

Review

# Performance of Mushrooms in Fermented Beverages: A Narrative Review

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**Abstract:** Mushrooms are indeed gaining attention for their unique therapeutic and nutritional qualities, especially in fermented drinks. This trend builds on their historical use in traditional medicine, especially within Eastern practices, where mushrooms like reishi, chaga, shiitake, oyster, lion's mane, and cordyceps are known for their immune-boosting, anti-inflammatory, and adaptogenic properties. This narrative review highlights the growing interest in the use of mushrooms as functional ingredients in fermented beverages, emphasizing their technological and functional advantages. Fermentation significantly enhances the nutritional content and bioavailability of mushrooms, making it an ideal method to maximize the health benefits and sensory appeal of mushroom-based beverages. Microbial activity breaks down complex compounds in mushrooms, making their bioactive components more accessible for absorption; bringing unique flavors, aromas, and textures; and creating a rich-sensory experience while offering potential health benefits. Mushrooms can also improve the stability and shelf life of fermented beverages due to the presence of antimicrobial and antioxidant compounds, adding another valuable benefit to their use in functional beverages. However, despite their potential, further research is needed to fully understand their impact on health and to refine production techniques for optimal quality and consistency. This review provides a comprehensive overview of the current knowledge of mushroom-fermented beverages, highlighting both the known benefits and research gaps that require further investigation. Given the early stage of this field, the review emphasizes the importance of the additional investigation to unlock the full potential of mushrooms in functional beverage applications.

**Keywords:** *Ganoderma lucidum*; *Lentinula edodes*; *Pleurotus ostreatus*; *Cordyceps militaris*; *Hericium erinaceus*; wine; beer; kombucha; non-alcoholic beverages



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## 1. Introduction

Functional beverages are increasingly recognized for their health benefits beyond basic nutrition, particularly those produced through fermentation, a key process for enhancing sensory, nutritional, and functional properties. Fermented beverages can be produced from a wide range of raw materials, reflecting cultural traditions and local resources, such as fruits for the production of wines and ciders, cereals for beer, herbs for kombucha, milk for

kefir, seeds or legumes (nut, pistachio, chickpea, fava bean) for beverage alternatives to milk [1–4]. In addition, the development of new fermented mushroom-based beverages, which combine the benefits of fermentation with the bioactive properties of mushrooms, has been receiving much attention in recent years [5,6].

Edible and medicinal mushrooms have been part of the human diet for centuries, appreciated not only for their taste but also for their considerable nutritional benefits [7]. They are particularly valued as a source of protein, making them an excellent substitute for meat, especially in vegetarian and vegan diets [8]. In addition to protein, mushrooms offer a rich supply of carbohydrates, dietary fibre, minerals, fatty acids, essential amino acids, and vitamins [9,10]. Moreover, mushrooms are a natural source of numerous bioactive compounds, including polysaccharides, polyphenols, terpenoids, lectins, sterols, alkaloids, glucoproteins, sesquiterpenes, and ergosterols [11]. Thanks to these bioactive compounds, mushrooms demonstrate a wide range of health-promoting properties, including antioxidant, anticancer, antidiabetic, immunomodulatory, antihypertensive, neuroprotective, hepatoprotective, antifungal, antimicrobial, and antiviral activities [12,13]. Today, approximately 3000 species of edible mushrooms have been identified, with 40–50 varieties extensively cultivated on a commercial scale [14]. Among these, some edible mushroom species, such as *Ganoderma lucidum* (reishi), *Lentinula edodes* (shiitake), and *Pleurotus ostreatus* (oyster mushrooms), are increasingly being explored as substrates for the production of fermented beverages. Fermented mushroom beverages are generally produced by fermenting mushroom fruiting bodies or their extracts using microorganisms such as bacteria, yeasts, or symbiotic cultures [15,16].

Several lactic acid bacteria (LAB) species have been used in the fermentation of mushroom beverages, including *Lactiplantibacillus plantarum* [15,17], *Levilactobacillus brevis* [18], *Lacticaseibacillus casei* [19], *Limosilactobacillus fermentum* [16,20], *Pediococcus acidilactici* [21], and *Lactiplantibacillus pentosus* [22]. Besides LAB, yeasts and moulds are also commonly used as starter cultures for the fermentation of edible mushrooms [23]. Microbial fermentation, in particular through the use of LAB, plays a crucial role in the enhancement of fermented beverages [24,25]. This process also offers multiple advantages in the production of mushroom-fermented beverages, i.e., it imparts unique flavours, enhances nutritional value, and may increase the bioavailability of beneficial compounds such as polysaccharides, antioxidants, and vitamins [15].

Different types of fermented mushroom beverages have been developed and studied on a laboratory scale, each focusing on different quality characteristics, sensory attributes, and health benefits. Among these, wine [26], beer [27], and kombucha [28] were the most studied mushroom-fermented drinks.

Although the use of mushrooms in fermented beverages offers numerous advantages, there are significant challenges associated with their application. A key concern is the risk of contamination by pathogens in the raw materials. Poor-quality mushrooms or sub-optimal production techniques can introduce harmful microorganisms, toxins, or other contaminants into the final product, potentially posing health risks to consumers [29]. Therefore, ensuring high-quality raw materials, observing strict hygiene standards during processing, and implementing robust quality control measures become essential requirements to mitigate these risks.

Based on these assumptions, this narrative review, drawing on recent original research and scientific reviews, highlights the versatility and potential of edible and medicinal mushrooms in the production of fermented beverages, emphasizing both their technological and functional advantages. Mushroom-based fermented drinks are seen as innovative products that combine the health benefits of bioactive compounds with the potential of fermentation, appealing to the increasing demand for functional foods.

## 2. Overview of Mushroom as Fermenting Agents and Their Application

The emerging research on the use of mushrooms as fermenting agents highlights their versatility and potential for food innovation. Both edible and medicinal mushrooms have shown significant promise in fermentation processes due to their rich biochemical profiles and functional properties.

Edible mushrooms, rich in nutritional components such as proteins, carbohydrates, dietary fibres, and fatty acids, are widely cultivated for culinary and dietary purposes [7]. In addition to their good protein content, mushrooms are a relatively good source of nutrients like phosphorus, iron, and vitamins, including thiamine, riboflavin, ascorbic acid, ergosterol, and niacin [10].

