritional significance of wild mushrooms [5].

Wild edible mushrooms are characterized by fatty acid profiles dominated by unsaturated fatty acids, particularly cis-linoleic and cis-oleic acids, which

contribute to their nutritional value, as demonstrated by analyses of various species from diverse regions [6].

In Romanian culinary heritage, *Armillaria mellea* is cherished as a foraged delicacy. The average annual quantity of wild edible mushrooms foraged and consumed gastronomically in Romania between 2012 and 2015 is approximately 500 t/year, with *Armillaria mellea* being the species most frequently collected from the forest [7].

Similarly, *Armillaria mellea* is primarily composed of carbohydrates (81.25 g/100 g), with low levels of lipids (1.97 g/100 g) and proteins (1.81 g/100 g), and its chromatographic fatty acid profile is predominantly characterized by polyunsaturated fatty acids (64.12%), followed by saturated fatty acids (18.42%) and monounsaturated fatty acids (17.47%) [8].

Given the significant trend of consuming various mushroom species across Romania, we aimed at determining their content, both for commercially sourced mushrooms and those foraged from the wild flora, in terms of total carbohydrates, proteins, lipids, and fatty acid profiles, to assess their gastronomic quality.

2. Materials and methods

2.1 Mushroom powder

The raw material of *Pleurotus ostreatus* (CPO) was purchased from the local market, wild *Armellaria mellea* (WAM) was collected from the southern region of Romania, in the Comana forest, Giurgiu County, while wild *Pleurotus ostreatus* (WPO) was gathered from the Bușteni area, Prahova County.

The raw mushrooms were dried at 38°C. Subsequently, the dried raw material was ground using a Retsch laboratory knife mill (Grindomix GM 200 model).

2.2. Total Lipid Content

A semi-automatic continuous extraction apparatus (Soxhlet -VELP) was used for the lipid extraction and quantification. Five grams of the sample weighed in a cellulose extraction thimble were immersed in boiling n-hexane and extracted for 60 min, followed by a rinsing phase with cold solvent. After the solvent recovery the extracted lipidic mass was weighed [9, 10].

2.3 Fatty Acid Profile

The fatty acid composition was determined by analyzing 0.1 g of lipid extract, accurately weighed using an analytical balance. For transesterification of triacylglycerols into fatty acid methyl esters (FAMEs), the lipid extract was dissolved in 0.5 mL of petroleum ether, followed by the addition of 9.5 mL of a 0.5M HCl solution in MeOH. The mixture was heated at 65°C until clear, with an additional 5 min of heating to ensure complete reaction. After cooling, the solution was transferred to a separatory funnel, and 20 mL of isooctane was added. The

organic (isooctane) phase was washed with distilled water until the aqueous wash reached a neutral pH. To remove residual water, the isooctane layer was dried over anhydrous sodium sulfate, filtered through glass microfiber filters (Whatman, Cat. No. 1822-070) and diluted 1:5 with isooctane. A 1µL aliquot of the resulting solution was injected into a gas chromatography-mass spectrometry (GC-MS) system for analysis.

The analysis was performed using a Thermo Scientific Focus GC gas chromatograph equipped with a Macropol 20,000 R column (60 m length, 0.25 mm inner diameter, 0.25 µm film thickness). Helium was used as the carrier gas at a constant flow rate of 1.5 mL/min. Detection was carried out with a Thermo Scientific DSQ II mass spectrometer, and peak identification was conducted by comparing mass spectra to the NIST spectral library [11].

2.4 Carbohydrate content

The total carbohydrate content was analyzed using the adapted phenol-sulfuric acid method described by Dubois et. all (1951). The glucose stock solution prepared for obtaining the calibration curve was made by weighing 100 mg of Glucose S.R. on an analytical balance, which was then dissolved in 100 mL of H_2O. 10 mL of this solution was diluted to 100 mL with H_2O (Glucose W.S.). From the Glucose W.S. solution, volumes of 0.2, 0.4, 0.6, 0.8, and 1 mL were pipetted (for volumes less than 1 mL, the volume was adjusted to 1 mL with H_2O). To each, 1 mL of 5% phenol solution and 5 mL of 96% H_2SO_4 were added, followed by agitation and incubation in a water bath at 100°C for 10 min, then mechanical agitation at 25°C for 20 min. The absorbance was measured at a wavelength of 490 nm against a blank prepared with 1 mL of H_2O [12, 13].

