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REVIEW ARTICLES

Edible macromycetes as an alternative protein source: advances and trends

Walter José Martínez-Burgos[a], Diego Ocán[a], Maria Clara Manzoki[a], Rafael Novaes Barros[a], Ricardo Vieira[a], Carlos Ricardo Soccol[a,*]

[a]*Department of Bioprocess Engineering and Biotechnology, Federal University of Paraná – UFPR, Polytechnic Center, CEP 81531-990, Curitiba, PR, Brazil*

Highlights

- *Lentinula*, *Pleurotus*, *Auricularia*, *Agaricus* and *Flammulina* are the main species of mushrooms consumed
- Solid and liquid fermentation are being used to produce edible mushroom biomass
- Edible mushrooms have an amino acid profile similar to meat of animal origin
- Edible mushroom proteins have functional properties
- Mushrooms are potential protein substitutes for traditionally consumed meat

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KEYWORDS
Edible mushrooms;
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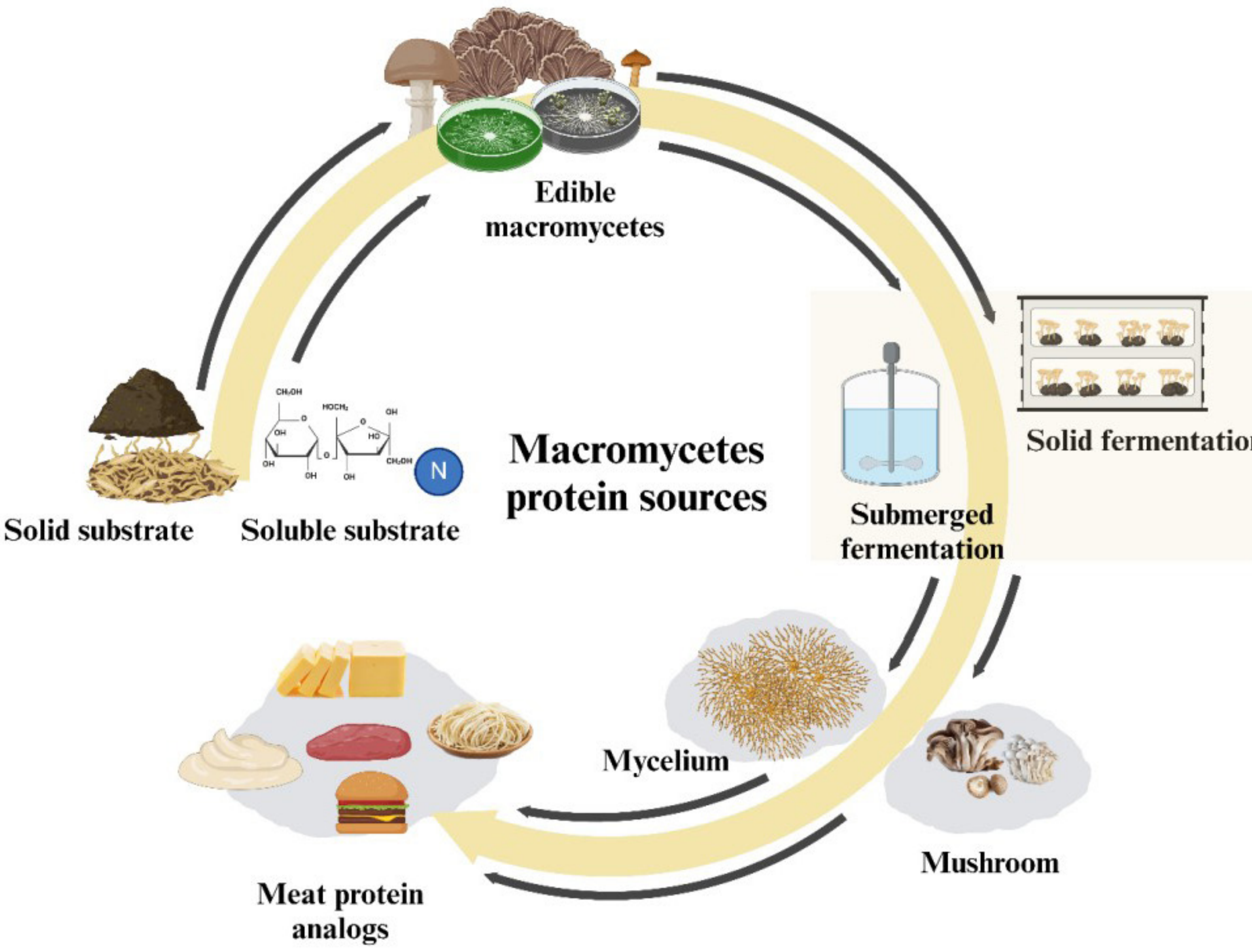
Protein;
Meat analogue.

Abstract: Overcoming the planet's food crisis is one of the greatest challenges today. The problem is even more acute when we think about the main foods consumed as a source of protein, such as meat, which is widely questioned because of the impacts its production generates. The world requires food that is nutritious, cheap, and has a low environmental footprint. One protein alternative that has been explored and is constantly increasing in production is mushroom biomass (approximately 44 million tons), which has a protein content up to three times higher than meat (up to 82%). It also contains all the essential amino acids and presents antimicrobial, antioxidant, and anti-cancer properties. Among the main species being produced are *Lentinula*, *Pleurotus*, *Auricularia*, *Agaricus* and *Flammulina*, which are produced via solid and submerged fermentation. Although mushrooms were first consumed fresh, they have now been developed as counterparts to meat and meat derivatives, such as sausages, pastes, and protein complexes. The aim of this review is to present the technological advances in mushroom production, the different derivatives, and the new trends in mushroom-derived products that are being consumed as alternative proteins.