Common examples of edible mushrooms used in fermentation include *L. edodes*, *P. ostreatus*, and *G. lucidum*, which have been utilized to create innovative food products such as fermented beverages (jiuqu, wine, kombucha, beer) and fermented food (sausage and soybean meal) [27,30–35].

Most edible mushrooms are also considered medicinal mushrooms since they are rich in bioactive compounds such as polysaccharides, amino acids, and phenolic compounds, which offer antioxidant, antimicrobial, and overall health benefits to consumers [29]. In addition to their health benefits, medicinal mushrooms like *Cordyceps* spp. and lion's mane (*Hericium erinaceus*) are also used as fermenting agents, contributing to the creation of new and innovative food products with added health benefits [36–39].

The use of *Cordyceps* spp. mushrooms as fermenting agents, particularly *C. militaris*, has demonstrated notable improvements in both the sensory and nutritional qualities of various fermented food products. The incorporation of these mushrooms can enhance the flavour, aroma, and bioactive compound content of the final products, making them more appealing and functional for consumers.

Also, the use of *H. erinaceus* as a fermentative agent has also shown promise in enhancing the physicochemical and nutritional properties of various substrates [38,39].

Mushrooms, particularly medicinal varieties, are not only valued for their high nutritional content and bioactive compounds but also for their ability to produce a wide range of enzymes. These enzymes can be harnessed for various applications, particularly in health and medicinal contexts, offering significant benefits for both food processing and therapeutic purposes [40–46] (Table 1).

A study by El-Fakharany et al. [41] explored the use of laccase enzyme extracted from *P. ostreatus*, which was found to inhibit the entry and replication of the hepatitis C virus in peripheral blood cells and hepatoma cells, suggesting a potential therapeutic action against hepatitis C. On the other hand, Takemoto et al. [43] examined the activity of alcohol dehydrogenase from 87 different mushroom strains by measuring the conversion rate of acetaldehyde to ethanol. Among these strains, *S. commune* (2 strains), *L. nuda* Cooke, *C. fuscus*, and *L. edodes* exhibited the highest NADPH levels in the conversion of acetaldehyde to ethanol. Additionally, Kim et al. [44] reported that tannase extracted from the fermentation of *Toxicodendron vernicifluum* (Stokes) with *Fomitella fraxinea* produced oligomeric galloylglucoses, whereas using *Aspergillus niger* resulted in gallic acid production.

The use of mushrooms as fermenting agents not only enhances the nutritional and medicinal value of the substrates they are used with but also provides significant antimicrobial benefits. The bioactive compounds present in medicinal mushrooms, such as polysaccharides, phenolic compounds, and secondary metabolites, contribute to their ability to fight off a wide range of pathogens [47–51] (Table 2).

**Table 1.** Application of enzymes extracted from mushrooms.

Mushroom Species	Enzyme	Uses	Reference
<i>A. cinnamomea</i>	2,3-Oxidosqualene Cyclase	Production of triterpenoids for medicinal use	[40]
<i>P. ostreatus</i>	Laccase	Inhibit Hepatitis C viral infection	[41]
<i>P. ostreatus</i> MBFBL400	Laccase Peroxidase Xylanase 1,4-b-xylosidase	Degrade lignocellulose to be used in mushroom fruit body production	[42]
<i>S. commune</i> <i>L. nuda</i> Cooke <i>C. fuscus</i> <i>L. edodes</i>	Alcohol dehydrogenase	Fermentation of alcoholic beverages	[43]
<i>F. fraxinea</i>	Tannase	Hydrolyses 1,2,3,4,6-penta-O-galloyl- $\beta$ -D-glucose (PGG) into oligomeric galloylglucoses	[44]
<i>B. edulis</i>	Nitrite reductase	Reduce nitrite content during fermentation and act as antidote in intoxicated mice	[45]
<i>A. bisporus</i>	Tyrosinase	Removal of phenolic compounds in wastewater	[46]

Abbreviations: *A. cinnamomea*, *Artrodia cinnamomea*; *P. ostreatus*, *Pleurotus ostreatus*; *P. ostreatus* MBFBL400, *Pleurotus ostreatus* MBFBL400; *S. commune*, *Schizophyllum commune*; *L. nuda* Cooke, *Lepista nuda* Cooke; *C. fuscus*, *Cyclomyces fuscus*; *L. edodes*, *Lentinula edodes*; *F. fraxinea*, *Fomitella fraxinea*; *B. edulis*, *Boletus edulis*; *A. bisporus*, *Agaricus bisporus*.

**Table 2.** Antimicrobial activities of different mushroom species on common pathogens.

Mushrooms Species	Pathogens	Reference
<i>P. ostreatus</i> <i>L. edodes</i> <i>H. tessulatus</i>	<i>S. aureus</i> , <i>B. subtilis</i> , <i>E. coli</i> , <i>P. aeruginosa</i> , <i>S. enterica</i> ser. Typhi, <i>K. pneumoniae</i> , <i>C. albicans</i> , <i>S. cerevisiae</i>	[47]
<i>G. lucidum</i>	<i>A. flavus</i> , <i>S. aureus</i>	[48]
<i>S. commune</i>	<i>S. aureus</i> , <i>B. cereus</i> , <i>Salmonella</i> spp., <i>E. coli</i>	[49]
<i>A. bisporus</i> (Champignon) <i>A. bisporus</i> (Portobello) <i>A. brasiliensis</i> <i>F. velutipes</i> <i>L. edodes</i>	<i>B. cereus</i> , <i>S. aureus</i> , <i>S. enterica</i> ser. Enteritidis, <i>E. coli</i>	[50]
<i>T. striatus</i>	<i>P. aeruginosa</i> , <i>E. coli</i> , <i>B. subtilis</i> , <i>S. aureus</i> , <i>C. albicans</i> , and <i>S. cerevisiae</i>	[51]
<i>L. edodes</i>	<i>S. aureus</i> , <i>L. monocytogenes</i> , <i>E. coli</i> O157	[30]

