Alternative substrates for the cultivation of macrofungi

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ABSTRACT

Several raw materials, or residues, can be used as alternative substrates, aiding in mushroom cultivation and reducing production costs by using resources that would otherwise be discarded. Agroindustrial residues can act as a base substrate supplementation and a carbon source, improving the substrate for fermentation or several other factors. This review highlights the importance and effectiveness of using such substrates, and the significant role it plays in contributing to a sustainable future. The socio-environmental benefits such as reducing pollution, reusing agro-industrial waste, and cutting production costs are not just advantages, but a contribution to a healthier planet.

Keywords: Mushrooms, Substrates, Supplementation.

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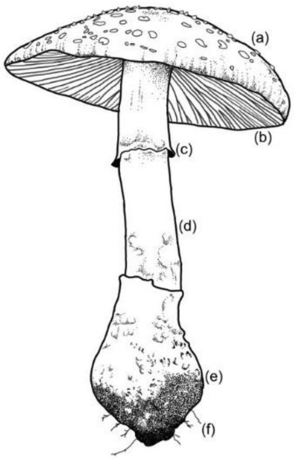
INTRODUCTION

Fungi are characterized by their heterotrophic nature, as they do not produce their food and obtain nutrients through hyphae, which are microscopic structures. Mushrooms are macrofungi part of the Fungi kingdom and can be epigeal (they grow above the ground) or hypogeal (they grow in the soil). They have a fruiting body visible to the naked eye and reproduce through spores. There is a wide morphological diversity among mushrooms, which can vary in the presence of cap, lamellae, stipe, ring, and volva, and also have a wide range of shapes and colors (Figure 1) (Cho & Quimio, 2004; Chang & Miles, 1992). The cap, or cap of the mushroom, is essential for identification, observing its color, shape, and size (Jordan, 2016).

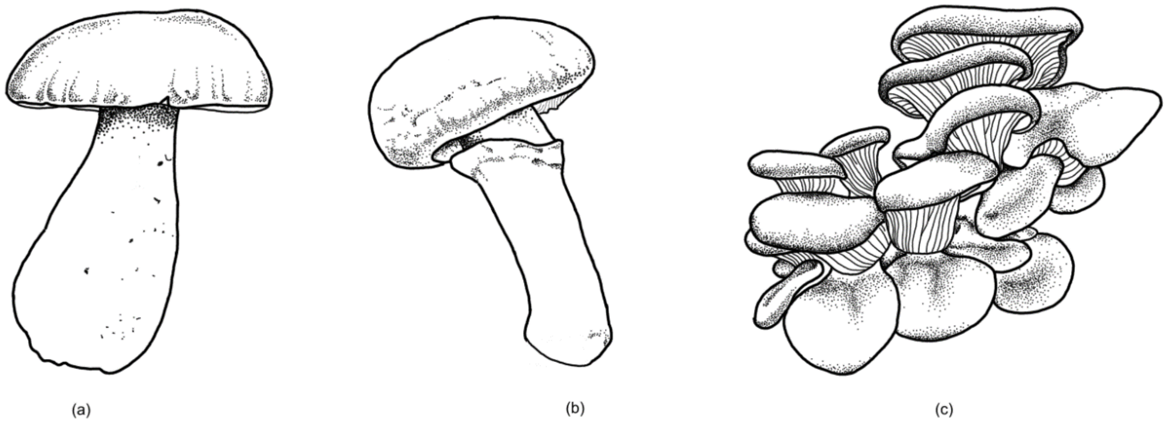
Figure 1. Morphological characteristics of *Basidiomycetes*.



Where: Species *Amanita muscaria*, Pileus (a), lamellae (b), ring (c), stipe (d), volva (e), hyphae (f).

Macrofungi can be divided into *Ascomycetes* and *Basidiomycetes*. *Basidiomycetes* are popularly known for belonging to the *Agaricaceae* and *Pleurotaceae* families. The *Agaricaceae* family can grow in sacs called volva and have a universal veil that covers it entirely in one of the phases of this macrofungus, and, as its development progresses, it breaks. The veil remains present in the *Agaricus* structure, and the partial veil later originates in the final phase of the mushroom's development. The ring is present in the stem (Jordan, 2016). Some morphological differences mentioned can be seen in Figure 2, with differentiation of stipe.