*Corresponding author.
E-mail: soccol@ufpr.br (C. R. Soccol).

Graphical Abstract



Introduction

Macromycetes, also known as mushrooms, are fungal organisms that play essential roles in various ecosystems, one of their main functions being to degrade dead matter and return nutrients to the soil. It is estimated that there are around 3 million species of fungi, but only about 2,000 have been identified as edible, of which only about 25 species are produced on an industrial scale (Furlani & Godoy, 2007; Kalač, 2016). Mushrooms are sources of different compounds such as proteins, polysaccharides, minerals, glycopeptide-protein complexes, triterpenes, ergosterol, cordycepin and non-cellulosic β-glucans with β (1-3) and β (1-6) bonds in the main chain. These compounds give mushrooms nutritional, antioxidant, anti-inflammatory, antimicrobial, anticancer and immunostimulant properties, which is why their consumption is increasing (Berovic et al., 2022). In fact, mushroom consumption is constantly on the rise, with 31.78, 38.66 and 44.2 tons produced in 2012, 2016 and 2021, respectively (Statista, 2023). One of the most important applications of mushrooms today is their use as an alternative source of protein, as they can have a protein content of up to 80% (Barros et al., 2008; Dilfy et al., 2020; Krishnamoorthi et al., 2022). In addition, fungal proteins have an amino acid profile similar to that of meat and the proteins are more digestible, healthier and have a lower environmental impact, since producing one kg of animal protein uses around 10 times more water than mushroom production (González et al., 2020).

Mushroom biomass is produced via solid or submerged fermentation. In the former, biomass with a higher protein content is obtained, while in the latter, fermentation time is reduced by at least 50% compared to solid fermentation. Mushroom biomass is processed to produce meat homologues, protein concentrates, pastes, protein drinks, among others. Considering the importance that the mushroom production and derivatives industry has gained, the aim of this review was to present an overview of edible mushrooms diversity, recent technologies, and innovations to produce mushrooms and mushroom derivatives, and the trends of new products currently used as alternative proteins.

Biodiversity and protein nutritional value

Macromycetes or macrofungi are a group of heterotrophic eukaryotic organisms belonging to the phylum Basidiomycetes and Ascomycetes of the kingdom Fungi, characterized by a visible fruiting body containing spores for their reproductive function (Zhou, 2020). It is estimated that there are between 2.2 and 3.4 million species belonging to this group, of which, many of them are still undiscovered or little studied, mainly for their edible potential (Hawksworth & Lücking, 2017). In this sense, more than 2000 species of this group of fungi have been identified as edible, of which 350 are cultivated for commercial distribution and only 25 are cultivated on an industrial scale (Furlani & Godoy, 2007; Kalač, 2016).

Among the most cultivated and industrially produced edible macromycetes are the genera *Lentinula*, *Pleurotus*, *Auricularia*, *Agaricus* and *Flammulina*. These genera also include many species that are distributed worldwide, such as *Agaricus bisporus* and *Agaricus campestris*, commonly known as "champignon"; *Lentinula edodes* or "shiitake"; *Pleurotus ostreatus*, also known as "hiratake" or in specific countries "shimeji"; *Auricularia auricula* or "wood ear"; and *Flammulina velutipes* or "enoki" (Das et al., 2021; Royse et al., 2017). Similarly, the extensive diversity of edible macrofungi positions these organisms as a promising and complementary alternative to conventional foods, particularly meat derivatives, since the amino acid composition of both sources can be almost equivalent in some species (Longvah & Deosthale, 1998). Moreover, the integration of macrofungi into dietary practices could potentially alleviate the ongoing negative impact resulting from the overexploitation of animal resources, as reported by Godfray et al. (2018). Figure 1 shows a graph of the diversity of edible species of high-protein macromycetes, obtained based on the number of occurrences in their wild form at the continental level, using data collected by the Global Biodiversity Information Facility (GBIF).

According to Boukid (2021), meat analogs should have a fat composition of 1 to 5%, polysaccharides of 2 to 30% and mainly proteins of 20 to 50%, which is one of the most difficult challenges to achieve from a non-animal protein source. In this context, the genus *Agaricus* contains about 300 known species, of which the species *A. bisporus* is the most representative. The presence of this genus is mainly distributed in the regions of Europe and North America, but it is extensively cultivated worldwide, generating a significant impact on the economy of the food sector. Productions can reach up to almost 4 million tons per year, with China being the main global producer (Mleczek et al., 2018; Royse et al., 2017).

At the nutritional level, it has been reported in several studies that macromycetes present a high protein value with concentrations ranging from 29.6 to 39.8% in dry weight and up to 80% when it is a wild and non-commercial crop (Barros et al., 2008; Dilfy et al., 2020; Krishnamoorthi et al., 2022). Likewise, the essential amino acids lysine and leucine usually have the highest proportion with values of 6.1 and 6.0%, respectively (Vetter, 1993b). *Marasmius oreades* is a species of edible macromycete that, according to studies reported by Mos Vetter (1993), presents one of the highest percentages of crude protein reported, with concentrations ranging from 36 to 58%. This fungus has a worldwide distribution, with a higher incidence in tropical regions of Europe, North America, and Oceania, showcasing a good adaptation to different ecosystems, which is why it can be exploited for cultivation at a potential industrial level. Likewise, it contains all the essential amino acids, with leucine being the most abundant, comprising 6.76% of the total protein content (Stoyanova et al., 2020). It should also be noted that the presence of essential amino acids distinguishes macromycetes from other types of alternative proteins, such as those based on plants. Another important group of macromycetes to be mentioned are the fungi of the genus *Tricholoma*, most of which are mycorrhizal species with a worldwide distribution, especially in Europe, North America, Asia and Oceania. Several species are of great economic importance, as is the case of *Tricholoma matsutake*, whose production generates profits of up to 50 million dollars a year in the Japanese market; it is also considered one of the

