Proceeding Paper

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

Ana Saldanha 1,2,3, Leonardo Corrêa Gomes 1,2, José Pinela 1,2, Manuel A. Coimbra 3, Lillian Barros 1,2, Maria Inês Dias 1,2,* and Carla Pereira 1,2

1 Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal; ana.saldanha@ipb.pt (A.S.); leonardocorrea@ipb.pt (L.C.G.); jpinela@ipb.pt (J.P.); lillian@ipb.pt (L.B.); carlap@ipb.pt (C.P.)
2 Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
3 LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal; mac@ua.pt
* Correspondence: maria.ines@ipb.pt

**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 affect their availability. Controlled cultivation on forest substrate can contribute to species preservation, and a comprehensive review of nutritional and chemical composition is essential to ensure quality and consumer confidence, 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

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 fiber, 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, for example [4,5]. Consequently, the food, pharmaceutical, and nutraceutical sectors extensively harness these 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 further exacerbate 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 chypeatum; (h) Marasmius oreades; (i) Cerioporus leptocephalus; (j) Pseudoclitocybe cyathiformis.

2. Mushroom 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 all 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 [14]. 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 their significant presence of carbohydrates, fiber, proteins, essential amino acids, unsaturated fatty acids, vitamins, and low-calorie content, as well as 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 [3,15–18].

2.2. Mushroom Cultivation

Today, there is a growing demand for nutritionally balanced options, increased sensitivity to environmental pollution issues, and heightened concern about the availability of raw materials in general, which has remained limited due to obvious associated costs [19,20]. 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 [21]. Therefore, mushroom cultivation, in addition to preserving species’ characteristics, can also contribute to reducing atmospheric pollution, and the byproducts can
still be utilized [12]. Mushrooms can be cultivated in structures built specifically with controlled environmental conditions. However, macrofungi cultivation is extremely challenging, and, so far, only a small number of species have been cultivated commercially [22]. *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 [23]. 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), that may vary in some specific points depending on the type of mushroom cultivated, but overall follows as: (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 and distribution.

(i) Strain selection

The strain mushroom selected is aimed for a specific purpose, whether it’s for culinary, medicinal, or other applications.

(ii) Substrate preparation

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

(iii) Sterilization or pasteurization

All the substrate 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

The mushroom spores or mycelium are introduced into the sterilized or pasteurized substrate. This can be done through 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, as it can also requires casing techniques.

(vi) Fruiting conditions

Once the substrate is fully colonized with mycelium, it needs to be placed under light, moisture, and air conditions that allows fruiting of the mushrooms. Typically involves lower temperatures, higher humidity, and fresh air exchange, as also physical triggers. These conditions trigger mushroom formation.

(vii) Harvesting
The harvesting depends on the specific requirements of each mushroom species. The size, color, and maturation process of each mushroom are some of the specifications to be considered. At this stage the collection of the spores and tissue is recommended if the producer intends to preserve the strain and to continue the cultivation cycle. The resulting substrate can also be reused after sterilization or pasteurization. It is a crucial check point for scaling up the production, by increasing the size of the growing area, improve automation, and optimize the processes above described.

(viii) Quality control

Monitor for contaminants, pests, or diseases throughout the production process and take appropriate measures to mitigate them.

(ix) Market and distribution

Mushrooms are prepared for sale, developing post-harvesting strategies to ensure the high quality of the produced mushrooms. The sale of the product can occur directly to the final consumers, but in can also be direct to restaurants, or other marketplaces.

Scheme 1. Key-points for mushroom cultivation aiming market distribution.

Ex-vitro mushroom production can be a complex process that requires careful attention to environmental factors, substrate preparation, and disease management. It’s essential to research the specific requirements of the mushroom species intended to cultivate 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 [24]. 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, 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 in turn include polymers such as cellulose, hemicellulose, and lignin, in varying percentages. Their decomposition, carried out by both mushrooms and earthworms, microfungi, and bacteria, plays an important role in the terrestrial carbon cycle [25,26]. 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, 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 [24]. The choice of substrate can directly influence the growth of each mushroom species, as each one has distinct nutritional and environmental requirements (Table 1).

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

<table>
<thead>
<tr>
<th>Mushrooms</th>
<th>Substrate</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Agaricus bisporus</em></td>
<td>Sunflower seed husk</td>
<td>[27,28]</td>
</tr>
<tr>
<td><em>Pleurotus ostreatus</em></td>
<td>Wheat straw</td>
<td>[26,27]</td>
</tr>
<tr>
<td><em>Hericium erinaceus</em></td>
<td>Sunflower hulls; Wheat straw; Rice straw</td>
<td>[26,27,29]</td>
</tr>
</tbody>
</table>
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 these lignocellulosic residues, as mushrooms have the capability to produce enzymes that degrade these materials [27].

2.3. Preserving Biodiversity and Ensure 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 [31]. Overharvesting wild mushrooms can disrupt these delicate ecosystems, leading to imbalances and biodiversity loss.

Sustainable cultivation practices:
(i) provides 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 these mushrooms, cultivators can contribute to their conservation while also meeting the demand for these unique culinary treasures;
(ii) 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, enhancing their dining experience;
(iii) 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 product. This not only benefits consumers but also aligns with the global push for healthier food options [32–35].

3. Conclusions

Through this comprehensive analysis, it has become 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 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.

Author Contributions: Writing—original draft preparation, A.S., L.C.G., J.P. and M.I.D.; writing—review and editing, M.A.C., L.B., M.I.D. and C.P.; visualization, C.P.; supervision, M.A.C., L.B. and M.I.D.; project administration, C.P. and L.B.; and funding acquisition, C.P. and L.B. All authors have read and agreed to the published version of the manuscript.
Funding: The Safe2Taste project (MTS/BRB/0056/2020) received financial support from the Foundation for Science and Technology (FCT, Portugal).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement:

Acknowledgments: The authors would like to thank the Foundation for Science and Technology (FCT, Portugal) for the financial support from national funds to CIMO (UIDB/00690/2020 and UIDP/00690/2020), SUSyTEC (LA/P/0007/2021), LAQV-REQUIMTE (UIDB/50006/2020), Safe2Taste project (MTS/BRB/0056/2020), the A. Saldanha Doctoral Scholarship (2021.08346.BD), and the national funding from FCT, P.I., through the institutional contract of scientific employment for L. Barros, M.I. Dias, J. Pinela, and C. Pereira. Thanks to BL3-Campus de Tecnologia e Inovação, partners of the Safe2taste project, for providing the the photographic record of saprophytic mushrooms found in Montesinho Natural Park.

Conflicts of Interest: The authors declare no conflict of interest.

References


Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.