Figure 2. Morphological differences in the stipe of different mushroom species.

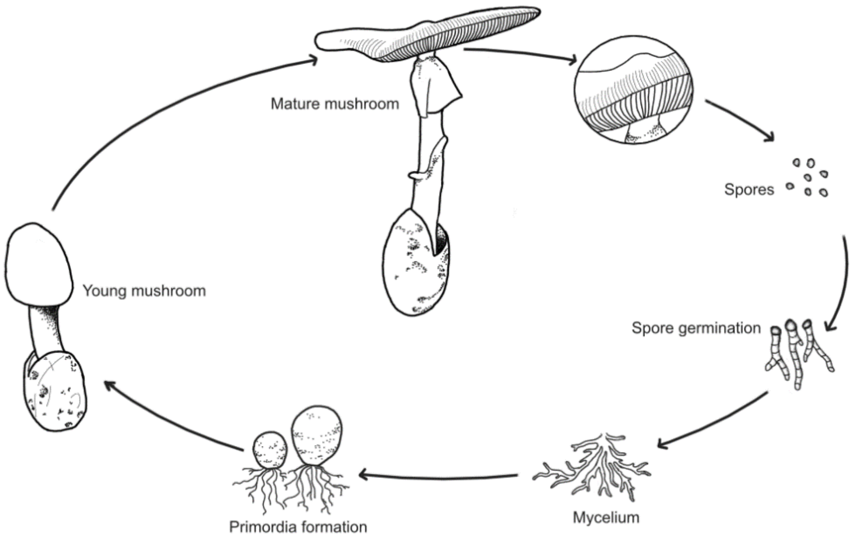


Where: (a) *Boletus edulis*, (b) *Agaricus bisporus*, (c) *Pleurotus ostreatus*.

The *Pleurotaceae* family includes the genus *Pleurotus*, which provides representatives popularly known as oyster mushrooms, one of the most popular edible mushrooms. They are cosmopolitan and act as primary decomposers of hardwoods. They have hyphae that have clamp-shaped connections and a monomictic hyphal system, a type of hyphal organization in which there is no morphological differentiation (Webster, 2007; Cho & Quimio, 2004).

In mushroom cultivation, several substrates can be used, with emphasis on alternative substrates coming from agro-industrial waste such as wheat, rice and corn straw, cotton waste, sawdust, and bagasse (Jahedi et al., 2024; Ekun et al., 2024). These substrates are used sustainably to dispose of and reduce waste, making it more economical to grow mushrooms for commercial purposes (Ekun et al., 2024). Mushroom cultivation does not vary, as the macrofungi cycle is always the exact (Figure 3), not changing according to the species. The milestone of the reproductive process is the release of spores on a substrate suitable for each species. This substrate will require specific conditions, such as nutrients, humidity, and temperature, thus initiating germination. Germination is a complex process, and the growth time will depend on the growing species. New cells are formed that will create filaments called hyphae. The set of hyphae is mycelium, the vegetative part of the cycle (Figure 3). Mycelium cultivation is commonly done with hydrated grains and vermiculite with sterilization in the containers used (Russel, 2014).

Figure 3. The life cycle of mushrooms.



Mushrooms are used as food, possess good nutritional qualities, and are beneficial to health, given that they are rich in proteins, fibers, and several vitamins and have few calories (Singh, 2017; Ukwuru, 2018). Furthermore, mushrooms have medicinal uses due to their anti-inflammatory, immunomodulatory, antimicrobial activity and acting as a liver protector (Wang et al., 2014; Yao et al., 2015, Li et al., 2016, Cui et al., 2016; Liu et al., 2016).

The popularity of mushrooms is mainly due to their different and exotic flavor. The protein content of mushrooms may be lower than that of meat and eggs, but it is similar to that of milk and corn. Therefore, they are considered superior to most fruits and certain vegetables, except for beans and peas. They also have a high content of lysine, essential amino acids, vitamin C, cyanocobalamin, thiamine, riboflavin, iron, calcium, potassium, sodium, phosphorus, and folic acid (Cho & Quimio, 2004).

The objective is to group information about various alternative substrates, showing their advantages and disadvantages. The aim is to highlight the importance and effectiveness of using such substrates, helping with socio-environmental problems such as reducing pollution, reusing agro-industrial waste, and reducing production costs.