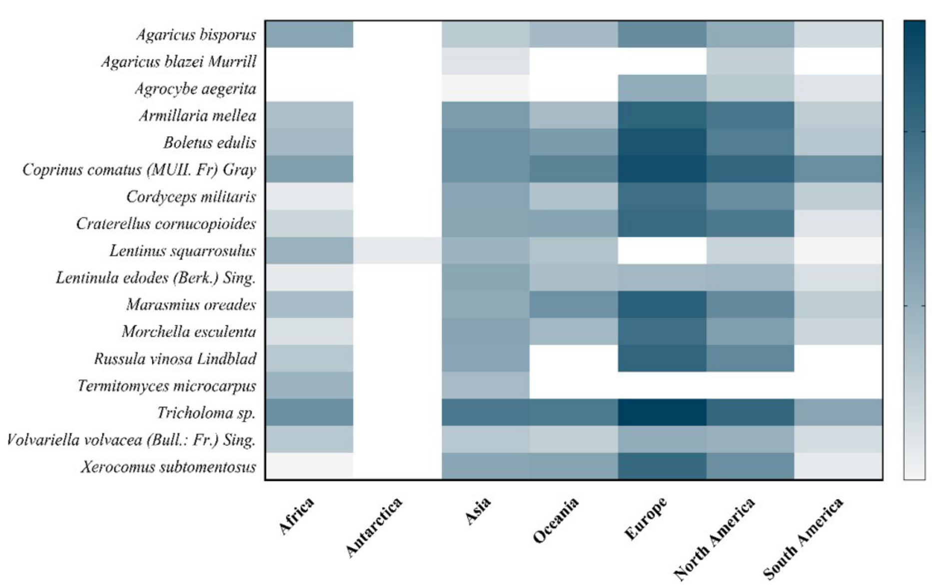


Figure 1. Distribution of species in the wild form of edible Macromycetes with a high percentage of crude protein across various continental regions (data are presented as the number of occurrences according to the GBIF database, expressed in Log10).

most important edible ectomycorrhizal fungi in the world (Aoki et al., 2022). The fruiting body of this species develops on the periphery of a structure called 'shiros,' which forms an association between the roots of coniferous trees and the soil, thereby optimizing the uptake of nutrients by plant cells. For this reason, it is currently a difficult species to cultivate in vitro, especially for commercial purposes, and the current demand is met by direct harvesting (Yamanaka et al., 2020). At the nutritional level, values between 20 and 36.8% crude protein in dry weight have been reported, making it even more valuable for the consumer (Díez & Alvarez, 2001; Yu et al., 2020). As well as those already mentioned, there are reports of other species that have significant crude protein concentration and essential amino acids proportion, in addition to other beneficial properties, which further strengthens the interest in promoting their cultivation on an industrial scale as alternative sources to traditional foods. In Table 1, reports on various species of edible macromycetes and meat products are presented, detailing the approximate crude protein content in grams per 100 g of dry weight, as well as the amino acid percentages relative to the total protein content.

Fermentative processes with macromycetes

The production of macromycetes on an industrial scale can be done using mainly two types of processes: solid fermentation and submerged fermentation (Letti et al., 2018). According to Rothmann et al. (2023), solid fermentation is the most efficient method for applying macromycetes in delignification, conversion, and addition of nutritional value. In submerged fermentation mycelial biomass is mainly cultivated to produce bioactive compounds, while in solid fermentation the fruiting bodies are used mainly for food or medicinal products.

Solid fermentation for macromycetes production

Solid fermentation (SF) is characterized by being a process that occurs on the surface of materials or substrates in solid and semi-solid states, which have the ability to contain or absorb water and nutrients. Solid supports or materials may be biodegradable or non-biodegradable in nature, however, to produce macromycetes it is more common to use biodegradable substrates (Couto & Sanromán, 2006). On some occasions, nutrients are solubilized in water to become available for use by organisms. According to Viniegra-Gonzàlez (1997), for microbial growth to occur in solid fermentation, nutrients must be diffusible under or over the liquid-solid interphase. Among the advantages of solid fermentation, the following stand out: the costs of the media used are generally cheap (the substrates used are agroforestry residues), and substrate treatments are easy, with short cooking being the most applied method to humidify or dilate the biomass, and fractionations being applied to facilitate the accessibility of microorganisms to the nutrients (Chang & Wasser, 2017). Furthermore, SF in the production of macromycetes is a selective process in which the available humidity is low, meaning that these systems can hardly be contaminated with bacteria (Vandenberghe et al., 2021). According to Rothmann et al. (2023), solid fermentation takes place in the absence or almost absence of free water.

