



Proceeding Paper

# Sustainable Cultivation of Edible Mushrooms: Preserving Biodiversity and Ensuring Product Quality †

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**Abstract:** Mushrooms have long been valued for their taste and numerous health benefits. The Montesinho Natural Park is home to over two hundred edible mushroom species, yet climate change and unsustainable practices have affected their availability. Controlled cultivation on forest substrates can contribute to species preservation, and a comprehensive review of nutritional and chemical composition is essential for ensuring quality and consumer confidence, while supporting biodiversity and sustainability. By responsibly meeting the demand for mushrooms, it is possible to protect natural habitats and promote global ecosystem sustainability.

**Keywords:** mushrooms; mycological diversity; dietary benefits; sustainable cultivation; biodiversity production



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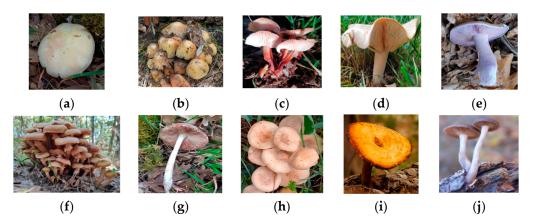
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### 1. Introduction

Mushrooms are macrofungi that have sparked significant interest since ancient times, both for their sensory characteristics and the numerous health benefits they offer, as well as the many dietary advantages for consumers [1,2]. Their classification as a healthy food stems from the fact that mushrooms have a low fat and calorie content and are rich in dietary fibers, proteins, and minerals [3]. In addition to their gastronomic importance, many edible species have medicinal properties and are used for therapeutic purposes as they are composed of biologically active compounds, such as polyphenols and polysaccharides [4,5]. Consequently, the food, pharmaceutical, and nutraceutical sectors extensively harness mushrooms due to their various properties [6]. The Agaricaceae family includes various types of mushrooms, which can be distinguished from each other by color, shape, and activity. Among the approximately 1.5 million estimated fungi, about 14,000 globally cataloged species develop fruiting structures that reach a considerable size to be recognized as mushrooms. Among these, at least 2000 species are known to be edible [7].

The Montesinho Natural Park, a Portuguese mountainous region known for its mycological diversity (Figure 1), hosts over two hundred species of edible mushrooms that are rich in proteins, carbohydrates (including polysaccharides and fibers), and minerals [8–11]. However, the availability of these fungi is compromised by seasonality and the impacts of climate change on forest composition, resulting in a decrease in mushroom diversity. Unsustainable harvesting practices and illicit trade have further exacerbated the limited availability of these fungi, posing risks to the ecosystems. To address these challenges, the

cultivation of edible mushrooms in controlled environments could preserve the unique attributes of different species. A comprehensive review of their nutritional, chemical, and bioactive characteristics will ensure the quality of cultivated species, enhance consumer trust, and drive sustainable mushroom cultivation [12,13]. By responsibly meeting the demand for mushrooms, it is conceivable to safeguard natural habitats and promote global ecosystem sustainability.



**Figure 1.** Endemic saprophytic mushrooms from the Montesinho Natural Park, Portugal: (a) *Boavista plúmbea*; (b) *Coprinus angulatus*; (c) *Flammulina velutipes*; (d) *Calocybe gambosa*; (e) *Clitocybe nude*; (f) *Armillaria mellea*; (g) *Entoloma clypeatum*; (h) *Marasmius oreades*; (i) *Cerioporus leptocephalus*; and (j) *Pseudoclitocybe cyathiformis*.

#### 2. Mushrooms as Food

#### 2.1. Nutritional, Chemical, and Bioactive Properties

Different types of cuisine around the world use edible mushrooms in many recipes, primarily due to their unique flavor and the vast array of ways they can be prepared and consumed. In addition to these characteristics, mushrooms are considered a delicacy with high nutritional and functional value, making them a part of a balanced diet essential for preventing numerous health deficiencies [1]. Mushrooms are known for their high levels of moisture (between 85% and 95%), carbohydrates (ranging from 35% to 70%), proteins (with contents between 15% and 34.7%), fats (making up approximately 10% of their composition), and minerals (with ranges from 6% to 9.9%). Furthermore, mushrooms also stand out as a rich source of various vitamins, such as thiamine, riboflavin, niacin, biotin, ascorbic acid, pantothenic acid, and folic acid. Regarding minerals, mushrooms contain calcium, iron, manganese, magnesium, zinc, and selenium. Due to the significant presence of carbohydrates, fibers, proteins, essential amino acids, unsaturated fatty acids, and vitamins, their low calorie content, as well as the presence of minerals such as potassium, iron, copper, zinc, and manganese in their composition, mushrooms are widely recognized as a healthy food with nutritional benefits in their fruiting bodies [3,14–17].