METHODOLOGY

This study used a systematic review methodology to find alternative substrates for mushroom cultivation. A literature survey was conducted with a wide range of substrates using the Springer, SciELO, ScienceDirect, and JABB (Journal of Applied Biology & Biotechnology) databases. Articles and books published since 2001 were used, all international. In addition, books in digital format were used, and they were not limited to the year of publication as previously mentioned. All the information found in this study was gathered, discussed, interrelated, and compared using tables and images to facilitate the comparison of the data obtained.

TYPES OF ALTERNATIVE SUBSTRATES AND THEIR COMPOSITIONS FOR MUSHROOM CULTIVATION

SAWDUST

Sawdust is a substrate with a high lignin content, and the wood from which it is made results in different growth rates for the same mushroom species (Zervakis et al., 2001). For *Pleurotus ostreatus* and *Pleurotus eryngii*, as well as for other *Pleurotus* spp., the high levels of lignocellulose, hemicellulose and cellulose can result in a delay in the incubation period, as well as in the branching of the mushroom on the substrate; this delay may be due to the difficulty in metabolizing these organic materials by the extracellular enzymes of the mushrooms (Adebayo et al., 2021).

The wood used for sawdust will result in different rates of carbohydrates, nitrogen, dry matter, proteins, minerals, moisture, crude fiber, and lipids, among others; thus, prior analysis of the substrate serves as an aid in choosing the best substrate, that is, with the best component indexes, for the selected mushroom (Bhattacharjya et al., 2015)

For some mushrooms, such as *L. edodes*, the use of live pieces of wood leads to delayed growth, whereas when previously dried sawdust is used, effective growth of the mushroom is obtained (Shinomura & Hasebe, 2004).

However, supplemented with wheat and rice bran, sawdust-based substrates can produce *P. ostreatus* mushrooms with higher nutritional and medicinal quality than mushrooms generated only from sawdust substrate (Elkanah et al., 2022).

STRAW

Among several types of substrates, wheat straw has a high amount of lignin, cellulose, and hemicellulose, which can provide high biological efficiency. It is widely used as a control substrate in cultivating *Pleurotus* spp. (Yildiz et al., 2002). The agricultural residue from which the straw will be used and the amount to be used influences the mushroom yield (Deora et al., 2021).

Culturing *Pleutorus* sp. in wheat straw results in a mushroom with a higher final weight than when it is grown in other substrates (Dedousi et al., 2024; Melanouri et al., 2022).

Mushrooms such as *Pleurotus* sp. grown on wheat straw substrate have lower yields and lower biological efficiency when compared to those grown on fruit residues. This may be due to the low amount of nutrients that wheat straw still has. (Otieno et al., 2023).

COFFEE RESIDUE

Coffee residues have a high C:N ratio, which may be responsible for the high growth rates that this substrate causes in some mushrooms, such as *Pleurotus* spp. (Akcay et al., 2023; Melanouri et al., 2022). However, the coffee residue substrate has lower lignin levels than in its SMS. Furthermore, *Pleurotus* spp. Mussolini grown on this substrate generates mushrooms with lower weights than other substrates (Melanouri et al., 2022). Juárez et al. (2019) reported that such residue is a good alternative for producing *Pleutorus ostreatus*.

According to Juárez et al. (2019), the protein content of coffee residue can vary from 17 to 35%, which indicates that the place of origin of the substrate, as well as the conditions used in its cultivation, may be able to influence the nutritional composition of the macronutrients. Coffee beans can increase the spawning time, which may occur due to the shape and size of the coffee particles (Membrillo et al., 2011).

Juárez et al. (2019) observed that after the completion of mushroom growth, using coffee residue substrate, 50% caffeine was still present, indicating that the mushroom is not capable of degrading caffeine, as previously observed by Fan et al. (2006) and Ramírez et al., (2007).

GRAIN BRAN

Wheat bran used in substrates serves as a protein source for mushrooms, increasing their crude protein (Olakanmi et al., 2024; Elkanah et al., 2022). In addition, it is a good source of hydroxycinnamic acids, which produce laccase, which in turn is associated with better use of the solid substrate during substrate colonization (Ballentine et al., 2019; El-Batal et al., 2015). Another benefit of wheat bran is the substrate's ability to retain water, thus reducing the mortality of young fruiting bodies due to lack of water (Zakil et al., 2022). Rice bran, on the other hand, contains vitamins such as thiamine, which is necessary for the production of some mushrooms such as *Flammulina velutipes* (Chang and Miles, 2004). According to Masevhe et al., 2016, using substrates such as rice bran, wheat, and oats as an additional nitrogen source for the substrate to produce high-quality mushrooms is possible. Furthermore, substrates supplemented with wheat and rice bran can produce mushrooms with better nutritional and medicinal values than those made on a single substrate, such as sawdust (Elkanah et al., 2022).