The sample was processed by weighing the sample mass in a test tube. Then, 5 mL of 2.5 N HCl was added, and the mixture was maintained in a water bath at boiling temperature for 3 h (acid hydrolysis). After boiling, the solution was cooled to room temperature and neutralized with solid Na_2CO_3, added until effervescence ceased. The volume was brought to 100 mL with H_2O. The solution was centrifuged and filtered. Subsequently, the reaction and reading of the samples were performed as described for the reference solution used to obtain the calibration curve.

2.5 Total Protein content

The total protein content was determined using an adapted Kjeldahl method, weighing 0.5 g of the sample, and employing the KjelMaster K-375 from BÜCHI. Proteins in a sample were digested with H_2SO_4 in the presence of a catalyst, converting total organic nitrogen into ammonium sulfate. The digest was neutralized with NaOH and distilled into a boric acid solution. The resulting borate anions were titrated with standardized HCl. The concentration of hydrogen ions (in moles) is equivalent with the nitrogen content in the sample. The analysis yields the

crude protein content of the sample, as the nitrogen originated from both protein and non-protein components [14].

3. Results and discussion

Regarding carbohydrate content, we observed that CPO exhibited the highest value at 51.8 g/100g, while the two types of wild mushrooms collected from the flora showed similar values, with lower contents of 29.95 g/100g total carbohydrate for WAM and 25.23 g/100g total carbohydrate for WPO. It is possible that the substrate and cultivation technology applied in the CPO cultivation regime favored the accumulation of these compounds in the commercial species, whereas the environmental conditions experienced by the two wild mushroom types may have been more conducive to the accumulation of other compounds. Our result for WAM is notably lower compared to the value of 81.25 g/100g reported by Kostic et al. (2017) for *Armillaria mellea* [8]. Regarding the two *Pleurotus ostreatus* types (wild and commercial), our result for CPO is fairly close to those reported by Fogarasi et al. (2018) and Zouhour Ouali et al. (2023), who found values of approximately 62 g/100g for *Pleurotus ostreatus* and *Pleurotus pulmonarius*, though our values for WPO are significantly lower [15, 16]. This discrepancy may be attributed to differences in the methodology used to obtain these results, as their studies estimated carbohydrate content as the difference between total mass and the content of lipids and proteins, whereas we employed the UV-Vis colorimetric method.

The protein and lipid content as shown in Fig. 2, was consistent across the two wild mushroom species, whereas CPO displayed a notably low protein content of 13.00 g/100g.

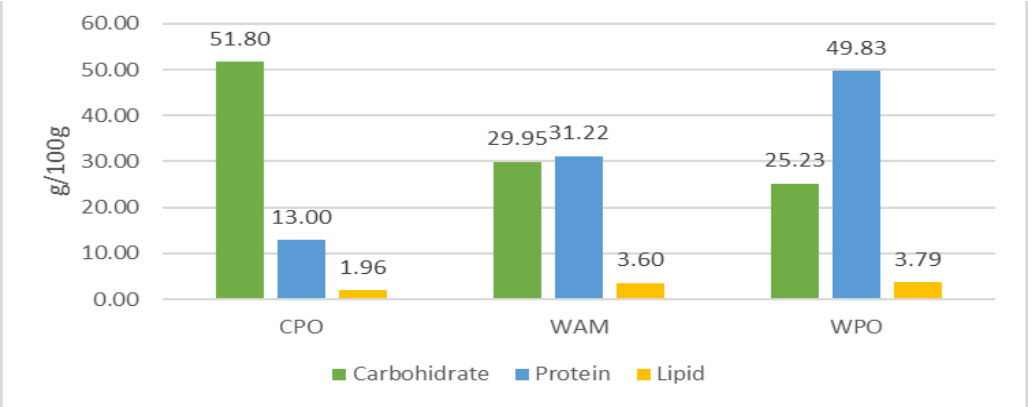

Fig. 2. Carbohidrate, protein and lipid content of CPO, WAM and WPO species, expressed as g/100g

Our findings for the protein content of CPO align with those reported by Fogarasi et al. (2018) and Zouhour Ouali et al. (2023), who obtained comparable values for total protein content in the same species. However, discrepancy emerges when comparing our results for WPO and WAM with those of Kostic et al. (2017), who reported a protein content of 1.81 g/100g for *Armillaria mellea*. Nevertheless, their reported value is considerably lower than those found in other studies we reviewed, which generally indicate much higher protein contents for various mushroom species, ranging from 4.80 g/100g to 36.24 g/100g [8, 15, 16].