Although various types of bioreactors are used in SF for the production of macromycetes, static bioreactors are generally preferred. These mainly consist of a chamber with temperature and relative humidity control, along with shelves where trays or polyethylene bags containing the substrate and macromycetes are placed (Figure 2A) (Sánchez, 2010). Advances in this regard are mainly focused on the automation of processes to control the conditions of temperature, relative humidity, and CO_2 concentration in the fermentation chamber, thus optimizing the growth of the macromycetes' fruiting

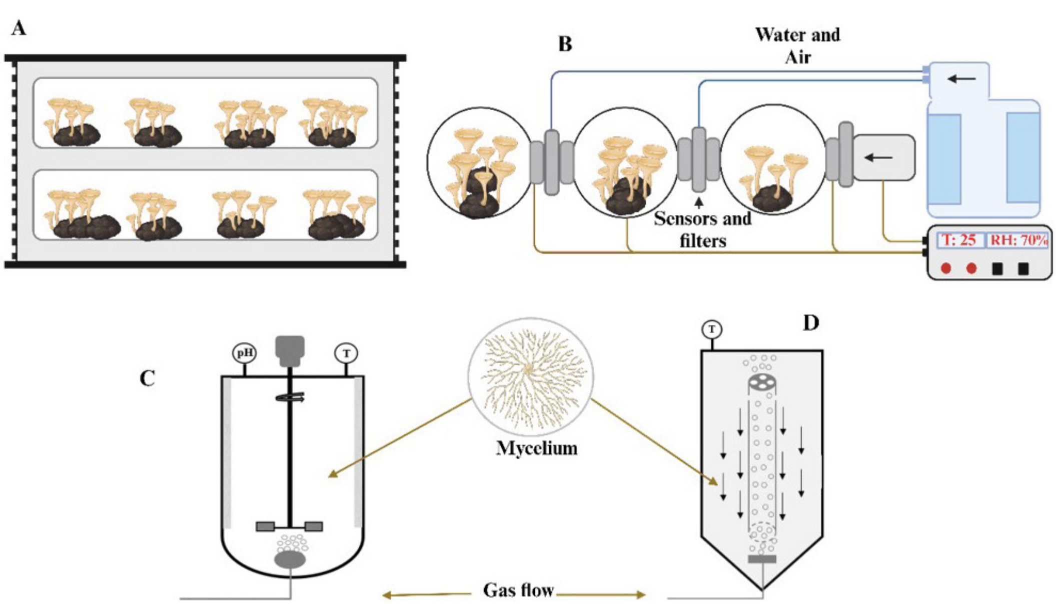


Figure 2. Different cultivation systems applied for edible macromycetes production. (A) Solid fermentation chamber; (B) Modular chain bioreactor; (C) Stirred tank bioreactor; (D) Airlift bioreactor for mycelium production.

Table 1. Edible macromycete species and conventional meat products with high crude protein content, and essential amino acids percentage proportion.

Product	Crude protein (g/100 g dry matter)	Essential amino acids content (% of total amino acids)								Reference
		Val	Leu	Ile	Thr	Met	Lys	Phe	Trp	
Edible Macromycete										
Agaricus bisporus	29.64 – 39.8	3.9	6	3.1	3.4	1.4	6.1	3.1	-	Barros et al. (2008); Hamza et al. (2024); Krishnamoorthi et al. (2022); Vetter (1993)
Agaricus blazei Murrill	26.6 – 28.4	2.8	3.7	2.1	3.0	2.0	3.0	4.7	1.1	Henriques et al. (2008); Zied et al. (2017)
Agrocybe aegerita	24.79 – 26.1	6.70	7.90	5.20	4.86	1.56	5.96	4.04	-	Azeez et al. (2020); Li et al. (2024); Yu et al. (2020)
Armillaria mellea	22.3 – 24.4	7.2	-	7.8	10.5	-	-	6.6	-	Erbiai et al. (2021); Rai et al. (2007)
Boletus edulis	29.15 – 33.1	2.75	1.74	2.56	24.68	4.21	2.51	4.55	0.77	Petrovska (2001); Rasalanavho et al. (2020); Tan et al. (2022)
Coprinus comatus (MUll. Fr) Gray	23.07 – 30.1	4.48	3.82	2.99	2.93	0.25	3.07	2.83	0.86	Nowakowski et al., (2020; Reyes et al. (2009); Petrovska & Bauer Petrovska (2001); Stilinović et al. (2020)
Cordyceps militaris	25.56 – 59.8	4.99	2.06	1.67	8.64	0.26	21.73	1.66	-	Chan et al. (2015); Hur (2008)
Craterellus cornucopioides	22.3 - 69.4	0.57	9.76	9.76	3.01	0.143	5.45	2.44	-	Barros et al. (2008); Colak et al. (2009); Radović et al. (2022)
Lentinus squarrosulus	30.1	1.10	2.74	1.33	3.53	-	2.22	1.53	-	Ayimbila et al. (2022); Zhou et al. (2015)
Lentinula edodes (Berk.) Sing	23.2	3.8	6.4	3.3	5.6	2.2	5.0	3.8	1.9	Li et al. (2018, 2021); Manzi et al. (1999); Yu et al. (2020)
Marasmius oreades	36 – 52.8	4.0	7.2	4.8	3.6	2.5	4.4	6.8	-	Stoyanova et al. (2020); Uzun et al. (2017); Vetter, (1993a)
Morchella esculenta	25.85 – 39.3	6.34	9.17	4.84	5.00	2.00	7.17	5.34	-	Li et al. (2022) Zhang et al. (2017)
Russula vinosa Lindblad	27.2	6.9	8.4	5.3	5.3	1.4	6.8	5.3	-	Kalač (2009); Vetter (1993); Yu et al. (2020)
Termitomyces microcarpus	30.7	5.74	8.27	3.91	3.91	-	7.04	7.04	-	Gunasekara et al. (2021); Teke et al. (2020)
Tricholoma sp.	36.8	7.8	9.4	3.7	9.5	3.0	8.6	4.4	1.0	Díez & Alvarez (2001); Petrovska & Bauer Petrovska (2001); Yu et al. (2020)
Volvariella volvacea (Bull.: Fr.) Sing.	28.1	3.69	7.39	7.39	4.78	-	5.09	6.09	14.40	Torres-Lopez & Hepperly (1988); Zakhary et al. (1984)
Meat product										
Beef	29.5	4.75	7.88	4.49	4.32	2.45	8.45	4.01	0.73	Yang et al. (2023)
Chicken	27.4	4.80	7.50	4.33	4.15	2.31	8.16	4.25	0.81	
Pork	28.2	4.82	7.72	4.42	4.18	2.49	8.26	4.11	0.80	
Fish (Tuna)	29.0	2.56	3.93	3.29	2.40	1.36	3.77	1.92	0.24	Saidi et al. (2014)