#### 2.2. Mushroom Cultivation

Today, there is a growing demand for nutritionally balanced options, an increased sensitivity to environmental pollution issues, and a heightened concern about the availability of raw materials in general, which have remained limited due to obvious associated costs [18,19]. Regarding the availability of mushrooms, in mountainous regions like the Montesinho Natural Park, a significant limitation has been observed, primarily caused by climatic differences, such as intense droughts. This seasonality restricts the availability of mushrooms throughout the year and hinders their continuous supply to markets and restaurants [12]. Therefore, mushroom cultivation, in addition to preserving different species' characteristics, can contribute to reducing atmospheric pollution, and their byproducts can also be utilized [12]. Mushrooms can be cultivated in structures built specifically with controlled environmental conditions. However, macrofungal cultivation

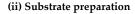
is extremely challenging, and thus far, only a small number of species have been cultivated commercially [20]. *Lentinus edodes* is the most widely cultivated mushroom globally, with notable mentions for *Pleurotus* spp., *Auricularia* spp., and *Agaricus bisporus*. Most cultivated mushroom species are saprophytic, meaning they play the role of decomposers of organic matter [21]. On the other hand, many of the finest gourmet mushrooms are mycorrhizal, growing in symbiotic relationships with plants and cannot yet be cultivated in the same way as other mushrooms.

Mushroom cultivation typically follows several key steps (Scheme 1), which may vary in some specific points depending on the type of mushroom being cultivated; but overall, the process is as follows: (i) strain selection; (ii) substrate preparation; (iii) sterilization or pasteurization; (iv) inoculation; (v) incubation; (vi) fruiting conditions; (vii) harvesting; (viii) quality control; and (ix) market distribution.



#### (i) Strain selection

A mushroom strain is selected that is aimed for a specific purpose, whether for culinary, medicinal, or other applications.





The substrate or growth medium is commonly straw, sawdust, wood chips, or compost, depending on the mushroom species. However, materials obtained from forest clearing are also being studied from the perspective of circularity and sustainability of ecosystems.

#### (iii) Sterilization or pasteurization



All substrates used must undergo sterilization or pasteurization procedures. Sterilization involves heating to high temperatures (e.g., autoclaving), while pasteurization involves heating at lower temperatures to preserve some beneficial microorganisms. This step needs to be optimized depending on the mushroom species.

#### (iv) Inoculation

Mushroom spores or mycelia are introduced into the sterilized or pasteurized substrate. This can be performed using various methods, such as agar plugs, liquid inoculants, or grain *spawn*.



#### (v) Incubation

The inoculated substrate is placed in a controlled environment with optimal temperature and humidity conditions. This encourages mycelial growth and colonization of the substrate. The incubation period varies depending on the mushroom species, and it may also require casing techniques.



#### (vi) Fruiting conditions

Once the substrate is fully colonized with mycelia, it needs to be placed under light, moisture, and air conditions that allow the fruiting of mushrooms. These conditions typically involve lower temperatures, higher humidity, and fresh air exchange, as well as physical triggers. These conditions trigger mushroom formation.

Scheme 1. Cont.

#### (vii) Harvesting



Harvesting depends on the specific requirements of each mushroom species. The size, color, and maturation process of each mushroom species are some of the specifications to be considered.

At this stage, the collection of spores and tissues is recommended if producers intend to preserve the strain and continue the cultivation cycle. The resulting substrate can also be reused after sterilization or pasteurization.

It is a crucial checkpoint for scaling-up production, by increasing the size of the growing area, improving automation, and optimizing the processes described above.

## (viii) Quality control



This involves monitoring for contaminants, pests, or diseases throughout the production process and taking appropriate measures to mitigate them.

#### (ix) Market distribution



Mushrooms are prepared for sale, and post-harvesting strategies are developed to ensure the high quality of the produced mushrooms. The sale of products can occur directly to final consumers, but it can also be indirect via restaurants or other marketplaces.

**Scheme 1.** Key points for mushroom cultivation aiming at market distribution.

Ex vitro mushroom production can be a complex process that requires careful attention to environmental factors, substrate preparation, and disease management. It is essential to research the specific requirements of the mushroom species selected to be cultivated and adapt the approach accordingly.