Abbreviations: *P. ostreatus*, *Pleurotus ostreatus*; *L. edodes*, *Lentinula edodes*; *H. tessulatus*, *Hypsizigus tessulatus*; *G. lucidum*, *Ganoderma lucidum*; *S. commune*, *Schizophyllum commune*; *A. bisporus* (Champignon), *Agaricus bisporus* (Champignon); *A. bisporus* (Portobello), *Agaricus bisporus* (Portobello); *A. brasiliensis*, *Aspergillus brasiliensis*; *F. velutipes*, *Flammulina velutipes*; *T. striatus*, *Termitomyces striatus*; *S. aureus*, *Staphylococcus aureus*; *B. subtilis*, *Bacillus subtilis*; *E. coli*, *Escherichia coli*; *P. aeruginosa*, *Pseudomonas aeruginosa*; *S. enterica* ser. Typhi, *Salmonella enterica* serovar Typhi; *K. pneumoniae*, *Klebsiella pneumoniae*; *C. albicans*, *Candida albicans*; *A. flavus*, *Aspergillus flavus*; *B. cereus*, *Bacillus cereus*; *S. enterica* ser. Enteritidis, *Salmonella enterica* serovar Enteritidis; *B. subtilis*, *Bacillus subtilis*; *L. monocytogenes*, *Listeria monocytogenes*; *E. coli* O157, *Escherichia coli* O157.

This makes mushroom fermentation a promising approach for developing functional foods with added health benefits, particularly in promoting antimicrobial activity against harmful microorganisms.

The antimicrobial activity of mushrooms has been a subject of interest in various studies, with a focus on the bioactive compounds present in different mushroom species. Research has shown that these compounds, particularly phenolic compounds, play a significant role in the antimicrobial effects of mushrooms. Chowdhury et al. [47] investigated the antimicrobial activity of extracts from *P. ostreatus*, *L. edodes*, and *H. tessulatus* (Beech Mushroom). The researchers found that the extracts from these mushrooms, rich in bioactive compounds, exhibited antimicrobial effects against eight microbial strains. In 2019,

Bach et al. [50] discussed the antimicrobial properties of mushroom extracts, emphasizing the role of phenolic compounds in these activities. They found that mushrooms with high phenolic content were effective in inhibiting the growth of various pathogens, whereas mushrooms with lower phenolic content did not exhibit the same inhibitory effects. More recently, Sitati et al. [51] investigated the antimicrobial activity of *T. striatus* extracts using different solvents for extraction (aqueous, methanol, and dichloromethane). Their study revealed that *T. striatus* extracts from all solvents showed significant microbial inhibition against pathogens like *P. aeruginosa*, *E. coli*, *B. subtilis*, and *S. aureus*. Additionally, the extracts exhibited antifungal activity against *C. albicans* and *S. cerevisiae*.

The use of edible and medicinal mushrooms in fermentation showcases their potential to revolutionize functional food and beverage production. As research continues to expand, the integration of mushrooms into fermentation processes is set to become a cornerstone of innovative and sustainable food technologies.

### 3. Alcoholic Mushroom Fermented Beverages

Fermented alcoholic beverages are usually produced through the fermentation process of raw materials such as grapes, cereals such as barley or wheat, and tea, where sugars are converted mainly into alcohol and carbon dioxide by the metabolic activities of microorganisms. There is a wide range of fermentation processes used in the food industry in which the microorganisms responsible are bacteria and yeasts [52].

Recently, mushrooms have emerged as a fascinating alternative in the production of alcoholic beverages, especially wine and beer, achieving notable alcohol concentrations. Different species of mushrooms can be used for fermentation, offering unique flavors and potential health benefits. Furthermore, considering the rich nutritional content of mushrooms, they can serve as a prebiotic source, promoting the development and balance of beneficial gut bacteria known as probiotics, leading to various health advantages [53].

Here is an overview of the main types of mushroom-based alcoholic beverages currently available.

#### 3.1. Wine

The process of wine fermentation is a fascinating blend of natural biology and human intervention, with a history rooted in both ancient practices and modern science. Traditionally, the primary agents of wine fermentation are yeasts, predominantly of the *Saccharomyces* genus, such as *S. cerevisiae*, which is the most widely used yeast due to its high fermentation rate and ethanol tolerance [54]. These microorganisms are naturally present on grape skins and in winery environments and are adept at converting sugars in grape must into ethanol and carbon dioxide. This conversion is catalyzed by a series of enzymes, including alcohol dehydrogenase (ADH), which plays a crucial role in the final steps of alcoholic beverage production.

Recent studies indicate that some mushrooms, including species like *P. ostreatus*, *Flammulina velutipes* (enoki mushroom), *Agaricus blazei* (himematsutake mushroom), and *Tricholoma matsutake* (matsutake mushroom), can also produce alcohol dehydrogenase enzymes. Mushrooms capable of producing alcohol dehydrogenase could play a role in fermentation dynamics, possibly altering alcohol levels or contributing to specific flavour transformations. Okamura et al. [55] described an innovative approach to exploit fungal cultures and their metabolic capabilities in wine production. Using sonication, a technique that uses sound waves to disrupt cell structures, they were able to introduce bioactive fungal compounds into the wine without physical fungal biomass, providing interesting insights into the potential of mushrooms in wine fermentation. In detail, the species *A. blazei* exhibits strong alcohol dehydrogenase (ADH) activity, which plays a key role in converting