Val: valine; Leu: leucine; Ile: isoleucine; Thr: threonine; Met: methionine; Lys: lysine; Phe: phenylalanine; Trp: tryptophan. -: Unrecorded data.

bodies (Higgins et al., 2017). The main challenges in the production of fruiting bodies are controlling oxygen levels, the substrate's internal temperature, and pH. The temperature should be maintained between 25 to 30 °C, and the pH should be kept between 5.0 and 7.0. To address these challenges, innovations are being developed. For example, Kırdök et al. (2022) established a solid fermentation system with plastic bags integrated into a chain that circulates air and moisture through filtered connections, called modular chain bioreactor (MCB) (Figure 2B). The system contains sensors to control the internal temperature and humidity of the substrate in each bag and optimizes air circulation, improving macromycete growth.

Submerged fermentation for macromycetes production

Submerged fermentation of the mycelial form of mushroom-producing fungi has garnered special attention due to its advantages over solid fermentation (Dudekula et al., 2020). It stands out for its process homogeneity and significant improvement in oxygen transfer, even with increased viscosity of the fermentation broth, which facilitates scaling up the processes. According to Dudekula et al. (2020) high homogeneity improves the development of mycelium pellets. Furthermore, in submerged processes metabolites are produced in a more controlled way (Perveen et al., 2023). In these processes, primarily stirred tank bioreactors and airlift bubble columns have been used. Stirred tank bioreactors are mainly composed of a tank, a mechanical agitation system, an aeration system and temperature and pH controllers (Figure 2C). In these, the agitation and heat transfer processes are facilitated by the impeller and aeration. These are the main systems used in the production of mycelia via submerged fermentation because they are more efficient than airlifts.

Airlift bioreactors are the second most used in the production of mycelia. In these bioreactors, agitation and homogenization are carried out by injection of gas (air/oxygen) (Dudekula et al., 2020). The system mainly consists of a column with an internal tube that directs the fermentation broth to rise and fall, and the gas sprinkler (Figure 2D). The fermentative broth is moved from bottom to top, driven by gas bubbles fed at the bottom of the column, returning from top to bottom through a region different from the rising region. The difference between gaseous retention produces variability in dispersion densities, causing the circulation of the fermentation broth and the mycelia (Chisti & Moo-Young, 1987; Dudekula et al., 2020).

In the submerged mycelium biomass production process, aeration and agitation must be controlled to prevent the deterioration of the mycelium. According to Papaspyridi et al. (2013), in these processes dissolved oxygen must have at least 20 to 30% saturation (Tang et al., 2007). In the case of airlifts the flow must be higher, which can vary between 1 and 2 vvm (Dudekula et al., 2020; Feng et al., 2010). Agitation should be maximum between 150-200 rpm (Dudekula et al., 2020; Papaspyridi et al., 2013). Furthermore, in submerged fermentation, the *kLa* parameter must be controlled, which affects the growth of mycelia and the production of metabolites (Tang et al., 2007). This parameter is also related to the oxygen dissolved in the fermentation broth (Mello et al., 2024).

A drawback of submerged fermentation in protein production is that the levels of this compound are typically lower compared to those achieved in the fruiting bodies during solid fermentation. For example, Manu-Tawiah & Martin (1987) reported a maximum crude protein content for *P. ostreatus* biomass of 36 and 25.7% in the fruiting bodies and mycelium, respectively. Papaspyridi et al. (2013) also reported a higher protein content for fruiting bodies (36.4%), while for mycelia the protein content reached 32%. However, the process time for submerged fermentation is significantly shorter when compared to solid state fermentation, by at least 50%. While solid fermentation takes place in between 15-25 days (De Carvalho et al., 2010), submerged fermentation occurs in 6 to 10 days (Dudekula et al., 2020). Finally, the inoculation process in solid fermentation is more expensive than in submerged fermentation, in which an inoculum volume of 5 to 10% (v/v) is used (Dudekula et al., 2020).

Substrates and circular economy

Alternative protein sources need to be low-cost to meet the needs of the population. Therefore, from the perspectives of economic viability and global feasibility, the production of mushrooms as an alternative protein source depends, especially, on the substrates used for their growth (González et al., 2020). These substrates, besides being accessible, should facilitate high mushroom growth with a rich biochemical composition, and possibly enhance the flavor and aroma of mushrooms, making them more appealing to human taste. *Flammulina velutipes*, one of the most appealing edible mushrooms, presented enhanced umami taste and nutrient contents (higher crude fiber, crude fat, soluble protein, total aminoacids and soluble sugar) by cultivating it in mushroom root fermentation broth (MRFB) - a medium composed of byproducts from the edible mushrooms industry itself (mushroom root and fixing water from mushroom blanching procedure) (Wang et al., 2023).

