

Characterization of Bioactive Compounds in Biomass of *Coriolus versicolor* (Coriolus-MRL)

Helena Araújo-Rodrigues, Freni Tavarina, João Relvas, and Manuela Pintado

Centro de Biotecnologia e Química Fina, ESB, Universidade Católica Portuguesa (Porto, Portugal)

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Table 1 presents the centesimal and chemical characterization of *Coriolus versicolor* mushroom biomass. The biomass powder has a water content of less than 8% on a fresh weight basis (FW). The water activity (a_w) is approximately 0.33, which reflects the amount of free water available for chemical reactions and microbiological growth, confirming that it is a stable and safe dry product. 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 indicated that *C. versicolor* possesses 91.5% carbohydrates on a dry weight basis (DW), which includes monosaccharides, disaccharides, and polysaccharides. Among these, glucans are the most abundant, comprising 76.2% of carbohydrates. Glucans are known for their various biological functions, including their potential to modulate gut microbiota. Mushrooms are alternative and sustainable protein sources, and the analysis showed that *C. versicolor* contains 6.2% DW of protein and 2% DW of soluble protein. Concerning fat and ash content, this species possesses approximately 0.7% DW and 1.5% DW, respectively. From a nutritional perspective, *C. versicolor* has low energy values: 375 kcal/ 100 g DW.

Table 1. Chemical composition of *Coriolus versicolor* mushroom biomass (MB).

	Parameter	Content	
Centesimal composition	Water (% fresh weight-FW)	7.59±0.10	
	Carbohydrates (% of dry weight-DW) ⁺	91.49±0.37	
	Protein (% DW)	6.15±0.37	
	Soluble protein (% DW)	1.99±0.16	
	Ash (% DW)	1.48±0.01	
	Fat (% DW)	0.74±0.01	
	a_w	0.33±0.01	
	Energy (kcal/ 100 g DW)	375.02±0.22	
	Carbohydrate profile	Total glucose (% DW)	66.23±6.84
		Total free sugars ⁺⁺ (% DW)	1.19±0.24
Total glucans (% DW)		76.15±5.06	
(1→3)(1→6)-β-glucans (% DW)		4.67±1.28	
α-glucans and β-glucans with other linkages (% DW)		71.49±7.26	

⁺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 the mushroom biomass's free (FAA) and total amino acids (TAA) profiles. The amino acid (AA) composition reveals a wide diversity, with the presence of almost all essential

AAs. Gamma-aminobutyric acid (Gaba), an important neurotransmitter, was also quantified in significant amounts in *C. versicolor* (24.27 mg/ 100 g DW). The most prevalent AAs are glutamic (Glu) and aspartic acid (Asp), asparagine (Asn), arginine (Arg), alanine (Ala), and leucine (Leu). In addition to the key role of essential AAs, the identified AAs possess several health benefits, including the promotion of normal central nervous system function and enhancement of memory.

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

AA	FAA content (mg/ 100 g DW)	TAA content (mg/ 100 g DW)
Asp	24.47±2.82	845.62±82.71
Glu	42.48±6.16	939.24±66.87
Asn	18.40±2.69	N.D
His [*]	0.00±0.00	0.01±0.00
Gln	38.95±4.27	N.D.
Gly	21.18±3.26	317.33±64.18
Thr [*]	20.33±2.96	348.74±40.20
Arg	28.31±1.49	638.32±61.63
Ala	34.06±0.99	615.74±50.21
Tyr	5.15±0.05	374.56±54.99
Val [*]	17.32±0.04	460.37±47.11
Met [*]	N.D.	31.62±1.43
Phe [*]	18.32±1.03	325.55±12.07
Ile [*]	17.25±0.89	495.60±37.64
Leu [*]	20.38±2.15	540.79±67.20
Gaba	24.27±3.99 ^a	24.27±3.99 ^a
Total [*]	349.75±21.86	6059.47±286.11

^{*}Essential amino acids. ^aTotal 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 *C. versicolor* mushroom biomass. The mineral profile suggests a very interesting mineral profile. This species is a good source of phosphorus (P), magnesium (Mg), calcium (Ca), and potassium (K), exhibiting a sodium (Na): K ratio of approximately 0.06 (which has been associated with cardiovascular health preservation). These minerals play essential biological roles, including involvement in enzymatic and metabolic processes, as well as cardiac and nervous system functions. Selenium (Se) was also quantified in considerable amounts (104.6 μg/ 100 g DW). This mineral is involved in the immune system support and metabolism of thyroid hormones.

Table 3. Mineral composition of *Coriolus versicolor* mushroom biomass (MB).