When comparing the components in sawdust, wheat bran, rice bran, and palm stem substrates, sawdust has the highest amount of cellulose and hemicellulose. In contrast, wheat and rice bran have the lowest values for these components. Furthermore, palm stem has more lignin than wheat bran (Adebayo et al., 2021). When choosing the best substrate for cultivating the mushroom, these factors should be considered.

SUGARCANE BAGASSE

Sugarcane bagasse is a raw material capable of producing large quantities of value-added products because it contains cellulose and hemicellulose. However, because it includes lignin, saccharification is inhibited, leading to low yields of value-added products (Alokika et al., 2021).

The mushroom *Ganoderma lucidumpoderia* can be grown on substrates where the only carbon source is sugarcane bagasse. The proteins secreted by this mushroom can produce lignocellulosic enzymes, which release monosaccharides from sugarcane bagasse, improving its effectiveness as a substrate (Manalavan et al., 2012).

Sugarcane bagasse and sawdust serve as a good source of organic biomass in cultivating *P. eryngii* (Li et al., 2014). Bagasse generates higher biological yields when supplemented with wheat bran (Hasam et al., 2015).

CORN COB

Corn cob is a substrate with low lignin content, thus favoring the growth of mushrooms such as *P. ostreatus*, *P. eryngii*, and *P. pulmonarius*; this occurs because the low lignin content makes cellulose more available as a carbon source (Zervakis et al., 2001). In addition, corn cob is rich in potassium but poor in sodium, phosphorus, calcium, and magnesium (Ikeda et al., 2021), so the needs of the mushroom you want to grow must be evaluated.

OTHER SUBSTRATES

According to Nerender et al. (2017) and Olakunler et al. (2019), fruit residue substrate has antifungal compounds that can act by slowing down the growth of mushrooms, a fact observed by Otieno et al. (2022) who reported a delay in the appearance of pinheads in some fruit residue substrates.

Agaricus bisporus grown on rice straw and reeds decrease production with each fruiting phase. When cultivated on wheat straw, there is a smaller decrease, and the fruiting body develops better for this substrate. Mushrooms grown on reed straw tend to have low weights and a softer texture that is likely to open the cap. In addition, *A. bisporus* cannot adequately degrade reed straw, making it unavailable as a carbon source. Reed straw ensures high porous ventilation of the substrate during the composting process due to its texture. Although it aids in fermentation, this condition can also result in a decrease in the substrate temperature (Wang et al., 2021).

Hazelnut shells, as well as hazelnut branch residues, are lignocellulose materials that contain cellulose, hemicellulose, and lignin and can serve as a substrate for lignocellulose mushrooms (Guney et al., 2013; Piliga et al., 2022). The yield of *Pleurotus ostreatus* mushrooms grown on hazelnut-based substrates is higher than those grown on wheat straw. Thus, hazelnut residue substrate becomes an alternative to wheat straw-based substrate (Akcay et al., 2023).

Pineapple contains high lignocellulosic content, such as cellulose, lignin, and hemicellulose, making it an effective substrate for the growth of *Volvariella volvacea* (Munir et al., 2013; Narh et al., 2018). Like pineapple, empty oil palm bunches also contain cellulose, lignin, and hemicellulose (Narh et al., 2018).

The pineapple leaf substrate, when compared to empty oil palm bunches, has a higher amount of crude fiber, which contains cellulose, hemicellulose, and lignin, and is therefore essential for the growth of mushrooms (Munir et al., 2023; Carrasco et al., 2018; Hamalatha & Anbuselvi, 2013; Vos et al., 2017). Because empty oil palm bunches have a low crude fiber content, they become a substrate with a low lignocellulosic component. Furthermore, the pineapple leaf substrate has a higher mineral content than the empty oil palm bunch, although both contain essential minerals for the growth of mushrooms. For cultivating *V. volvacea*, the substrate with pineapple peel has higher amounts of essential minerals (Munir et al., 2023).