Synthetic media for mushroom cultivation are often costly and time-consuming to prepare, making them preferable for small-scale productions, particularly in laboratory settings (Chang, 2008). On a large scale, agricultural or agro-industrial by-products with low or no economic value are exploited as substrates in mushroom production. Some examples are sugarcane bagasse, coffee husks, rice bran, wheat straw and bran, sawdust, corncobs, corn stems, cotton waste from textile industry, animal manure, bean straw, cocoa shell waste, banana leaves, oil palm pericarp waste, cassava bagasse, olive mill and winery wastes (Pandey et al., 2008). The utilization of such substrates can contribute to enhancing cultivation performance and elevating the nutritional value of mushrooms. Depending on the combination, higher protein contents can be achieved (Martín et al., 2023; Salami et al., 2016). Furthermore, cheap lignocellulosic residues positively impact substrate costs, providing an eco-friendly solution for their efficient handling and utilization in a circular economy (Martín et al., 2023).

In solid-state mushroom cultivation, such agro-industrial byproducts can serve as substrate after simple particle size reduction and moisture adjustment. Finding a balanced particle size is essential for optimal results in mushroom growth. Smaller substrate particles offer a larger surface area for fungi vegetative mycelium to access nutrients and water. However, excessively small particles may lead to substrate agglomeration, potentially hindering fungal aeration in solid-state (Pandey et al., 2008). The water content in the substrate is fundamental for achieving an optimal water activity (a_w). Water activity value fluctuates due to evaporation and metabolic processes (Pandey et al., 2008).

In submerged mushroom cultivation, it is common to use biomass hydrolysates to provide nutrients more efficiently. For example, *P. ostreatus* mycoprotein was obtained by submerged fermentation on 4-liters stirred-tank bioreactors using aspen wood hydrolysate as culture media. This hydrolysate provided glucose and xylose sugars, and the ratio between them was shown to be closely linked to the amino acid composition of the resulting protein. A value of 54.5 ± 0.5% (g protein/100 g sugars) was reached (Bakratsas et al., 2023a). In patent CN111771621A, *P. ostreatus* was successfully cultivated in bioreactors in a medium composed of 200 mL L^{-1} of *Sophora flavescens* alcohol precipitate hydrolysate, 4 g L^{-1} of rice bran, 4 g L^{-1} of yeast extract and 4 g L^{-1} of malt extract (Jin, 2020). In Chinese traditional medicine, *Sophora flavescens* is used in the production of extracts or medicinal compounds, and during the alcohol precipitation process, a precipitate is formed, which is normally discarded as waste. As described in the patent, it contains nutrients and bioactive compounds that are useful for the culture of oyster mushroom mycelium.

Experimental design strategies can be applied to enhance protein content and mushroom growth, helping to identify the ideal cultivation conditions. In the study by Bakratsas et al. (2023b), the production of single-cell protein (SCP) from *P. ostreatus* by submerged cultivation was optimized. An initial screening of different parameters affecting protein production has been conducted, considering carbon sources, organic and inorganic nitrogen sources, initial pH and cultivation time. Concentrations of carbon and nitrogen sources in the culture medium were found to be the two most significant factors, and after response surface methodology optimization a value of 44.8 ± 0.8% of proteins (dry weight) was attained, with 10.0 ± 0.9 g L^{-1} of proteins in 3.5-liters stirred tank reactors (Bakratsas et al., 2023b).

Nitrogen plays a crucial role in synthesizing numerous organic compounds such as proteins, nucleic acids, and cell wall components in fungi. Mushroom substrates normally contain organic nitrogen sources and are low in free ammonium since the excess can inhibit growth or fruiting of mushrooms. For fruiting body induction, the balance between C and N sources is essential, being a parameter that can be studied and optimized to improve a specific fungal species growth yield (Pandey et al., 2008). Furthermore, inorganic salts have been shown to increase the biochemical potential of mushrooms. In the study by Madaan et al. (2024), *Pleurotus* spp. was cultivated on substrate organically enriched with Se and Zn, which could increase total soluble proteins and flavonoids/phenolic content on the mushrooms, with suitable characteristics for the development of dietary supplements (Madaan et al., 2024). On upscaled mushroom cultivations, large quantities of by-products consisting of spent substrate are generated, known as spent mushroom substrate (SMS) or spent mushroom compost (SMC). It is estimated that after several cycles of mushroom harvesting approximately 5 kg of wet by-products are generated for every kg of fresh mushroom produced (Gao et al., 2021; Leong et al., 2022). Those spent substrates may be used in various applications following the concept of circular economy, which include their use as biofertilizers, soil amendment, bio-control agent, alternative feed for poultry, ruminant and pig, feedstock for production of second-generation biofuel and bioremediation agent for heavy metals, polycyclic aromatic hydrocarbon (PAHs), pesticides, etc. (Leong et al., 2022).

Safety of mushroom production as alternative protein source

The use of agricultural materials to cultivate edible mushrooms must be performed with care due to food safety concerns. Mushrooms are known to accumulate substances, such as the heavy metal arsenic (As) depending on the metalloid residue level in the substrate (Mleczek et al., 2016). This holds true for pesticides as well, making it imperative, particularly at an industrial scale, to characterize and monitor potential contaminants in the substrate. Nevertheless, the ability of fungi to absorb and bioaccumulate molecules can also be used to favor their nutritional composition (Madaan et al., 2024; Oliveira & Naozuka, 2019). In the study by Oliveira & Naozuka (2019), selenium (Se) in culture media was shown to favor the formation of Se-proteins, which are more bioaccessible species, in white and pink oyster mushrooms (*P. ostreatus* and *P. djamor*). Especially with the focus on edible mushrooms production, sterilization or disinfection of the substrate is an important step required for eliminating microbial forms such as fungi, bacteria, viruses and spores, ensuring food quality and safety. Normally, treatments using high temperatures and/or pressures are performed, such as pasteurization, autoclaving, or immersion in hot water (Atila, 2023; Macias González et al., 2022). Although highly efficient at large scale, those methods often demand specific equipment, with substantial initial investment cost, and require high energy for operating (Macias González et al., 2022).