acetaldehyde to ethanol during fermentation. Its strong ADH activity likely explains its ability to produce alcohol under both aerobic and anaerobic conditions, a trait also found in traditional yeast like *S. cerevisiae*, which starts fermentative activity when the concentration of glucose in the substrate exceeds, the so-called crabtree effect [56]. This aspect makes *A. blazei* a versatile fermentation agent, potentially suitable for environments where oxygen levels fluctuate. Wines fermented with *P. ostreatus* have also been shown to have the highest alcohol levels, suggesting the presence of efficient metabolic pathways for ethanol production despite its ADH activity being weaker than that of *A. blazei*. It is possible that *P. ostreatus* has other enzymes or metabolic strategies that compensate for its relatively lower ADH activity, enabling higher ethanol yields. Finally, the study highlighted that *F. velutipes* produces wines with the lowest alcohol levels due to its limited ethanol production during fermentation. This characteristic could make it suitable for the production of low-alcohol or non-alcoholic wines, which are increasingly in demand in health-conscious markets [26]. The incorporation of mushrooms like *A. blazei* and *F. velutipes* into wine production offers a remarkable opportunity to create functional wines and fermented beverages that provide not only pleasure but also potential health benefits. In this regard, the study showed that the wine produced with *A. blazei* contained  $\beta$ -D-glucans. These polysaccharides, derived from the fungal cell wall, are known for their anti-cancer properties and immune effects. The wine produced with *F. velutipes*, on the other hand, showed thrombosis-preventing activity. Compounds present in this mushroom may improve blood circulation and reduce the risk of blood clots. This property makes wine made with *F. velutipes* a heart-friendly beverage, potentially complementing the cardiovascular benefits traditionally associated with moderate wine consumption. Furthermore, several authors [55,57] have pointed out that these mushrooms provide vitamins such as thiamine (B1) and riboflavin (B2), as well as dietary fibre and protein, improving the nutritional profile of a wine. Their involvement in the fermentation process could potentially introduce distinctive flavour traits, adding new dimensions to the taste profiles of wine.

It has also been seen that mushrooms can play an important role in grape wine production, where sometimes a lack of nitrogen sources in grape juice leads to a limitation of yeast growth and fermentation rate [58]. Different studies highlighted that *L. edodes* can be used as an easily accessible nitrogen source during the alcoholic fermentation process due to their high protein content [59].

Lin et al. [60] showed that shiitake stipe, a by-product of the mushroom industry, could be used to improve the technological performance of *S. cerevisiae* strains during the production of a new alcoholic beverage also containing cane sugar, with positive consumer acceptance.

In addition, *L. edodes* are highly valued for their health benefits due to their rich composition of bioactive compounds, including phenolic compounds and ergothioneine (with antioxidant activity), ergosterol,  $\beta$ -glucans, and erythadenine (known for cholesterol-lowering properties), as well as antihypertensive peptides and lentinine (which exhibits antithrombotic activity).  $\beta$ -glucans extracted from *L. edodes* demonstrate potent antitumor activity, exhibiting cytotoxic effects on breast cancer cells. They also possess immunomodulatory and antiviral properties, such as reducing the secretion of IL-1 $\beta$  and IL-6 [61].

In a recent study by Geng et al. [27], different amounts of *L. edodes* powder, known for its rich umami flavour and medicinal properties, were incorporated into the fermentation process of Huangjiu, a traditional Chinese rice wine. The mushroom powder was mixed with Jiuku wheat and water to initiate fermentation, and the influence of shiitake mushrooms on the microbial dynamics of the fermentation process and volatile compounds was investigated. The study highlighted that the addition of *L. edodes* influenced the microbial population during fermentation. More precisely, the richness and uniformity of

the microbial community composition of Jiuqu were higher when the amount of shiitake mushroom powder was lower, and *Aspergillus* and *Paecilomyces* were the most dominant microorganisms at the genus level. The most abundant volatile compounds found in the wine were ketones, alcohols, and esters, which contributed to aromas of malt, roasted hazelnut, almond, mushroom, and fresh fruit, as well as a slightly creamy aroma.

Another species of mushroom used in winemaking is *G. lucidum*, commonly known as reishi or lingzhi, an edible and healing fungus native to China but also imported to European and North American countries for its countless health benefits [62]. This mushroom is rich in bioactive compounds, including polysaccharides, triterpenoids, and antioxidants. These compounds are believed to boost immunity, reduce inflammation, and provide anti-ageing effects. Furthermore, its potential role in winemaking is particularly intriguing, as the mushroom has an earthy and slightly bitter flavour, which could add depth to wines. Fermentation with *G. lucidum* may, therefore, also influence the flavour profile, potentially creating a different product from conventional grape wines. *G. lucidum* has indeed been utilized in innovative ways across different winemaking traditions, showcasing its versatility and health-promoting attributes. *G. lucidum* has been effectively utilized in the production of functional beverages, where its powder form or extracts derived from the fruiting body and mycelia are incorporated during fermentation. These forms of *G. lucidum*, obtained through extraction with alcohol and hot water, are rich in bioactive compounds and have already been used in the production of classic Australian wine [63] or Korean rice wine (Yakju) [64]. In both cases, through small- and large-scale fermentations, *G. lucidum* demonstrated its potential to transform traditional alcoholic beverages into functional and healthy products. In detail, Kim et al. [64] evaluated the effects of the fruiting body of *G. lucidum* on the alcohol fermentation of yakju, a traditional Korean rice wine. The effects of the amount of *G. lucidum* added on the acceptability and functionality of *G. lucidum*-yakju were investigated. The authors demonstrated that the fermentation of a yakju rice wine with *G. lucidum* extract mixed in the rice mash improved the consumer acceptability of the product. Furthermore, the alcohol content of the beverage was slightly higher or similar to that of yakju produced without *G. lucidum*, suggesting that its fruiting body did not influence ethanol production. Furthermore, *G. lucidum*-yakju showed high angiotensin I-converting enzyme (ACE) inhibitory activity, suggesting its use in the formulation of a new functional Korean traditional rice wine with antihypertensive properties. Nguyen et al. [62] evaluated the impact of *G. lucidum* on the primary alcoholic fermentation and secondary, malolactic fermentation of shiraz wine, finding that the addition of its extract had no impact on wine fermentation but influenced the sensory profiles and chemical composition of the resulting wines. Using HS-SPME-GC-MS and sensory analysis, the authors defined the aromas perceived in wines containing *G. lucidum* extracts. A woody aroma, pepper, mushroom, spice, or floral flavours, as well as sweetness, are the main sensory characteristics found. In addition, wines produced with *G. lucidum* had a red to brownish colour depending on the amount of extract used [62]. Nguyen et al. [65] also evaluated consumer acceptance of this wine, finding a notably positive attitude among both Australian and Asian consumers. This suggests that there is a promising market for innovative wines that appeal to health-conscious consumers, offering not only traditional enjoyment but also additional health benefits.