Mineral	Content (mg/ 100 g DW)
Mo	0.04±0.01
Zn	1.85±0.05
Cd	0.01±0.00
P	410.67±8.41
Ni	0.01±0.00
Mn	3.87±0.08
Fe	2.03±0.09
Mg	163.70±2.12
Ca	24.41±0.37
Cu	0.40±0.01
Na	14.51±0.37
K	242.70±2.17
Se	0.10±0.01
Total ⁺	864.19±9.93

⁺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 *C. versicolor* mushroom biomass. The analysis indicates the presence of numerous FAs, such as oleic and linoleic acids, which are linked to normal brain and cardiovascular functions. The monosaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), both known for their health benefits, were found in higher concentrations than saturated fatty acids (SFAs).

Table 4. Fatty acid composition of *Coriolus versicolor* mushroom biomass (MB).

Fatty acid	Content (mg/ 100 g DW)
Myristic acid (C14:0)	3.58±0.14
Pentadecanoic acid (C15:0)	3.22±0.25
Palmitic acid (C16:0)	178.12±18.43
Palmitoleic acid (C16:1 c9)	1.29±0.07
Heptadecanoic acid (C17:0)	2.83±0.21
Stearic acid (C18:0)	24.57±0.99
Elaidic acid (C18:1 t9)	1.68±0.05
Oleic acid (C18:1 c9)	293.74±25.55
cis-vaccenic acid (C18:1 c11)	8.94±0.77
c18:1 c4/t6	1.75±0.00
Linoleic acid (LA) (C18:2 c9c12)	265.76±22.52
Arachidic acid (C20:0)	5.18±0.11
γ-linolenic acid (C18:3 c6c9c13)	6.23±0.69
Paullinic acid (C20:1 c13)	8.82±0.94
α-Linolenic Acid (ALA) (C18:3 c9c12c15)	3.59±0.38
C18:2 t9t11	1.43±0.02
Heneicosanoic acid (C21)	1.21±0.06
Behenic acid (C22:0)	1.38±0.09

C20:3 c11c14c17	13.70±1.17
α-eleostearic acid (C18:3 c9t11t13)	1.45±0.13
Eicopentaenoic Acid (EPA) (C20:5 c5c8c11c14c17)	1.12±0.06
Catalpic acid (C18:3 t9t11t13)	3.16±0.30
Tricosanoic acid (C23)	1.35±0.11
C22:2 c13c16	1.97±0.17
Lignoceric acid (C24:0)	33.53±3.13
Nervonic acid (C24:1 c15)	0.83±0.01
Docosahexaenoic acid (DHA) (C22:6 c4c7c10c13c16c19)	3.15±0.05
Total SFAs⁺	254.96±22.12
Total MUFAs⁺	310.34±26.55
Total PUFAs⁺	301.56±24.98
Total fatty acids (FAs)⁺	875.69±73.95
Ratio unsaturated fatty acids: SFAs	2.82
Ratio MUFAs: SFAs	1.45
Ratio PUFAs: SFAs	1.36

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

Bioactive macromolecules that play key roles in human health (e.g., antioxidant and anti-inflammatory), such as total free and bound phenolic compounds, carotenoids, ergosterol, D vitamin and tocopherols (vitamin E isomers), were also determined and described on **Table 5**.

Table 5. Content of different bioactive groups of *Coriolus versicolor* mushroom biomass (MB).

Bioactive group	Compound	Content
Phenolic	Total free phenolics ¹	19.19±1.47
	Total bound phenolics ¹	166.70±16.65
Antioxidant capacity	Total free fraction ²	7336.80±202.98
	Total bound fraction ²	1705.10±64.73
Carotenoids	Total carotenoids ³	18.05±0.74
Sterols	Ergosterol ⁴	8.97±0.68
	α-Tocopherol ⁵	94.20±2.58
E vitamin isomers	β-Tocopherol ⁵	7.04±1.24
	γ-Tocopherol ⁵	79.30±2.02
	δ-tocopherol ⁵	13.26±1.92
	Total tocopherols ^{5,+}	193.81±7.42
D vitamin	D2 (ergocalciferol) ⁵	11.46±1.55

¹mg gallic acid equivalent/ 100 g DW; ²mg ISH equivalent / 100 g DM; ³mg β-carotene equivalent/ 100 g DW; ⁴ mg/ 100 g DW; ⁵μg/ 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 antioxidant capacity and the presence of bioactive compounds in *C. versicolor* mushroom biomass such as key amino acids (e.g., Gaba), unsaturated fatty acids, Se, P, Mg, P and D vitamin. These compounds contribute to vital human health, including brain, cardiovascular, and kidney functions.