New disinfection methods are being explored, however. In the article by Atila (2023), chlorine dioxide was used to disinfect *Hypsizygus ulmarius* as an alternative to autoclaving. This treatment prevented substrate contamination by harmful microorganisms, permitting a shorter cultivation cycle from inoculation to the first flush and enhancing the mushroom yield and biological efficiency (Atila, 2023). Besides sterilization or disinfection, training personnel for microbiological and chemical assessments is also critical to ensure food safety and quality on mushroom production (Devi Tentu et al., 2021).

Macromycetes and their products

Macromycetes find application across various industries, with one of the most promising sectors being food, where they have been utilized in diverse product formulations. These can be grouped into four categories: dairy, beverages, pasta, and meat analogs (Figure 3). One category gaining increasing relevance is that of meat substitutes, given the high percentage of crude protein found in the biomass of macromycetes, as demonstrated in Table 1. Moreover, they exhibit an essential amino acid profile comparable to that of proteins from animal sources, positioning mycoproteins as promising substitutes for conventionally consumed proteins (Ayimbila & Keawsompong, 2023; González et al., 2020). Some of the most significant advantages of this alternative source of proteins over traditional meat are: (i) macromycete-based proteins are produced more quickly and with a significantly lower environmental impact compared to beef production.; (ii) mushroom proteins have functional properties, are highly digestible, healthy and are widely accepted by the population (Singh et al., 2023).

Many companies such as Kinoko Tech, Prime Roots, NaturesFynd, Better Meat, Enough Foods, Planetary Group, Mycotechnology, among others are engaged in producing macromycete-based foods. These products include: beef patties, pork sausages, frankfurter, beef patty, beef nuggets, meat emulsion, pork patty, chicken patty, beef burger, beef patty, fishless seafood, etc. (Singh et al., 2023; Wang & Zhao, 2023). Some relevant patents have been developed with products based on macromycetes. For example, Kim & Kim (2006) in patent KR10076284848B1 presented a process for producing mycoproteins and meat analogs as substitutes for conventional protein and meat. Meat analogs can be defined as products obtained from mushroom proteins or textured vegetable proteins (Wang & Zhao, 2023). In the case of the protein concentrate, mycelium, corn husk and egg white were mixed, the first component in a maximum proportion of 40% and the other two in a maximum proportion of 30% each. Subsequently, the mixture was extruded under conditions of 100-1000 psi and in a temperature range between 100 and 170 °C. To manufacture the meat analog, the protein concentrate was mixed with emulsifiers, water and starch, and the traditional method of artificial meat production was used to obtain a fibrous structure. At the end of the process, macromycete proteins were found to have excellent texture and even better taste than soy protein.

Patents have also been developed focusing on fermentation methods for producing macromycete fungi. This is as the case of patent US11432574B2, filed by Patillo (2022), which presents several types of improved aerobic fermentation procedures to produce a mushroom-based protein food ingredient for meat hybrids or meat analogues. In this work, 150 mL of a 2-day culture of *Cordyceps militaris* were inoculated in a pulse-fed aerobic fermentation. An optimized culture medium was used (17 L deionized water, 30 g L^{-1} light malt extract prepared from the hydrolysis of spent malted barley, 10 g L^{-1} glucose, 5 g L^{-1} yeast extract, 0.5 g L^{-1} $NH_4H_2NO_3$, 0.2 g L^{-1} $MgSO_4$, 3 g L^{-1} safflower oil, and 0.0025 g L^{-1} biotin). Pulses were injected after 48 and 96 hours of incubation (20 g L^{-1} light malt, 10 g L^{-1} glucose, and 20 g L^{-1} yeast extract - all previously autoclaved). The biomass was then recovered by pressing and decanting the supernatant to give a final yield of 41 g L^{-1} dry biomass. The product obtained can be used as a mushroom-based protein food ingredient that can supplement or complement a conventional meat product.

In general, meat-like products produced from edible macromycete cultures, as described above, are usually mycelial or fruiting body fungal biomass. Although various fungi can mimic the texture, flavor, and odor of animal meat, they typically need to be blended with other ingredients like flavorings or pigments in different proportions to increase their appeal and achieve a closer resemblance to conventional meat products. For instance, Yuan et al. (2021) developed a meat analogue based on macromycetes mixed in equal proportions (*L. edodes*, *P. ostreatus*, *C. comatus*) and soybeans, using mainly an extrusion process. The product

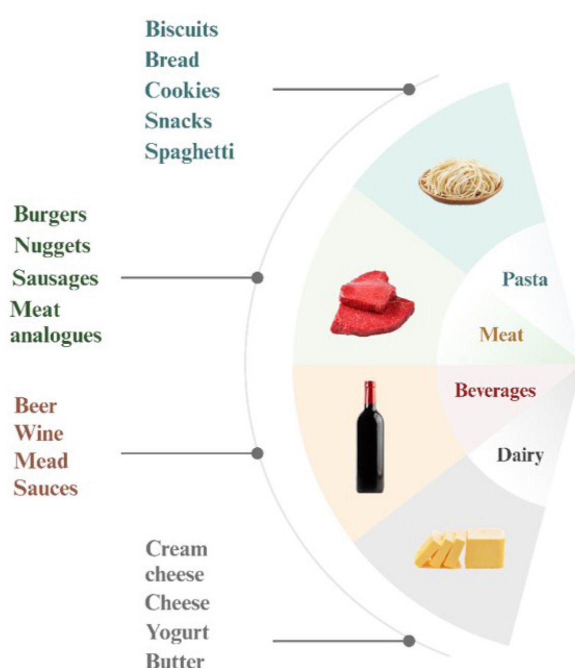


Figure 3. Categories of edible macromycetes products applied in the food industry.