Therefore, the pineapple leaf substrate is more suitable for the cultivation of *V. volvacea* than the empty oil palm bunch because it has a higher yield and biological efficiency, as well as a shorter time for mycelial colonization and formation of the pinhead and fruiting bodies (Munir et al., 2023).

Table 1. Alternative substrates used in the growth of mushroom fruiting bodies.

Substrate	**Number of uses**	**Substrate**	**Number of uses**	**Substrate**	**Number of uses**
Sawdust	18	Grape pomace	1	Wheat grains	1
Wheat straw	9	Banana	1	Common reed	1
Coffee residues	7	Bean cake	1	Dried mahogany chips	1
Wheat bran	6	Cocoa powder	1	Palm oil (stalk and bunch)	1
Rice straw	6	Avocado peel	1	Reed straw	1
Sugar cane bagasse	5	Pineapple peel	1	Lentil straw	1
Empty palm kernel bunch	5	Peanut shell	1	Quinoa straw	1
Corn cob	5	Banana peel	1	Sorghum straw	1
Cotton residue	4	Cottonseed peel	1	Sawdust	1
Rice husk	3	Orange peel	1	Olive pulp	1
Corn straw	3	Mango peel	1	Banana pseudostem	1
Forestry residues	3	Watermelon peel	1	Diaper waste	1
Corn cob	3	Eggshell	1	Cedrela odorata wood waste	1
Sugar cane	2	Solid digestate of biogas	1	Gliricidia sepia wood waste	1
Rice bran	2	Corn flour	1	*Mangifera indica* wood waste	1
Coconut peat fiber	2	Palm oil fiber	1	Paper waste	1
Banana leaf	2	Pineapple leaf	1	Pueraria root waste	1
Oat straw	2	Palm oil leaf	1	Date palm waste	1

Barley straw	2	Leucaena foliage	1	Hazelnut waste	1
Tea residue	2	Fragments of face mask	1	Soybean powder	1
Corn stubble	2	Mulberry twig	1	SMS ADR*	1
Agar agar	1	Barley grain	1	Peat	1
Beechwood shavings	1	Sorghum grain	1	Total substrates	68

*Spent mushroom substrate from anaerobic digestion residue

EDIBLE MUSHROOMS

PLEUROTUS OSTREATUS AND PLEUROTUS ERYNGII

Pleurotus spp. can grow on various substrates and is popularly known for having enzymes capable of degrading lignin, cellulose, and hemicellulose (Chang & Miles, 2004).

Pleutorus ostreatus is a primary wood decomposer widely distributed in temperate zones. It is the most frequently cultivated species of the genus due to its easy cultivation. There are several strains of the species, each with different degrees of tolerance to cold or heat. The strains have different colors, such as pink, blue, and yellow (Kong, 2004; Russell, 2014).

For cultivation, it is necessary to carry out a low-temperature treatment, around 2°C to 10°C, to form fruiting bodies. The ideal fruiting temperature, the growth of fruiting bodies, is 18°C to 21°C, and the perfect humidity for this period is 90-95%. Fruiting time varies from 2 to 3 weeks (Kang, 2004; Russell, 2014).

Plerotus eryngii, like *Pleurotus ostreatus*, is a primary wood decomposer; however, it is more sensitive to diseases and climatic conditions and grows more slowly than *P. ostreatus* (Cho & Quimio, 2004; Kang et al., 2004). *P. Eryngii* requires low-temperature treatment, and the ideal fruiting temperature is between 13°C and 18°C (Kang, 2004).