In addition to wine production, *G. lucidum* has also been successfully used as a raw material for the production of a special grain brandy [66]. The authors investigated the influence of extraction parameters (time and concentration) on the colour, total phenolic content, antioxidant capacity, sensory characteristics, and triterpenoid acid content in brandy. A total of 15 triterpenoid acids were found, with total contents ranging from 2.63 to 4.06 mg/100 mg. Among these, ganoderic acid A was the most frequently detected

triterpenoid acid. The total phenolic content of the analysed samples ranged from 34.07 to 118.1 mg/L GAE. Notably, the colour and sensory characteristics of brandies enriched with *G. lucidum* were significantly improved compared to samples without. These results suggest that the obtained samples represent a promising new product with enhanced antioxidant capacity and worldwide market potential [66].

The integration of mushrooms into the production of fermented alcoholic beverages clearly paves the way for the creation of hybrid products that combine the characteristics of traditional fermented alcoholic beverages with the nutritional and biochemical benefits of mushrooms. Future research will likely focus on optimizing these production methods and exploring their full potential for both industrial and artisanal alcoholic beverage applications.

### 3.2. Beer

Beer is one of the oldest alcoholic beverages and is the most consumed worldwide [67]. It is defined as the product obtained by the alcoholic fermentation conducted with strains of *S. cerevisiae* or *S. carlsbergensis* of a wort prepared from barley malt, wheat malt or their mixtures, and water, bittered with hops or their derivatives or both. This definition covers both conventional and commercialized beer [68].

This beverage contains numerous ingredients that exert physiological effects upon consumption, attributable to its diverse composition (water, malt, hops, brewer's yeast, non-malted cereals, starch, or starch syrups as adjuncts) [69]. Characterized by a high carbohydrate concentration and the presence of proteins, amino acids, vitamins, organic acids, more than 30 different micro- and macro-elements, and a range of antioxidants, beer is considered a product of great nutritional value [70]. Current research aims to improve its functional and technological features through the addition of natural compounds such as fruits, spices, plants, or herbal extracts [71]. Furthermore, there is much interest in the addition of raw mushroom powders or their extracts for the development of unconventional beers with the aim of adding unique flavors and improving the functional aspects of the beverages [72]. Mushrooms commonly used in beer to enhance its aroma include shiitake mushrooms that promote malty, roasted nut and honey-like flavors [27]; chanterelle mushrooms that can impart a slight buttery, umami-infusing texture to the beer, with an apricot-like aroma [73,74]; and porcini mushrooms that are praised since they can confer earthy and roasted notes to the products [75].

The analysis of volatile compounds in beers is a very important aspect of understanding how additives can affect the flavor profile of beers.

Cirlincione et al. [76] added two different amounts (5 and 10 g/L) of *Pleurotus eryngii* var. *eryngii* in powder form added at different production of beer and highlighted improved yeast viability during the alcoholic fermentation and enhanced the sensorial profile of the final product.

Mushrooms used in beer production also for their therapeutic and medical features include *G. lucidum*, a woody *Basidiomycotina* fungus belonging to the *Ganodermataceae* family of *Polyporales* [72]. *G. lucidum* is an edible mushroom that has been used for thousands of years in Traditional Chinese Medicine due to the belief in its ability to reduce the risk of cancer and the incidence of heart disease, as well as to boost the human immune system [62]. It contains a wide variety of bioactive components in the fruiting body, mycelium, and spores, such as triterpenoids, which are characterized by their antioxidative, immune-modulating, and antitumor effects. They are also characterized by the presence of polysaccharides, as well as phenols, steroids, amino acids, lignin, mycines, vitamins, nucleosides, and nucleotides [77]. The mushroom addition can be achieved during wort boiling, fermentation, maturation, and packaging of the beer [71]. The aim of this addition



is to provide bioactive compounds in order to improve the antioxidant activity and sensory characteristics of the beer [78].

Leskosek-Cukalovic et al. [72] added *G. lucidum* mushroom extract in powder form in the range of 0.1–1.5 mL/L of beer, adhering to the recommended daily dose of 1.8–4.8 g established by the FDA (FDA, memorandum no. 953–03 16, 1999, FDA no. 955-0316, 2003) and demonstrated that triterpenes and fatty acids, bioactive compounds present in *G. lucidum*, were successfully incorporated into the beer.

In some cases, mushrooms are also added to replace fermenting yeasts for the production of unconventional beers. The highest alcohol concentrations in beer were obtained with edible and medicinal mushrooms *P. eryngii*, *T. matsutake*, and *F. velutipes* [55,57]. *P. eryngii* var. *eryngii* also contains various bioactive compounds such as polyphenols, polysaccharides, peptides, and dietary fibres. It was added in powder form in both pre-alcoholic and post-alcoholic fermentation in combination with yeasts. This addition improved yeast viability due to carbohydrates in the fruiting body of *P. eryngii* that may be available to the yeast [76]. Mushroom-enriched beers showed higher alcohol content than the control beer. In terms of sensory evaluation, the mushroom-enriched beers showed a cocoa and mushroom taste and aroma [76]. *T. matsutake* and *F. velutipes* are edible mushrooms rich in fibre, protein, and vitamins, in addition to having a preventive effect on cancer and thrombosis [55,57].

In summary, the scientific literature highlighted that brewing beer with mushrooms offers a unique blend of enhanced flavors and health benefits and is in line with current market trends favoring functional beverages. These unconventional beers are an exciting option for both brewers and consumers alike. Certainly, further research is needed to assess the possible applications of different edible mushrooms to different beer styles.

