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Characterization of Bioactive Compounds in Biomass of *Pleurotus ostreatus* (Pleurotus-MRL)

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Table 1 presents the centesimal and chemical characterization of *Pleurotus ostreatus* mushroom biomass (MB). The biomass powder has a water content of less than 3% on a fresh weight basis (FW). Its water activity (a_w) is approximately 0.12, indicating the free water available for chemical reactions and microbiological growth, confirming that MB is a highly stable and safe dry product. *P. ostreatus* MB is under Food Safety Certification (ISO 22000:2018; Registration Number: PT- 2019/GSA.0263PT-BIO-05.620-0008110.2023.001; GB-ORG-04; Serial Number: 01850/24). The centesimal analysis revealed that *P. ostreatus* possesses 92.8% carbohydrates on a dry weight basis (DW), which includes monosaccharides, disaccharides, and polysaccharides. Glucans are the most abundant carbohydrates, comprising 80.5% of carbohydrates. Glucans are known for their various biological functions, including the potential to modulate gut microbiota. The analysis also indicated that *P. ostreatus* contains 4.6% DW of protein and 1.6% DW of soluble protein, being an alternative and sustainable source of proteins. In terms of fat and ash content, this species has approximately 1.3% DW and 1.4% DW, respectively. From a nutritional perspective, *P. ostreatus* MB has low energy values: 378 kcal/ 100 g DW.

Table 1. Chemical composition of *Pleurotus ostreatus* mushroom biomass (MB).

	Parameter	Content
Centesimal composition	Water content (% fresh weight-FW)	2.98±0.04
	Carbohydrates (% of dry weight-DW)[+]	92.75±0.01
	Protein (% DW)	4.62±0.00
	Soluble protein (% DW)	1.58±0.02
	Ash (% DW)	1.36±0.02
	Fat (% DW)	1.28±0.00
	a_w	0.12±0.01
	Energy (kcal/ 100 g DW)	377.97±0.36
Carbohydrate profile	Total glucose (% DW)	64.78±5.16
	Total free sugars[++] (% DW)	0.34±0.04
	Total glucans (% DW)	80.45±5.05
	(1→3)(1→6)-β-glucans (% DW)	2.96±0.52
	α-glucans and β-glucans with other linkages (% DW)	77.49±2.22

[+]Carbohydrates were calculated by 100 minus the humidity, ash, total fat, and protein difference. [++]Total sugars were calculated by the sum of quantified sugars. All determinations were carried out in triplicate.

Table 2 displays *P. ostreatus* MB's free (FAA) and total amino acids (TAA) profiles. The amino acid (AA) composition reveals a wide diversity, with several essential AAs. Gamma-aminobutyric acid (Gaba), an important neurotransmitter, was also quantified in this species (3.33 mg/ 100 g DW). The most prevalent AAs are aspartic acid (Asp), glutamic acid (Glu), arginine (Arg), alanine (Ala), isoleucine (Ile) and leucine (Leu). In addition to the key role of essential AAs, the identified AAs possess several health benefits, such as promoting muscle health and normal central nervous system function as well as enhancing metabolism, energy homeostasis, immunity responses, and memory.

Table 2. Free (FAAs) and total amino acids (TAAs) composition of *Pleurotus ostreatus* mushroom biomass (MB).

AAs	FAA content (mg/ 100 g DW)	TAA content (mg/ 100 g DW)
Asp	3.51±0.41	806.65±38.07
Glu	14.42±1.53	861.08±66.66
Gly	1.07±0.18	331.20±3.19
Thr[*]	0.67±0.04	473.93±31.11
Arg	16.98±2.18	626.03±63.10
Ala	5.07±0.16	603.51±68.11
Tyr	1.30±0.17	365.16±44.71
Val[*]	0.47±0.04	490.78±29.87
Met[*]	N.D.	34.35±9.51
Phe[*]	1.15±0.06	337.91±41.07
Ile[*]	N.D.	590.31±82.28
Leu[*]	2.70±0.35	560.78±51.31
Gaba	3.33±0.04	3.33±0.04
Total[+]	63.78±2.02	6164.94±384.17

[*]Essential amino acids. [+]Total AAs were calculated by the sum of quantified FAAs and TAAs. N.D. not determined. All determinations were carried out in triplicate.

Table 3 shows the mineral composition of *P. ostreatus* MB. The mineral profile reveals that this species is a valuable source of phosphorus (P), magnesium (Mg), and potassium (K). The European Food Safety Authority proposes that the adequate intake (AI) of P, Mg, and K for adults is 550, 300, and 3500 mg/ day, being the MB a good source of these minerals. *P. ostreatus* MB is also suitable for low-sodium (Na) diets, as the Na: K ratio is approximately 0.003, associated with preserving cardiovascular health. Safe and AI of Na is 2 g/ day. These minerals are involved in signaling, enzymatic, and metabolic processes, as well as immune, muscle, cardiac, and nervous system functions. Selenium (Se) was also quantified in considerable amounts (87.7 µg/ 100 g DW). This mineral is involved in numerous biological roles such as the immune system enhancement and metabolism of thyroid hormones.

Table 3. Mineral composition of *Pleurotus ostreatus* mushroom biomass (MB).