#### Substrates for Mushroom Cultivation

Due to their saprophytic nature, mushrooms acquire the necessary nutrients by absorbing dissolved organic matter present in decomposing wood and other degraded materials [22]. The quality of edible mushrooms can be influenced by the type of substrate used for their cultivation. In the process of mushroom cultivation, it is crucial to first determine the species to be produced. This requires evaluating its characteristics, substrate availability, and optimal environmental conditions for growth and fruiting. Mushroom cultivation often involves the use of large quantities of agricultural waste, which significantly increases their volume when they have no other use. These waste materials are primarily composed of lignocellulosic materials, which include polymers such as cellulose, hemicellulose, and lignin in varying percentages. Their decomposition, which is carried out by mushrooms, earthworms, microfungi, and bacteria, plays an important role in the terrestrial carbon cycle [23,24]. The main lignocellulosic byproducts include materials such as rice straw, wheat straw, barley straw, corn and sorghum stalks, coconut husks, sugarcane bagasse, oil palm residues, pineapple husks, and banana leaves.

Nutrients for mushrooms primarily come from the substrate used to cultivate them, which affects their chemical, functional, and sensory characteristics. Minerals are essential for the growth of macrofungi and can be supplemented in the substrate to improve incubation and fruiting speed [22]. The choice of substrate can directly influence the growth of different mushroom species, as each species has distinct nutritional and environmental requirements (Table 1).

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Mushrooms	Substrate	References
Agaricus bisporus	Sunflower seed husk	[25,26]
Pleurotus ostreatus	Wheat straw	[24,25]
Hericium erinaceus	Sunflower hulls; wheat straw; rice straw	[24,25,27]
Lentinus sajor-caju	Sugarcane bagasse; rice straw	[24,28]
Auricularia polytricha	Hardwood sawdust; corn stalk; rice bran	[24,25]

**Table 1.** Substrates used for the cultivation of various species of edible mushrooms.

The physical and chemical characteristics of the used residues provide a significant opportunity for their exploration, offering substantial value in the field of biotechnology. Mushroom cultivation emerges as a practical alternative for the utilization of lignocellulosic residues, as mushrooms have the capability to produce enzymes that degrade these materials [25].

#### 2.3. Preserving Biodiversity and Ensuring Product Quality

One of the most significant advantages of sustainable mushroom cultivation is its potential to preserve biodiversity. Wild mushrooms play a crucial role in ecosystems, forming symbiotic relationships with trees and plants while breaking down organic matter [29]. Overharvesting wild mushrooms can disrupt these delicate ecosystems, leading to imbalances and biodiversity loss.

Sustainable cultivation practices have the following characteristics:

- (i) They provide a solution by reducing the reliance on wild mushroom harvesting. Additionally, sustainable mushroom cultivation can help protect threatened and endangered mushroom species. By replicating the natural habitat and growth conditions of mushrooms, cultivators can contribute to their conservation while also meeting the demand for these unique culinary treasures.
- (ii) They have a direct impact on the quality of edible mushrooms. A controlled environment allows for consistent growth conditions, resulting in mushrooms that are free from contaminants, pests, and diseases. This quality control ensures that consumers receive safe and flavorful mushrooms, thereby enhancing their dining experience.
- (iii) They can improve the nutritional value of mushrooms. By optimizing growing conditions, cultivators can enhance the content of essential nutrients like vitamins, minerals, and antioxidants in the final products. This not only benefits consumers but also aligns with the global push for healthier food options [30–33].

#### 3. Conclusions

Through this comprehensive analysis, it becomes evident that mushrooms play a crucial role both in human nutrition and in the environmental context. The various research findings presented in this article reveal that mushrooms not only provide a valuable source of nutrients and bioactive compounds but also play a significant role in recycling organic matter and maintaining ecosystem health. By highlighting the nutritional, chemical, and bioactive characteristics of mushrooms, this study emphasizes their importance in promoting human health and in facilitating the potential development of new functional foods and medicines. Furthermore, by exploring mushroom cultivation and the substrates used, we pave the way for sustainable and economically viable practices that can contribute to food security and the conservation of natural resources. In summary, mushrooms represent an extremely promising field of research and application with significant benefits for both human health and the environment. The intersection of food science, sustainable agriculture, and environmental conservation offers exciting opportunities for innovation and future development.

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