#### 4. Low- and Non-Alcoholic Mushroom Fermented Beverages

Fermented low- (LAFB) and non-alcoholic beverages (NAFB) are usually produced through the fermentation of milk, cereals, fruits, and vegetables but do not lead to significant alcohol content production, making them suitable for a wider audience, including those who avoid alcohol for health, personal, or cultural reasons. These beverages have been incorporated into European diets for several years and are gaining popularity with consumers because of their prolonged shelf-life and nutrient composition, contributing to food security and improving food safety. There is a variety of traditional LAFB and NAFB consumed in European regions, such as *kefir*, *kvass*, *kombucha*, *amazake*, and *hardaliye* or others from fresh fruits [79,80]. In addition, in the last few years, edible and medicinal mushrooms have also been increasingly incorporated into non-alcoholic fermented beverages, leveraging their unique flavors and potential health benefits. Lao et al. [81] investigated the application of *C. militaris*, an Ascomycete that originated in East Asia, to create a non-alcoholic fermented beverage. *C. militaris* is rich in proteins, amino acids, and bioactive compounds such as polysaccharide cordycepin, cordycepic acid (D-mannitol), and superoxide dismutase (SOD). Cordycepin has several functions that impact health, such as anti-tumor, hypoglycemic, immunomodulatory, antibacterial, and anti-inflammatory action [82]. However, this mushroom has an unpleasant smell and taste, which obviously makes the drink unacceptable to the consumer. To improve the sensory aspects of the beverage, Lao et al. [81], other than the powder of *C. militaris* fungus, added hydrolytic enzymes (cellulase, pectinase) and exploited the fermentative properties of lactic acid bacteria (*L. plantarum*, *L. acidophilus*), bifidobacteria (*B. lactis*, *B. longum*) and yeasts (*S. cerevisiae*). The authors verified that this fermentation and the enzymatic process improves the sensory quality and bioavailability of nutrients. The enzymatic activity of cellulase and pectinase destroys *C. militaris* cells and releases reducing sugars and polysaccharides [83], providing

nutrition for lactic acid bacteria and protecting cordycepin from decomposition. With fermentation, sourness gradually increases, while bitterness gradually decreases due to the acids masking the other flavors. In addition, yeast fermentation produces a small amount of alcohol, which imparts a smooth taste. The fermentation with LAB and yeasts produces a pleasantly fragrant and aromatic odor, then a pleasant flavor and enzyme treatments significantly enhance the antioxidant activity of fermented *C. militaris*. Another non-alcoholic beverage fermented with mushrooms was exploited by Wang et al. [84]. In this study, cane sugar was processed into juice, which was then fermented with *G. lucidum* mycelium. Sugar cane (*Saccharum officinarum* L.) is a raw material typical of tropical and sub-tropical areas of the world and is used for the production of sugar and ethanol [85]. The sugarcane juice was collected after washing and pressing, then diluted and used as a fermentation medium. Sugar cane juice provides nitrogen, carbon, inorganic salt, and other factors for the growth of *G. lucidum*, as well as polysaccharides, proteins, and other functional compounds available after fermentation. *G. lucidum* mycelium was inoculated into the sugarcane juice medium and allowed to ferment for 10 days. After fermentation, the result was a non-alcoholic fermented beverage. Other authors have also evaluated the addition of *L. edodes* in a beverage containing a mixture of Pilsner, Munich, and wheat malt type [86], forming the must. In this study, a pre-culture of shiitake mushroom mycelia was added to the must in different amounts and allowed to ferment at different agitation rates, providing a different amount of oxygen. Analyses carried out during fermentation showed that as the inoculum concentration increased, the concentration of dissolved oxygen in the medium decreased, reducing biomass productivity and the final biomass concentration. In addition, the effect of fermentation on the composition of volatile compounds was also evaluated, showing the predominance of a fruity odor resulting mainly from the production of 2 methylbutanoate. In this way, by incorporating *L. edodes* into the fermentation process at a low agitation rate, a new fermented non-alcoholic beverage with a fruity, slightly acidic, sweetish, fresh flavour and a reddish-brown colour was produced. Also, Aljumayi et al. [87] demonstrated that fresh white mushrooms (*A. bisporus*), added as a powder in kefir milk, can positively affect cholesterol-related and metabolism-related pathologies, such as hypercholesterolemia, hyperlipidemia, and metabolic syndrome.

They investigated the feeding of these mushrooms and kefir milk in rats with hyperlipidemia, demonstrating the hyperlipidemia anti-properties of this mixture. This study highlighted that the mixture of mushroom powder and kefir milk reduced body weight in rat models with hyperlipidemia, as well as glucose levels and lipid profile, and improved intestinal bacterial flora, creating a well-balanced composition of gut microbiota. These results highlighted that the combination of prebiotics and probiotics can positively affect cholesterol-related and metabolism-related pathologies, such as hypercholesterolemia, hyperlipidemia, and metabolic syndrome [87].

### *Kombucha*

Kombucha, a fermented, effervescent, low-alcohol, sweetened tea beverage, has grown in popularity due to its health benefits and unique flavor profile. Typically, sweetened black or green tea (with added sucrose) is used in kombucha preparation, but alternative substrates such as coconut water and grape juice were used [88,89].

Traditionally, kombucha fermentation relies on a symbiotic culture of bacteria such as acetic acid bacteria (AAB), lactic acid bacteria (LAB), and yeast (SCOBY). These microorganisms convert tea and sugar into a bioactive, slightly alcoholic, probiotic beverage. Together with bacteria, yeasts are fundamental to the fermentation process of kombucha, playing a pivotal role in its sensory, flavor development, carbonation, and bioactive compounds [90]. The ability of yeasts to efficiently ferment the sugars available in these substrates directly

influences the production of ethanol, which is critical for the subsequent oxidation by acetic bacteria into acetic acid and other acids, contributing to the flavor profile of kombucha. The yeasts component of kombucha comprises a diverse range of species across several genera, including *Zygosaccharomyces*, *Candida*, *Saccharomyces*, *Brettanomyces/Dekkera*, *Pichia*, *Schizosaccharomyces*, among others [90].

Several alternative substrates, such as cultivated or wild medicinal mushrooms, have been used to partially or totally replace tea with the aim of improving the aromatic and functional properties of this beverage [91,92].

The use of edible and functional mushrooms, such as *G. lucidum*, *L. edodes*, and *Coriolus versicolor* (turkey tail) in kombucha production, offers a novel approach that could potentially reshape the beverage profile. These mushrooms possess bioactive properties that may enrich kombucha with additional health benefits, such as anti-inflammatory, immunomodulatory, and antioxidant effects [93].

Currently, most of the available literature focuses on using mushroom extract as part of the fermentation substrate rather than incorporating it directly into the bacteria and yeast environment. This preference is likely due to the complexity of balancing the delicate dynamics between yeast and bacteria.