Mineral	Content (mg/ 100 g DW)
Mo	0.10±0.01
Zn	2.37±0.03
Cd	0.01±0.00
P	397.12±8.05
Ni	0.05±0.01
Mn	4.17±0.07
Fe	0.76±0.04
Mg	149.57±1.88
Ca	14.74±0.21
Cu	0.35±0.01
Na	0.94±0.10
K	285.10±3.49
Se	0.09±0.01
Total[+]	855.29±12.35

[+]Total minerals were calculated by the sum of quantified minerals. All determinations were carried out in triplicate.

Table 4 presents the fatty acid (FA) composition of *P. ostreatus* MB. This species possesses an interesting FA profile, with a higher concentration of monosaturated FAs (MUFAs) and polyunsaturated FAs (PUFAs), both known for their health benefits, than saturated fatty acids (SFAs), resulting in an unsaturated FAs (UFA): SFA ratio of 3.24. Oleic and linoleic acids, associated with normal brain and cardiovascular functions, are the most prevalent FAs.

Table 4. Fatty acid composition of *Pleurotus ostreatus* mushroom biomass (MB).

Fatty acid	Content (mg/ 100 g DW)
Caproic acid (C6:0)	0.72±0.08
Myristic acid (C14:0)	5.14±0.043
Pentadecanoic acid (C15:0)	2.57±0.01
Palmitic acid (C16:0)	281.62±15.43
Palmitoleic acid (C16:1 c9)	2.02±0.11
Heptadecanoic acid (C17:0)	2.14±0.09
Stearic acid (C18:0)	33.47±2.93
Elaidic acid (C18:1 t9)	1.95±0.13
Oleic acid (C18:1 c9)	539.07±15.81
cis-vaccenic acid (C18:1 c11)	14.90±0.46
c18:1 c4/t6	1.95±0.10
Linoleic acid (LA) (C18:2 c9c12)	475.24±24.37
Arachidic acid (C20:0)	4.45±0.01
γ-linolenic acid (C18:3 c6c9c13)	13.17±1.33
Paullinic acid (C20:1 c13)	11.55±0.43
α-Linolenic Acid (ALA) (C18:3 c9c12c15)	7.61±0.16
C18:2 t9t11	2.43±0.20c
Heneicosanoic acid (C21)	2.06±0.07
Behenic acid (C22:0)	0.74±0.04
Dihomo-γ-linolenic acid (DGLA) (C20:3 c8c11c14)	0.76±0.09
C20:3 c11c14c17	11.94±0.89
α-eleostearic acid (C18:3 c9t11t13)	4.12±0.20
Eicopentaenoic Acid (EPA) (C20:5 c5c8c11c14c17)	2.22±0.10
Catalpic acid (C18:3 t9t11t13)	1.45±0.06
Tricosanoic acid (C23)	5.14±0.18
C22:2 c13c16	5.46±0.21
Lignoceric acid (C24:0)	28.91±2.01
Docosahexaenoic acid (DHA) (C22:6 c4c7c10c13c16c19)	2.26±0.12
Total SFAs[+]	366.96±18.41
Total MUFAs[+]	559.89±16.09
Total PUFAs[+]	526.56±25.68
Total fatty acids (FAs)[+]	1464.96±58.63
Ratio unsaturated fatty acids: SFAs	3.24
Ratio MUFAs: SFAs	1.67
Ratio PUFAs: SFAs	1.55

[+]Total SFAs, MUFAs, PUFAs, and FAs were calculated by the sum of respective quantified FAs. All determinations were carried out in triplicate.

Table 5 displays the concentration of some bioactive macromolecules that play key roles in human health, such as total free and bound phenolics, carotenoids, ergosterol, and tocopherols (vitamin E isomers).

Table 5. Content of different bioactive groups of *Pleurotus ostreatus* mushroom biomass (MB).

Bioactive group	Compound	Content
Phenolic	Total free phenolics[1]	56.55±3.52
	Total bound phenolics[1]	24.48±2.39
Antioxidant activity	Total free fraction[2]	1665.75±67.63
	Total bound fraction[2]	1716.44±447.48
Carotenoids	Total carotenoids[3]	5.67±0.72
Sterols	Ergosterol[4]	5.20±0.34
E vitamin isomers	α-Tocopherol[5]	16.75±1.82
	β-Tocopherol[5]	0.46±0.01
	γ-Tocopherol[5]	52.69±5.21
	δ-tocopherol[5]	10.48±2.25
	Total tocopherols[5,+]	80.38±5.99

[1] mg gallic acid equivalent/ 100 g DW; [2] mg ISH equivalent / 100 g DM; [3] mg β-carotene equivalent/ 100 g DW; [4]mg/ 100 g DW; [5]µg/ 100 g DW. [+]Total tocopherols were calculated by the sum of quantified tocopherols. All determinations were carried out in triplicate.

In addition to the rich content of glucans, this report highlights the significant presence of bioactive compounds in this MB species, especially Asp, Glu, Arg, Ala, Ile, Leu, P, K, oleic, and linoleic acids. This bioactive profile underscores the potential health benefits of *P. ostreatus* MB, which contributes to vital human health.