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The lipid content was measured at 1.96 g/100g for WPO, 3.60 g/100g for WAM, and 3.79 g/100g for CPO. Fogarasi et al. (2018) reported a lipid content of 1.26 g/100g for *Pleurotus ostreatus*, while Kostic et al. (2017) found 1.97 g/100g for *Armillaria mellea*. Although the wild-collected species in our study showed nearly double the lipid content, our results are much closer to those of Fogarasi et al. (2018) and Kostic et al. (2017) compared to the findings of Zouhour Ouali et al. (2023), who reported values between 7.30 g/100g and a maximum of 61.65 g/100g across the nine mushroom species in their study [8, 15, 16].

The chromatographic profile of fatty acids from the three samples is shown in Figs. 3, 4, and 5, with the results presented as area percentage detailed in Table 1.

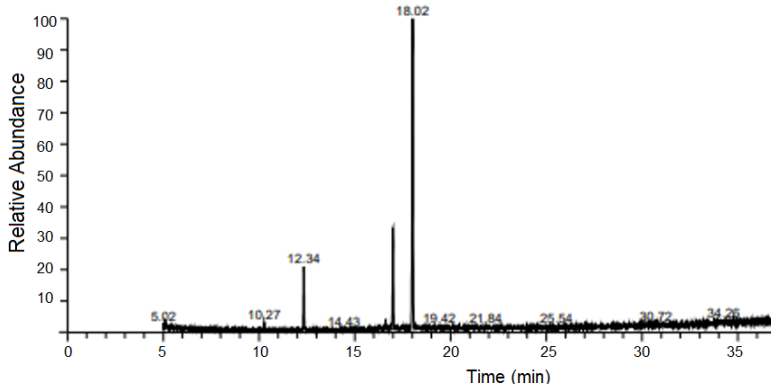

Fig. 3. CPO Fatty acid chromatographic profile (Pentadecanoic acid RT 10.27 / Palmitic acid RT 12.34 min / Stearic acid 16.61 min/ Oleic acid 16.99 min/ Linoleic acid 18.02 min)

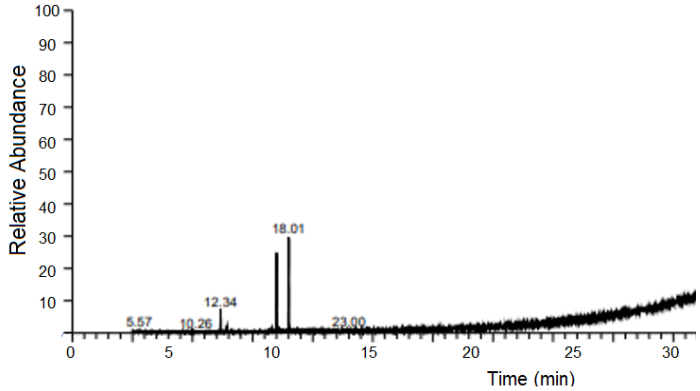

Fig. 4. WAM Fatty acid chromatographic profile (Lauric acid RT 5.57 min / Pentadecanoic acid RT 10.27 min / Palmitic acid RT 12.34 min / Palmitoleic acid RT 12.87 min/ Stearic acid 16.61 min/ Oleic acid 16.99 min/ Cis-Vaccenic RT 17.14 min / Linoleic acid 18.02 min)