Pleurotus eryngii can be grown on substrates based on wheat straw (Deora et al., 2021; Otieno et al., 2022; Dedousi et al., 2024; Melanouri et al., 2022), rice straw, corn, and sorghum (Deora et al., 2021), barley straw, oat straw, rice husk (Dedousi et al., 2024; Melanouri et al., 2022), fruit waste (Otieno et al., 2022), rice husk, coffee waste (Dedousi et al., 2024; Melanouri et al., 2022), sawdust (Dedousi et al., 2024; Li et al., 2024; Melanouri et al., 2022), peanut shell (Li et al., 2024), cotton waste (Sardar et al., 2022; Melanouri et al., 2022), olive pulp, grape pomace, corn cob (Melanouri et al., 2022). Furthermore, as a supplement to the substrates, for better cultivation efficiency, rice bran, wheat bran (Deora et al., 2021), spent mushroom substrate (SMS), commercial compost of fresh and used mushroom (SMS) (Dedousi et al., 2024) and moringa leaf powder (Sardar et al., 2022) can be used. The cultivation of *Pleurotus ostreatus* can be carried out on substrates such as wheat straw (Akcay et al., 2023; Otieno et al., 2022; Dedousi et al., 2024; Melanouri et al., 2022), rice straw, corn straw (Salazar et al., 2020), barley straw, oat straw (Dedousi et al., 2024; Melanouri et al., 2022); 2024; Melanouri et al., 2022), rice husk (Akcay et al., 2023; Dedousi et al., 2024;

Melanouri et al., 2022; Chouhan et al., 2022), wheat grain (Chouhan et al., 2022), coconut shell (Savón et al., 2020), banana leaf (Richard et al., 2020, Chouhan et al., 2022), paper waste, corn stalk (Tesfay et al., 2020), solid biogas digestate (Hultberg et al., 2022), fruit residue (Otieno et al., 2022), cotton residue, grape pomace, corn cob, olive pulp (Melanouri et al., 2022), oil palm residue (Zakil et al., 2020). al., 2022; Adebayo et al., 2021), hazelnut residue (Akcay et al., 2023), sugarcane bagasse, corn cob (Zakil et al., 2022), dried mahogany chips, wheat bran, facial mask fragment (Olakanmi et al., 2024) and palm residue (Elkanah et al., 2022). Calcium carbonate supplements (Zakil et al., 2022; Chouhan et al., 2022), gypsum (Tesfay et al., 2020), lime (Hultberg et al., 2022), spent mushroom substrate (SMS), fresh and used mushroom compost (SMS) (Dedousi et al., 2024), wheat bran (Zakil et al., 2022; Elkanah et al., 2022; Adebayo et al., 2021) and rice bran (Elkanah et al., 2022; Adebayo et al., 2021).

AGARICUS BISPORUS

Agaricus bisporus is the most productive mushroom in India (Maheshwari, 2013), and it requires a large amount of nitrogen, with a C:N (Carbon:Nitrogen) ratio of 17 to 18 during mycelial growth. It also requires fermented compost substrates, such as wheat straw and horse manure (Chang & Miles, 2004).

The ideal temperature for fruiting is 14°C to 18°C , requiring a high percentage of relative humidity. Growth should occur in the dark for good stem and cap development (Chang & Miles, 2004).

Agaricus bisporus can be cultivated on substrates such as wheat straw (Wang et al., 2021; Dedousi et al., 2024), rice straw, reed straw (Wang et al., 2021), barley straw, oat straw, sawdust, rice husk, coffee residue (Dedousi et al., 2024), sugarcane with dairy wastewater (Kumar et al., 2021). Several supplements can be added to the substrate chosen for use, among which we can mention the use of chicken feces, soybean meal, gypsum, and grain vinasse (Wang et al., 2021), spent mushroom substrate (SMS) and fresh and spent mushroom compost (SMC) (Dedousi et al., 2024).

HERECIUM ERINACEUS

Hericium erinaceus belongs to the *Hericiacea* family and has medicinal properties. Its cultivation began in Shanghai. The mushroom can be cultivated on substrates such as sawdust, corncobs, rice straw, and sugarcane bagasse. Gypsum, rice bran, wheat bran, and sucrose can be used as supplements (Chang & Miles, 2004).

The ideal temperature for *H. erinaceus* to fruit is 20°C. After the fruiting bodies appear, the ideal is to raise the temperature to around 25°C. Very low temperatures below 14°C are not recommended, as this may result in the non-formation of fruiting bodies (Chang & Miles, 2004).

Some of the substrates in which *H. erinaceus* can develop are sawdust, rice bran (Chutimanukul et al., 2023; Jahedi et al., 2024), corn cob (Chutimanukul et al., 2023), wheat straw, sugarcane bagasse, wheat bran, soybean powder, and corn flour (Jahedi et al., 2024), mulberry tree branches and puerparia root residue (Fan et al., 2021). Wheat bran, CaSO4, CaCO3 (Fan et al., 2021), and lime (Chutimanukul et al., 2023) can be used to obtain better growth results.