Edible mushrooms, especially those with recognized bioactive compounds, could enhance kombucha's functional properties. The inclusion of mushrooms in kombucha fermentation could introduce unique bioactive properties into the beverage.

*C. versicolor* is a medicinal mushroom with immunomodulatory activities [94] since it stimulates the production of interferons like IFN- $\gamma$ , interleukins (IL-2, IL-1 $\beta$ ), and tumour necrosis factor (TNF- $\alpha$ ). In a study by Sknepnek et al. [93], the cytotoxic activity and immunomodulatory effects of polysaccharides formed during the fermentation process of kombucha were found with both *C. versicolor* and *L. edodes*. The Kombucha extract of *C. versicolor* showed a more complex and higher total polysaccharide content but also higher levels of phenols and flavonoids than the kombucha extract of *L. edodes*. Both mushrooms also stimulated yeast ethanol production and AAB activity, reducing fermentation time.

Morales et al. [95] developed a beverage using two different truffle species in combination with three different symbiotic consortia of bacteria and yeasts. The two truffle species used are *Tuber melanosporum* and *Tuber aestivum*. Their use improved the chemical-physical, biochemical, and sensory parameters of the beverage; reduced the sugar content, minimized alcohol, increased protein, and phenolic compounds compared to traditional kombucha; and also achieved antioxidant, antiproliferative, and antidiabetic effects [91]. Truffle kombucha fermented for up to 14 days exhibited a more complex volatile organic compound (VOC) profile, with *T. melanosporum* producing a richer VOC profile than *T. aestivum* [95].

In another study, to improve both the antioxidant and organoleptic properties of kombucha, Sittisart et al. [28] added hemp leaves to milky mushroom flour (*Calocybe indica*). Hemp leaves contain many bioactive compounds, such as flavonoids, phenols, and cannabinoids, that promote antioxidant activity, while *C. indica* has several nutritional and beneficial properties for humans since it is rich in proteins, lipids, fibre, carbohydrates, vitamins, essential amino acids, and low-fat products [96].

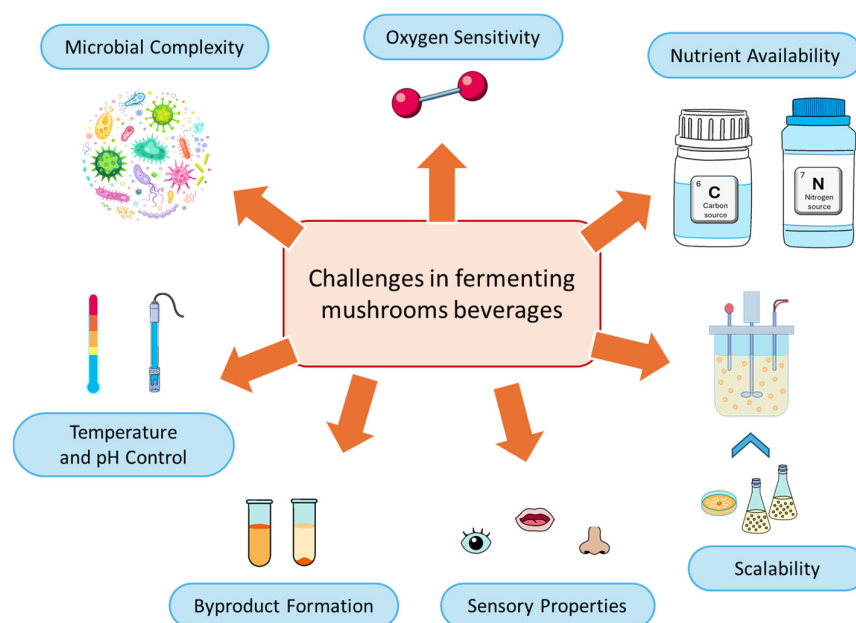
The use of *G. lucidum* for kombucha production was also evaluated. Its addition significantly improved antioxidant activity and showed strong antibacterial properties, with a distinctive chemical profile [93,97], but it also provided new phenolic compounds and flavonoids [97].

The mushroom addition also influenced microbial dynamics, such as the appearance of unique microbial genera such as *Setophoma* (25.91%) and *Macrocybe* (94.55%) at the beginning, but also *Dekkera*. The promotion of the growth of beneficial LAB and the

modification of the microbial composition, supporting fermentation, acidification, and antioxidant activity, highlight their functional potential [28].

## 5. Challenges and Future Perspectives

Mushroom-fermented beverages present a unique and promising area for experimentation, combining the flavors and functional properties of mushrooms with the potential benefits of fermentation. However, critical challenges must be overcome to ensure their successful development and commercialization (Figure 1). For mushroom-fermented beverages to be successful, it is essential to address these key challenges: selecting the right mushroom strains, optimizing fermentation conditions, and ensuring product stability and safety [98]. Although these mushrooms are generally considered safe for most people when used properly, some of the substances they contain may pose risks or cause side effects to the health of consumers. Furthermore, mushrooms are susceptible to chemical contamination. Some authors, in fact, found traces of heavy metals such as arsenic, cadmium, mercury, and plumb in mushrooms [99].



**Figure 1.** Critical challenges in fermented mushroom beverages production.

Also, Ab Rhaman et al. [100] observed that *G. lucidum* can accumulate heavy metals from the environment in which it is grown, especially if it is grown in polluted soil or water. Heavy metal contamination, such as lead, arsenic, or cadmium, can be toxic when consumed.

Advances in microbiology, fermentation technology, and quality control can help overcome these challenges. By focusing on these areas, mushroom-fermented beverages could find a strong place in the market, offering consumers a unique combination of taste and health benefits.

The microbial diversity that is naturally present in mushrooms adds complexity to their use as a fermentation substrate. This diversity includes a range of bacteria, yeasts, and fungi, which can vary depending on the mushroom species, growing environment, and post-harvest handling. While this microbial community offers potential for innovation in mushroom-fermented beverages, it also presents significant challenges in ensuring consistency, safety, and quality during fermentation [101]. In fact, microorganisms present in the substrate can impact mushroom cultivation both in positive and negative ways.

By using strategies such as pre-treatment, selective fermentation conditions, and robust quality control measures, it is possible to harness the complex microbial community of mushrooms while minimizing the risks. The application of advanced microbial and fermentation technologies, as highlighted by Suwannarach et al. [101], will be instrumental in overcoming these challenges and unlocking the full potential of mushroom fermentation.

