Bioaccessibility and Bioavailability of Bioactive Compounds from Mushroom Biomass Following *in vitro* Gastrointestinal Digestion

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Abstract:

Mushrooms represent a promising source of bioactive compounds for functional food applications due to their rich nutritional profile, containing α-glucans, β-glucans, lectins, and other bioactive macromolecules. These macromolecules exhibit numerous health-promoting properties, such as prebiotic, immunomodulatory, anti-inflammatory, and antioxidant activities. While previous research has primarily focused on polysaccharide fractions from mushroom fruiting bodies[1,2], this study explores the biochemical potential of complete mushroom biomass (MB). The present study investigated the bioaccessibility and bioavailability of bioactive compounds present in *Trametes versicolor* (TV), *Hericium erinaceus* (HE), and *Pleurotus ostreatus* (PO) species, following simulated gastrointestinal digestion (GID), with a special focus on polysaccharide and protein fractions.

MB was biochemically characterized and subjected to *in vitro* GID using the standardized INFOGEST protocol. Dialysis membranes (3.5 kDa) were used to mimic the passage throughout the duodenum and jejunum and predict the colon-available and serum-available fractions. Additionally, a Transwell assay with co-culture of Caco-2 and HT29-MTX cells was also carried out to validate permeability. Chromatographic and enzymatic assays were used to quantify the bioactive molecules throughout GID, while

the molecular weight (MW) distribution of proteins and peptides was determined by size exclusion chromatography. Total phenolic compounds (TPC) and antioxidant activity were evaluated through Folin-Ciocalteu, ABTS (2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid), and FRAP (ferric reducing antioxidant power) assays.

Biochemical characterization revealed that polysaccharides were the most prevalent, varying between 76.15 and 80.45 % of dry weight (DW) without significant differences between species. (1→4)-α-glucans were the most abundant, followed by (1→4)-β-glucans. A wide distribution of MW was found in the polysaccharide fraction, with a higher concentration of polysaccharides over 10^7 kDa. Protein content ranged from 4.08 to 6.28 % DW in the three species, with TV exhibiting significantly higher content of total and soluble protein. The three species predominantly comprised low MW proteins and peptides (<3 kDa)[3]. Other bioactive macromolecules were also identified and quantified, namely, gamma-aminobutyric acid, ergosterol, vitamins, organic acids, and phenolic compounds. Beyond nutritional properties, the biochemical profile of MBs suggests their rich bioactive potential. However, the bioaccessibility and bioavailability after GID are critical for deciphering health benefits.

Following simulated digestion, results revealed that (1→4)-α-glucans and (1→4)-β-glucans were the most prevalent groups in both colon-available and serum-available fractions (28-40 % DW), with results validated by permeability assays using Caco-2/HT29-MTX co-cultures. The colon-available fraction also contained proteins and peptides (<75 kDa) as well as fatty acids (oleic and linoleic acids), suggesting prebiotic activity. In contrast, the serum-available fraction was richer in low MW peptides (<1.2 kDa), amino acids (Tyr, Val, Phe, and Leu), and phenolic compounds (730-863 mg GAE/ 100 g DW). This fraction exhibited substantial antioxidant capacity using different methods (e.g., FRAP: 177-305 mg ISHE/ 100 g DW), indicating potential for systemic bioactivity.

These findings demonstrate that MB represents a valuable source of bioactive compounds after GID. Both colon-available and serum-available fractions showed potential for human health, expanding perspectives in the nutraceutical field and highlighting opportunities for future research into mushroom-based functional foods.

Keywords: Mushroom biomass; Bioactive macromolecules; Gastrointestinal impact; Bioaccessibility and bioavailability; Health benefits.

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