LENTINULA EDODES

Lentinula edodes is native to Asian countries and is known as Shitake (Cho, 2004). It is a fungus that grows on decaying tree trunks or stumps (Chang et al., 2017). It has medicinal properties by inducing the production of interferon and a homothallic life cycle, has a long cultivation, and has an ideal C:N ratio of 20 to 25 during the mycelial growth period (Chang & Miles, 2004; Cho & Quimio, 2004). During the fruiting phase, the C/N content must be balanced; if the nitrogen content is too high, the fruiting bodies will not be formed or developed (Chang et al., 2017). The ideal temperature for fruiting body formation is 10°C to 20°C, while for fruiting, it is 15°C. The perfect substrate pH is, on average, 5.0 to 5.5 (Chang & Miles, 2004; Stamets, 2000).

L. edodes can be cultivated on substrates such as coconut husks (Savón et al., 2020), sawdust (Atila & Cetin, 2024; Arenas et al., 2015), and corn stubble and cob (Arenas et al., 2015). One of the substrates that can be used is liquid biogas residue (Atila & Cetin, 2024).

VOLVARIELLA VOLVACEA

Volvariella volvacea, popularly called straw mushroom, is cultivated seasonally and grows rapidly. It grows best in compost with a lower nitrogen content (Chang & Miles, 2004). Its ideal temperature for mycelial growth is around 32°C to 35°C, and for fruiting, it is 28°C to 32°C. During mycelial growth, its perfect C:N ratio is around 40 to 60 (Chang & Miles, 2004).

V. volvacea can also be cultivated using different substrates; among the most studied are oil palm residues (Triyono et al., 2019; Kamaliah et al., 2021; Munir et al., 2023) and its association with pineapple leaves (Munir et al. 2023). NPK fertilizer, chicken feces, rice bran, and lime (Triyono et al., 2019) can be used together as supplementation.

Table 2. Mushroom species used in the study on the use of alternative substrates.

Mushroom species	Number of uses	Mushroom species	Number of uses
Pleurotus ostreatus	17	*Pleurotus floridanus*	1
Pleurotus eryngii	6	*Podoscypha petalodes*	1
Agaricus bisporus	3	*Pleurotus djamor*	1
Herecium erinaceus	3	*Pleurotus sajor-caju*	1
Lentínula edodes	3	*Flammulina velutipes*	1
Volvariella volvacea	3	*Auricularia cornea*	1
Pleurotus citrinopileatus	2	*Trametes versicolor*	1
Pleurotus pulmonarius	2	*Pleurotus eous*	1
Pleurotus florida	2	*Ganoderma lucidum*	1
Auricularia spp.	1	**Total species**	19

Table 3. Supplements used in conjunction with alternative substrates in the growth of mushroom fruiting bodies.

Supplement	Number of uses	Supplement	Number of uses
Gypsum ($CaSO_4$)	9	Sodium chloride (NaCl)	1
Lime (CaO)	7	Glycerol ($C_3H_8O_3$)	1
Rice bran	7	Betaine	1
Calcium carbonate ($CaCO_3$)	6	Urea	1
Wheat bran	6	Moringa leaf powder	1
Chicken manure	3	Thiourea (CSN_2H_4)	1
Lime	2	Fossil shells	1
Spent mushroom substrate (SMS)	1	Corn flour	1
Fresh and spent commercial mushroom compost (SMC)	1	Soybean meal	1
Liquid biogas residue from cattle manure	1	Grain vinasse	1
Rice straw	1	NPK fertilizer	1
Potassium chloride (KCl)	1	Corn cob	1
Total supplements used			**24**

EVALUATION OF PRODUCTION PARAMETERS: TOOLS TO OPTIMIZE CULTIVATION ON ALTERNATIVE SUBSTRATES

Spent mushroom substrate (SMS) and commercial fresh and used mushroom compost (SMC) are lignocellulosic by-products composed of a mixture of organic matter and mycelium remaining from mushroom cultivation (Economou et al., 2020; Guo et al., 2022).

SMS and SMC can be used in new mushroom cultivation cycles by providing sufficient carbon for growth (Phan et al., 2012; Zied et al., 2020). Primary decomposers such as *Pleurotus*, *Lentinula*, and *Ganodermas* species require substrates with higher C/N and lignin and lower nitrogen content, as in SMS. In contrast, secondary decomposers such as *Agaricus* species require a lower C/N ratio and are capable of growing on substrates already degraded by fungi or bacteria, such as SMC (Ahlawat et al., 2019; Philippoussis et al., 2001).