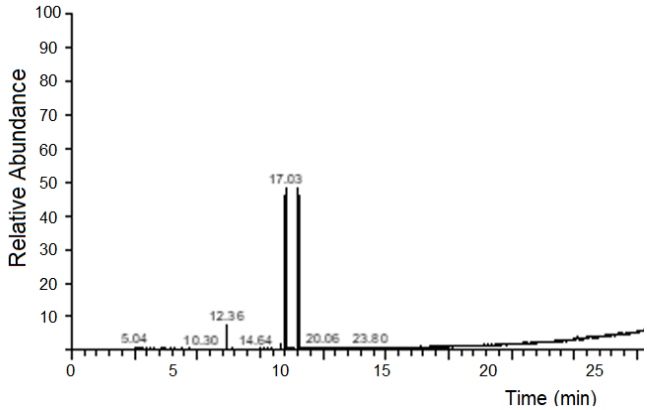

Fig. 5. WPO Fatty acid chromatographic profile (Palmitic acid RT 12.36 min / Stearic acid 16.62 min/ Oleic acid 17.03 min/ Linoleic acid 18.05 min)

Following the analysis, we determined that linoleic acid is the most important fatty acid across in all types of mushroom analyzed, accounting for 46.36% to 67.01% of the total fatty acid profile, depending on the mushroom type analyzed. Palmitic acid, stearic acid, oleic acid, and linoleic acid were detected in all three samples. Oleic acid, the second most abundant, was present in all three samples, with significantly higher levels in the two wild-collected mushroom types, reaching 43.79% in WPO and 36.20% in WAM, while in CPO its concentration its only 18.64%. Pentadecanoic acid was found only in CPO (1.30%) and WAM (0.24%). Cis-vaccenic acid and lauric acid were detected exclusively in WAM.

According to the results presented in Table 1, it is evident that essential polyunsaturated and monounsaturated fatty acids are present in the highest concentrations within the total lipid content of the three studied mushroom types, regardless of whether they were commercially sourced or collected from the wild.

Table 1

Fatty acid composition of CPO, WAM and WOP obtained by GC-MS

Sample	Fatty acid	Area %
CPO	Lauric acid	N.D.
	Pentadecanoic acid	1.30
	Palmitic acid	11.34
	Palmitoleic acid	N.D.
	Stearic acid	1.70
	Oleic acid	18.64
	Cis-Vaccenic acid	N.D.
	Linoleic acid	67.01
WAM	Lauric acid	0.71
	Pentadecanoic acid	0.24
	Palmitic acid	9.41
	Palmitoleic acid	2.79
	Stearic acid	2.57
	Oleic acid	36.20
	Cis-Vaccenic acid	1.72
	Linoleic acid	46.36
WPO	Lauric acid	N.D.
	Pentadecanoic acid	N.D.
	Palmitic acid	6.69
	Palmitoleic acid	N.D.
	Stearic acid	1.50
	Oleic acid	43.79
	Cis-Vaccenic acid	N.D.
	Linoleic acid	48.02

Our results align with those reported in the literature, specifically the studies by Fogarasi et al. (2018) and Kostic et al. (2017), which observed that polyunsaturated fatty acids are the most prevalent in *Pleurotus ostreatus* and *Armillaria mellea*, with linoleic acid identified as the most important fatty acid in the lipid content of these mushroom species [8, 15].

4. Conclusions

The novelty presented in this study lies in highlighting the different compositions of cultivated and wild-harvested *Pleurotus ostreatus*, while also characterizing *Armillaria mellea* grown in Romania's spontaneous flora, which has not been studied in this region.

In conclusion, this study underscores the compositional qualities of culinary mushrooms, whether commercially sourced or foraged from the wild. It provides a comparative analysis of their composition, particularly for *Pleurotus ostreatus* under two distinct conditions—industrial cultivation and natural growth—revealing a significantly higher carbohydrate content in commercially grown *Pleurotus ostreatus,* while wild-harvested samples exhibited elevated protein levels.

Our findings corroborate existing research on the nutritional composition of *Pleurotus ostreatus* and *Armillaria mellea*, specifically regarding their carbohydrate, protein, lipid, and fatty acid profiles. The lipid content and fatty acid composition appear relatively consistent across the three samples and align with studies of mushrooms from other European regions.

The substantial presence of essential polyunsaturated fatty acids, particularly linoleic acid, alongside notable protein and carbohydrate levels, highlights the nutritional benefits of consuming these mushrooms and their potential for applications in the cosmetics and dietary supplement industries.

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