obtained exhibited textural profiles close to those of beef, moreover, there was no significant difference in properties such as viscosity and elasticity between the meat and the analogue. Similarly, in the patent JP4628482B2 filed in Japan (Deok-Kim & Kim, 2006), a methodology was developed for the production of fungal mycelium from *Agaricus bisporus*, *Pleurotus ostreatus*, *Flammulina velutipes*, *Ganoderma lingzhi* and *Cordyceps* sp., and for the elaboration of meat analogues by mixing 40% of this mycelium with 30% of corn husk and 30% of egg white. Finally, the mixture is subjected to an extrusion process at 100-1000 psi and then cooled in a matrix. In addition, 50% of the meat analog product obtained was used to create substitute meat by adding a small amount of seasonings, spices, and pigments. Finally, a meat flavoring was also developed from meat analogs, which was made by mixing 65% of the pulverized meat analog product (previously obtained) with 10% anchovy powder, 10% kelp algae powder, and 5% seaweed powder. This resulted in several varieties and presentations of products based on edible fungal macromycetes that are more complete and similar to meat derivatives.

The “Alt protein” market is expected to grow from the current US$ 16.52 billion in 2024 to US$ 26.52 billion by 2030, which means an average growth rate of 8.82% per year (Grand-View-Research, 2023). In this scenario, mycoprotein represents a fraction of US$ 298 million in 2022 up to US$ 976 million in 2032, according to a report from Future-Market-Insights (2022). The budget has been substantial, and large companies are investing in meat substitutes. For example, Cargill’s budget for Enough Foods exceeds $100 million, doubling production in one year to 20,000 tons (Star-Tribune, 2024). The moment is heated by many start-ups flourishing around the world.

Table 2 shows some trends in products based on edible macromycetes as protein replacements and the industries responsible for their development.

Table 2. Trends in mushroom protein products.

Product	Proposal	Company	Reference
Eat Meati	Whole-food cutlets and steaks — made from mushrooms using a sustainable and clean process	Chipotle Mexican Grill	Jennings (2022)
Fish-Free Seafood	Out-flavors, out-textures, and out-nutritious anything else on the market. So, while the oceans rest, you can enjoy the freshest seafood of your life every night of the week	Aquaculture Foods	Aqua-Cultured-Food (2023)
Quorn®	“Mycoprotein” as various meat-cut products	Marlow Foods	AstraZeneca (2023)
Fermotein®	Food ingredient containing proteins, fibers and all those essential amino acids, vitamins, and minerals which a healthy, balanced diet needs	The Protein Brewery	Brewery (2023)
Koji-Meats	Various modalities of meat analogues, such as turkey, ham, salami and pate and bacon	Prime Roots	Prime-Roots (2023)
Fungi-powered Fy Protein	Meatless patties and including the first fungi yogurt	NaturesFynd	Natures-Fynd (2024)
Shroomacon	A simple product made from five ingredients: king oyster mushrooms, olive oil, natural smoke flavor, salt, and black pepper. Unlike many meat alternatives, Shroomacon is made from long strips of mushroom rather than processed and molded ingredients	Meat the Mushroom	Meat-Mushroom (2024)
FermentIQ™	FermentIQ™ plant protein is a superior protein source made by fermenting plant protein with shiitake mushroom mycelia. The process improves flavor, aroma, nutrition, and functionality	MycoTechnology	Myco-Technology (2021)
FermentIQ™ PEA	Pea protein fermented by shiitake mycelia, creamy mouthfeel and smooth texture with high solubility, heat stability and emulsion capacity, clean, neutral taste with a significant reduction in off-note attributes compared to other pea proteins	MycoTechnology	
Mycolein™	Is a fungi-stabilised fat ingredient developed with Mycorena’s proprietary emulsion technology. The fat solution displays properties more similar to animal fat than any other fat product on the market, produced entirely without animals. Mycolein™ is a Clean Label, low-fat product to be utilized in any type of product, whether plant-based, alternative protein or even meat	Mycorena	Mycorena (2020)
MICO	MICO is a third-generation mycelial protein, which stands out for its efficiency in production, complete nutritional profile, and commitment to sustainability	Done Properly	Done-Properly (2022)

Conclusions

Edible mushroom biomass holds significant potential to emerge as an important alternative protein source and a substitute for animal-based proteins, thereby fostering various environmental, economic, and dietary shifts. This potential is evident in the rapid growth of global production of macromycete biomass, which correlates with the increasing consumer preference for healthier and biologically rich foods. Mushroom biomass presents a high protein content and an amino acid profile comparable to traditional meats, further enhancing its appeal. Production of mushroom biomass occurs through both solid and submerged fermentation processes, which are constantly being improved and optimized to enable higher production with satisfactory protein content. The first process yields biomass with a higher protein content, reaching levels of up to 82%. Conversely, submerged fermentation offers shorter production cycles, taking less than half the time compared to solid fermentation. Advances in culture media and circular economy approaches are progressing, and the development of meat-like protein products or concentrates is an emerging application trend for mushroom biomass.

Conflict of interests

The authors declare no competing financial interests.

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