Thiourea is a sulfur-enriched compound that prevents protein oxidation and helps reduce oxidation catalyzed by copper ascorbate (Burman et al., 2004). In addition, thiourea acts on the homeostasis of cellular ions, increasing the intake and uptake of phosphorus and potassium and the concentration of ascorbic acid (Kaya et al., 2015). Substrates enriched with thiourea increase mycelial growth and biological efficacy (Fozia et al., 2022).

For mushroom cultivation, it is necessary to control several factors, such as temperature, humidity, light, ventilation, vitamins, minerals, and substrate acidity (Cho, 2004; Kang, 2004). Controlling these factors also influences diseases that can affect cultivated mushrooms, favored by high temperatures and humidity (Cha, 2004; Kang, 2004). Temperature and pH influence the need for vitamins, and mushrooms already require some essential vitamins, such as thiamine and biotin (Chang & Miles, 2004).

In the production of *Pleurotus* spp., the temperature during fruiting influences the color of the cap. Lower temperatures can be used to obtain mushrooms with colors such as light brown, while higher temperatures make them paler (Kong, 2004).

The cultivation temperature of mushrooms directly affects fungal growth; enzymatic activity is linked to increased temperature, which inactivates enzymes that result in mushroom growth (Chang & Miles, 2004).

Carbon dioxide (CO2) influences the amount of caps in mushrooms (Kong, 2004). These fungi obtain carbon through the catabolic breakdown of organic compounds, such as organic acids, cellulose, and lignin (Chang & Miles, 2004). CO2 influences the cap of *Pleurotus* spp. The mushrooms produce long stems with small caps at high concentrations of the compound, while at low concentrations, smaller stems are made with wider caps (Kong, 2004). Ventilation is used as a way to control CO2 during the growth of aerobic mushrooms, especially during the reproductive phase. Basidiomycetes may suffer from malformations if the environment they are being cultivated has a large amount of carbon dioxide (Chang & Miles, 2004).

For most *Basidiomycetes*, relative humidity between 95% and 100% and substrate humidity between 50% and 75% are efficient for cultivation, with the need for moisture for the initial, middle, and final stages varying (Cho, 2004).

The effect of light is variable, with some having a positive effect while others have a negative impact. For species that require light, such as *Basidiomycetes*, it triggers the formation of fruiting bodies; however, excessive lighting can cause damage to the mushrooms, destroying vitamins, possibly due to increased temperature (Cho, 2004; Chang & Miles, 2004). For species that do not require light, such as *Agaricus bisporus*, it inhibits the development of primordia, affecting the elongation of the stipes and the expansion of the cap (Chang & Miles, 2004). Liquid culture for mycelial spawning is advantageous for subsequent colonization due to its ease of dissemination. In the laboratory, glucose can be used as a carbon source with other sugars, which tends to be the first to be used. Some species of mushrooms grow better on different carbon sources, so the best carbon source to be used must be evaluated (Chang & Miles, 2004; Russel, 2014). Liquid cultures require agitation, for which different methods of implementation can affect the mass of mycelium, total glucans, α-glucans, and β-glucans, in addition to leading to varying levels of shear stress and aeration, affecting morphological, biochemical characteristics and final productivity (Pilafidis et al., 2024).

CONCLUSION

Several materials, some more popular than others, can be used as alternative substrates for mushroom cultivation. Each substrate has advantages and disadvantages due to the choice of mushrooms and cultivation conditions. Such substrates can increase the biological yield or quality of the mushroom and can be used as base substrates or supplements. Using alternative substrates positively affects the utilization of materials from discarded materials.

LIST OF ABBREVIATIONS

Not applicable

DECLARATIONS

Ethics approval and consent to participate

Not applicable

CONSENT FOR PUBLICATION

All authors agreed with this publication.

AVAILABILITY OF DATA AND MATERIALS

The datasets generated for this study are available upon request from the corresponding author.

COMPETING INTERESTS

There are no competing interests.

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AUTHORS' CONTRIBUTIONS

MB, SK, AFC: research and manuscript writing and discussion.

HT: research coordinator

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