Balancing the aerobic growth requirements of mushrooms with the anaerobic conditions needed for fermentation poses a unique challenge [102]. Mushrooms require oxygen for their metabolic activities, including the production of flavor precursors and bioactive compounds, while fermentation often thrives in low-oxygen or anaerobic environments. This conflict can complicate the development of mushroom-fermented beverages.

Furthermore, it is necessary to ensure that the substrate used for mushroom fermentation contains adequate nutrients both for the growth of the mushrooms and for the success of the fermentation process. Mushrooms and fermentation microbes require specific nutrients, particularly carbon and nitrogen sources, to thrive, metabolize effectively, and produce desired flavors, textures, and bioactive compounds [103]. If the substrate lacks these essential components, the growth and metabolic activities of both mushrooms and fermentative microorganisms may be compromised. Substrate enrichment, pre-treatment, and regular monitoring can optimize nutrient availability and enhance both the efficiency and quality of mushroom-fermented beverages. By addressing these nutritional needs, the substrate can support both mushroom metabolism and microbial fermentation processes effectively.

To ensure successful fermentation, maintaining ideal pH and temperature levels is crucial. Deviations from these conditions can result in spoilage, off-flavors, or reduced product quality [104].

Fermentation can produce numerous by-products, some of which may be undesirable. Therefore, careful monitoring and adjustment of fermentation conditions are essential to managing these by-products effectively [105].

Finally, as reported by several authors [106,107], scaling up mushroom fermentation for commercial production can be challenging due to the complexity of the process and the need for continuous quality control.

Therefore, future research should focus on optimizing fermentation processes to enhance flavor profiles, nutritional content, and the production of bioactive compounds. Exploring new mushroom strains and fermentation techniques could lead to the creation of unique and appealing beverages (Figure 2).

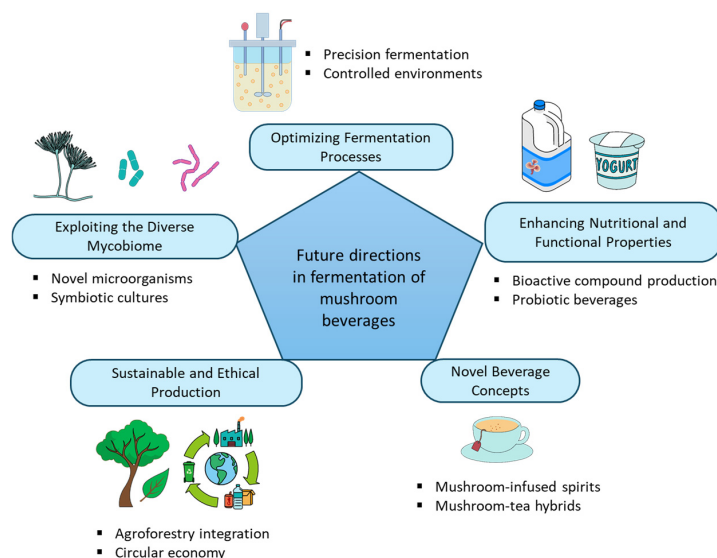


Figure 2. Future directions in fermentation of mushroom beverages.

Additionally, understanding the underlying mechanisms behind bioactive compound synthesis and their impacts on human health can guide the development of targeted products. Addressing these key challenges can position mushroom-fermented beverages as a valuable innovation in the food and beverage industry. Expanding the range of microorganisms used in fermentation, including bacteria and wild yeasts associated with different mushroom varieties, can enhance the functional properties and flavour profiles of the product. Therefore, developing bacterial and fungal co-cultures holds the potential for improving both their functional qualities and flavour attributes [108].

Chen et al. [23] studied the effects of fermentation on the umami taste substances of *L. edodes*, using different microorganism species (*S. cerevisiae*, *A. oryzae*, *A. niger*, and *L. plantarum*) and found that *L. plantarum* was the best species for fermentation providing the strongest umami flavor, highlighting a positive impact deriving from their co-inoculation. Ayar-Sümer et al. [109] demonstrated that lactic acid bacteria fermentation of mushrooms with high phenolic content leads to the liberation of bound phenolics, enhancing their bioactivity and bioaccessibility.

A novel beverage concept could be realized by integrating mushrooms into both alcoholic and tea beverages, representing an exciting opportunity to combine unique flavours with health benefits. By using distilled yeasts to ferment mushroom substrates and combining the revitalizing effects of tea with the functional properties of medicinal mushrooms, these beverage concepts offer consumers a new and health-conscious drinking experience. The innovation could appeal to health-conscious consumers while also appealing to those seeking new and interesting flavours in their alcoholic and non-alcoholic beverages [110]. Finally, by combining sustainable mushroom cultivation practices with innovative strategies for recycling and upcycling by-products, the mushroom industry can reduce waste, increase resource efficiency, and contribute to a more sustainable and circular economy [111]. Furthermore, by addressing these challenges, researchers and industry professionals can unlock the full potential of mushroom fermentation to create novel, nutritious, and environmentally friendly beverages that align with the growing demand for functional foods. The development of such products will represent a significant step forward in both environmental sustainability and the health-conscious beverage market.

## 6. Conclusions

From the in-depth literature analysis conducted within this review, it strongly emerges that the integration of mushrooms into fermented beverages represents a promising approach for enhancing the functional, health, and sensory properties of different fermented drinks.

However, the success of their incorporation depends on carefully managing fermentation conditions to ensure that the mushrooms' medicinal properties are preserved and the beverage maintains a desirable taste profile. Research into specific mushroom strains, fermentation techniques, and health benefits is ongoing. With advancements in fermentation technology and a deeper understanding of the interplay between mushrooms and microbes, the production of mushroom-based fermented beverages may become more refined, leading to better-tasting and more health-oriented products.

By exploiting the unique bioactive profile of mushrooms, beverages could be made that not only satisfy taste preferences but also provide a range of health benefits, from immune support to antioxidant protection to potential anti-cancer properties. This innovation is in line with the growing demand for functional foods and beverages that support holistic health and well-being.

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